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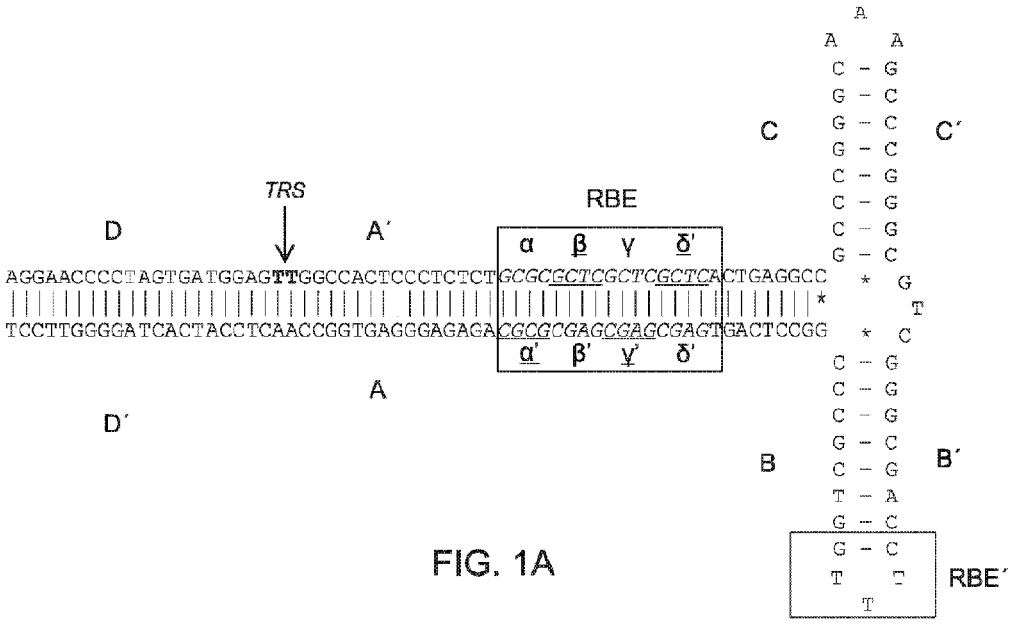


FIG. 1A

(57) Abstract: The application describes ceDNA vectors having linear and continuous structure for delivery and expression of a transgene. ceDNA vectors comprise an expression cassette flanked by two ITR sequences, where the expression cassette encodes a transgene encoding FVIII protein. Some ceDNA vectors further comprise cis-regulatory elements, including regulatory switches. Further provided herein are methods and cell lines for reliable gene expression of FVIII protein *in vitro*, *ex vivo* and *in vivo* using the ceDNA vectors. Provided herein are methods and compositions comprising ceDNA vectors useful for the expression of FVIII protein in a cell, tissue or subject, and methods of treatment of diseases with said ceDNA vectors expressing FVIII protein. Such FVIII protein can be expressed for treating disease, *e.g.*, hemophilia A.

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NON-VIRAL DNA VECTORS AND USES THEREOF FOR EXPRESSING FVIII THERAPEUTICS

RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 63/079,349, filed on September 16, 2020, and U.S. Provisional Application No. 63/132,838, filed on December 31, 2020, the contents of each of which are hereby incorporated by reference in their entireties.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on September 16, 2021, is named 131698-08220_SL.txt and is 1,915,851 bytes in size.

TECHNICAL FIELD

[0003] The present disclosure relates to the field of gene therapy, including non-viral vectors for expressing a transgene or isolated polynucleotides in a subject or cell. The disclosure also relates to nucleic acid constructs, promoters, vectors, and host cells including the polynucleotides as well as methods of delivering exogenous DNA sequences to a target cell, tissue, organ or organism. For example, the present disclosure provides methods for using non-viral ceDNA vectors to express FVIII, from a cell, *e.g.,* expressing the FVIII therapeutic protein for the treatment of a subject with a hemophilia A. The methods and compositions can be used *e.g.,* for treating disease by expressing the FVIII in a cell or tissue of a subject in need thereof.

BACKGROUND

[0004] Gene therapy aims to improve clinical outcomes for patients suffering from either genetic mutations or acquired diseases caused by an aberration in the gene expression profile. Gene therapy includes the treatment or prevention of medical conditions resulting from defective genes or abnormal regulation or expression, *e.g.,* underexpression or overexpression, that can result in a disorder, disease, malignancy, *etc*. For example, a disease or disorder caused by a defective gene might be treated, prevented or ameliorated by delivery of a corrective genetic material to a patient, or might be treated, prevented or ameliorated by altering or silencing a defective gene, *e.g.,* with a corrective genetic material to a patient resulting in the therapeutic expression of the genetic material within the patient.

[0005] The basis of gene therapy is to supply a transcription cassette with an active gene product (a transgene), *e.g.,* that can result in a positive gain-of-function effect, a negative loss-of-function effect, or another outcome. Such outcomes can be attributed to expression of a therapeutic protein such as an antibody, a functional enzyme, or a fusion protein. Gene therapy can also be used to treat a disease or

malignancy caused by other factors. Human monogenic disorders can be treated by the delivery and expression of a normal gene to the target cells. Delivery and expression of a corrective gene in the patient's target cells can be carried out via numerous methods, including the use of engineered viruses and viral gene delivery vectors. Among the many virus-derived vectors available (*e.g.,* recombinant retrovirus, recombinant lentivirus, recombinant adenovirus, and the like), recombinant adeno-associated virus (rAAV) is gaining popularity as a versatile vector in gene therapy.

[0006] Adeno-associated viruses (AAV) belong to the *Parvoviridae* family and more specifically constitute the *dependoparvovirus* genus. Vectors derived from AAV (*i.e.,* recombinant AAV (rAVV) or AAV vectors) are attractive for delivering genetic material because (i) they are able to infect (transduce) a wide variety of non-dividing and dividing cell types including myocytes and neurons; (ii) they are devoid of the virus structural genes, thereby diminishing the host cell responses to virus infection, *e.g.,* interferon-mediated responses; (iii) wild-type viruses are considered non-pathologic in humans; (iv) in contrast to wild-type AAV, which are capable of integrating into the host cell genome, replication-deficient AAV vectors lack the *rep* gene and generally persist as episomes, thus limiting the risk of insertional mutagenesis or genotoxicity; and (v) in comparison to other vector systems, AAV vectors are generally considered to be relatively poor immunogens and therefore do not trigger a significant immune response (see ii), thus gaining persistence of the vector DNA and potentially, long-term expression of the therapeutic transgenes.

[0007] However, there are several major deficiencies in using AAV particles as a gene delivery vector. One major drawback associated with rAAV is its limited viral packaging capacity of about 4.5 kb of heterologous DNA (Dong *et al.*, 1996; Athanasopoulos *et al.*, 2004; Lai *et al.*, 2010), and as a result, use of AAV vectors has been limited to less than 150,000 Da protein coding capacity. The second drawback is that as a result of the prevalence of wild-type AAV infection in the population, candidates for rAAV gene therapy have to be screened for the presence of neutralizing antibodies that eliminate the vector from the patient. A third drawback is related to the capsid immunogenicity that prevents re-administration to patients that were not excluded from an initial treatment. The immune system in the patient can respond to the vector which effectively acts as a "booster" shot to stimulate the immune system generating high titer anti-AAV antibodies that preclude future treatments. Some recent reports indicate concerns with immunogenicity in high dose situations. Another notable drawback is that the onset of AAV-mediated gene expression is relatively slow, given that single-stranded AAV DNA must be converted to double-stranded DNA prior to heterologous gene expression.

[0008] Additionally, conventional AAV virions with capsids are produced by introducing a plasmid or plasmids containing the AAV genome, rep genes, and cap genes (Grimm *et al.*, 1998). However, such encapsidated AAV virus vectors were found to inefficiently transduce certain cell and tissue types and the capsids also induce an immune response.

[0009] Accordingly, use of adeno-associated virus (AAV) vectors for gene therapy is limited due to the single administration to patients (owing to the patient immune response), the limited range of transgene genetic material suitable for delivery in AAV vectors due to minimal viral packaging capacity (about 4.5kb), and slow AAV-mediated gene expression.

[0010] There is large unmet need for disease-modifying therapies in hemophilia A. Current therapies are burdensome and require, *e.g.*, slow drip intravenous (IV) administrations. First, these Factor VIII injectables do not provide continuous delivery of factors, with trough levels allowing bleeding episodes. Second, there are no approved gene therapies for hemophilia A, and AAV based therapies cannot be used by 25% to 40% of patients due to pre-existing antibodies. AAV can only be administered once, and the resulting Factor VIII levels might not reach clinical significance, or may be supranormal, as dose levels cannot be titrated. Third, many hemophilia A patients cannot utilize these therapies because of the development of neutralizing antibodies to these exogenous, artificial clotting factors.

[0011] Accordingly, there is need in the field for a technology that permits expression of a therapeutic FVIII protein in a cell, tissue or subject for the treatment of hemophilia A.

BRIEF DESCRIPTION

[0012] The technology described herein relates to methods and compositions for treatment of hemophilia A by expression of Factor VIII (FVIII) protein from a capsid-free (*e.g.,* non-viral) DNA vector with covalently-closed ends (referred to herein as a "closed-ended DNA vector" or a "ceDNA vector"), where the ceDNA vector comprises a FVIII nucleic acid sequence or codon optimized versions thereof. These ceDNA vector can be used to produce FVIII proteins for treatment, monitoring, and diagnosis. The application of ceDNA vectors expressing FVIII to the subject for the treatment of hemophilia A is useful to: (i) provide disease modifying levels of FVIII enzyme, (ii) be minimally invasive in delivery, (iii) be repeatable and dosed-to-effect, (iv) have rapid onset of therapeutic effect, (v) result in sustained expression of corrective FVIII enzyme in the liver, (vi) restore urea cycle function, and/or (vii) be titratable to achieve the appropriate pharmacologic levels of the defective enzyme.

[0013] In embodiments, a ceDNA-vector expressing FVIII is optionally present in a liposome nanoparticle formulation (LNP) for the treatment of hemophilia A. The ceDNA vectors described herein can provide one or more benefits including, but not limited to providing disease modifying levels of FVIII, being minimally invasive in delivery, being repeatable and dosed-to-effect, providing a rapid onset of therapeutic effect, *e.g.,* in some embodiments, within days of therapeutic intervention, providing sustained expression of corrective Factor VIII levels in the circulation, being titratable to achieve the appropriate pharmacologic levels of the defective coagulation factor, and/or providing treatments for other types of hemophilia, including but not limited to Factor VIII deficiency (hemophilia A) or Factor IX deficiency (hemophilia B) or Factor XI deficiency (hemophilia C).

[0014] Accordingly, the disclosure described herein relates to a capsid-free (*e.g.*, non-viral) DNA vector with covalently-closed ends (referred to herein as a "closed-ended DNA vector" or a "ceDNA vector") comprising a heterogeneous gene encoding FVIII, to permit expression of the FVIII therapeutic protein in a cell (*e.g.*, hepatocytes of a human patient suffering from hemophilia A).

[0015] According to one aspect, the disclosure provides a capsid-free close-ended DNA (ceDNA) vector comprising at least one nucleic acid sequence, *e.g.*, heterologous nucleic acid sequence, between flanking inverted terminal repeats (ITRs), wherein at least one heterologous nucleic acid sequence encodes at least one FVIII protein, wherein the at least one nucleic acid sequence that encodes at least one FVIII protein is selected from any of the sequences in **Table 1A** (SEQ ID NOs: 71-183, 556 and 626-633).

[0016] In a first aspect, the disclosure provides a capsid-free close-ended DNA (ceDNA) vector comprising at least one nucleic acid sequence between flanking inverted terminal repeats (ITRs), wherein the at least one nucleic acid sequence encodes at least one FVIII protein, wherein the at least one nucleic acid sequence that encodes at least one FVIII protein is selected from a sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or least 99% identity to any sequence in **Table 1A** (SEQ ID NOs: 71-183, 556 and 626-633). According to some embodiments, the at least one nucleic acid sequence that encodes at least one FVIII protein is at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or least 99% identical to SEQ ID NO: 556. According to some embodiments, the at least one nucleic acid sequence that encodes at least one FVIII protein consists of SEQ ID NO: 556. According to some embodiments, the at least one nucleic acid that encodes at least one FVIII protein comprises SEQ ID NO: 556, wherein SEQ ID NO: 556 further comprises one or more modifications. According to some embodiments, the at least one nucleic acid comprising SEQ ID NO: 556, further comprising one or more modifications comprises or consists of SEQ ID NO: 627. According to some embodiments, the at least one nucleic acid comprising SEQ ID NO: 556, further comprising one or more modifications comprises or consists of SEQ ID NO: 628. According to some embodiments, the at least one nucleic acid comprising SEQ ID NO: 556, further comprising one or more modifications comprises or consists of SEQ ID NO: 628. According to some embodiments, the at least one nucleic acid comprising SEQ ID NO: 556, further comprising one or more modifications comprises or consists of SEQ ID NO: 630. According to some embodiments, the at least one nucleic acid comprising SEQ ID NO: 556, further comprising one or more modifications comprises or consists of SEQ ID NO: 631. According to some embodiments, the at least one nucleic acid comprising SEQ ID NO: 556, further comprising one or more modifications comprises or consists of SEQ ID NO: 632. According to some embodiments, the at least one nucleic acid comprising SEQ ID NO: 556, further comprising one or more modifications comprises or consists of SEQ ID NO: 633.

[0017] In some embodiments, the ceDNA vector comprises a promoter or promoter set operatively linked to the least one nucleic acid sequence that encodes at least one FVIII protein. According to some embodiments, the at least one nucleic acid sequence that encodes at least one FVIII protein is selected from any of the sequences in **Table 1A** (SEQ ID NOs: 71-183, 556 and 626-633). In some embodiments, the ceDNA vector comprises a promoter selected from the group consisting of human a1 antitrypsin (hAAT) promoter, minimal transthyretin promoter (TTRm), hAAT_core_C06, hAAT_core_C07, hAAT_core_08, hAAT_core_C09, hAAT_core_C10, and hAAT_core_truncated. In some embodiments, the ceDNA vector comprises a promoter selected from a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or least 99% identity to any one of SEQ ID NOs: 210-217. In some embodiments, the promoter set comprises a synthetic liver specific promoter set including enhancers and a core promoter, without a 5pUTR. In some embodiments, the promoter set is selected from a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or least 99% identity to any one of SEQ ID NOs: 184-197, 400, 401, 484, and 617-624.

[0018] According to some embodiments, the at least one nucleic acid sequence that encodes the at least one FVIII protein is selected from any of the sequences in **Table 1A** (SEQ ID NOs: 71-183, 556 and 626-633) and the promoter set is selected from a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or least 99% identity to any one of SEQ ID NOs: 184-197, 400, 401, 484, and 617-624. According to some embodiments, the at least one nucleic acid sequence that encodes the at least one FVIII protein is selected from a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or least 99% identity to any one of SEQ ID NOs: 556 or 626-633 and the promoter set is selected from a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or least 99% identity to any one of SEQ ID NOs: 184-197, 400, 401, 484, and 617-624.

[0019] In some embodiments, the ceDNA vector comprises an enhancer. In some embodiments, the enhancer is selected from the group consisting of: a Serpin enhancer (SerpEnh), the transthyretin (TTRe) gene enhancer (TTRe), the Hepatic Nuclear Factor 1 binding site (HNF1), Hepatic Nuclear Factor 4 binding site (HNF4), Human apolipoprotein E/C-I liver specific enhancer (ApoE_Enh), the enhancer region from Pro-albumin gene (ProEnh), a CpG minimized version of the ApoE_Enh (Human apolipoprotein E/C-I liver specific enhancer) (ApoE_Enh_C03, ApoE_Enh_C04, ApoE_Enh_C09, and ApoE_Enh_C10), and Hepatic nuclear factor enhancer array embedded in GE-856 (Embedded_enhancer_HNF_array). In some embodiments, the Serpin enhancer comprises a nucleic acid sequence at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or least 99% identical to SEQ ID NO: 198. In some embodiments, the enhancer is selected from a nucleic acid sequence set forth in **Table 7** (SEQ ID NOs: 198-209, 485 and 557-616). In some embodiments, the enhancer is selected from a nucleic acid sequence having at least 85%, at least 90%,

at least 95%, at least 96%, at least 97%, at least 98%, or least 99% identity to any sequence in **Table 7** (SEQ ID NOs: 198-209, 485 and 557-616).

[0020] According to some embodiments, the at least one nucleic acid sequence that encodes the at least one FVIII protein is selected from any of the sequences in **Table 1A** (SEQ ID NOs: 71-183, 556 and 626-633) and the enhancer is selected from a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or least 99% identity to any one of SEQ ID NOs: 198-209, 485 and 557-616. According to some embodiments, the at least one nucleic acid sequence that encodes the at least one FVIII protein is selected from a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or least 99% identity to any one of SEQ ID NOs: 556 or 626-633 and the enhancer is selected from a nucleic acid sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or least 99% identity to any one of SEQ ID NOs: 557-616.

[0021] In some embodiments, the ceDNA vector comprises a 5' UTR sequence. In some embodiments, the 5' UTR sequence is selected from a sequence having at least 85% identity to any sequence in **Table 10**. In some embodiments, the ceDNA vector comprises an intron sequence. In some embodiments, the intron sequence is selected from a sequence having at least 85% identity to any sequence in **Table 11**. In some embodiments, the ceDNA vector comprises an exon sequence. In some embodiments, the exon sequence is selected from a sequence having at least 85% identity to any sequence in **Table 12**. In some embodiments, the ceDNA vector comprises a 3' UTR sequence. In some embodiments, the exon sequence is selected from a sequence having at least 85% identity to any sequence in **Table 13**. In some embodiments, the ceDNA vector comprises at least one poly A sequence. In some embodiments, the ceDNA vector comprises one or more DNA nuclear targeting sequences (DTS). In some embodiments, the DTS is selected from a sequence having at least 85% identity to any sequence in **Table 14**. In some embodiments, the ceDNA vector comprises one or more of the following Ubiquitous Chromatin-opening Elements (UCOEs), Kozak sequences, spacer sequences or leader sequences.

[0022] In one embodiment of any of the foregoing aspects of embodiments, at least one nucleic acid sequence is cDNA.

[0023] In one embodiment of any of the foregoing aspects of embodiments, at least one ITR comprises a functional terminal resolution site and a Rep binding site.

[0024] In one embodiment of any of the foregoing aspects of embodiments, one or both of the ITRs are from a virus selected from a parvovirus, a dependovirus, and an adeno-associated virus (AAV). In some embodiments, the flanking ITRs are symmetric or asymmetric. In some embodiments, the flanking ITRs are symmetrical or substantially symmetrical. In some embodiments, the flanking ITRs are asymmetric. In some embodiments, one or both of the ITRs are wild-type, or wherein both of the ITRs are wild-type. In some embodiments, the flanking ITRs are from different viral serotypes. In

some embodiments, the flanking ITRs are from the same viral serotypes. In some embodiments, the flanking ITRs are from a pair of viral serotypes shown in Table 6 of International Publication No. WO/2019/161059 (incorporated by reference in its entirety herein). In some embodiments, one or both of the ITRs comprises a sequence selected from the sequences in **Table 2, Table 4A, Table 4B,** or **Table 5**. In some embodiments, at least one of the ITRs is altered from a wild-type AAV ITR sequence by a deletion, addition, or substitution that affects the overall three-dimensional conformation of the ITR. In some embodiments, one or both of the ITRs are derived from an AAV serotype selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, and AAV12. In some embodiments, one or both of the ITRs are synthetic. In some embodiments, one or both of the ITRs is not a wild-type ITR, or wherein both of the ITRs are not wild-type. In some embodiments, one or both of the ITRs is modified by a deletion, insertion, and/or substitution in at least one of the ITR regions selected from A, A', B, B', C, C', D, and D'. In some embodiments, the deletion, insertion, and/or substitution results in the deletion of all or part of a stem-loop structure normally formed by the A, A', B, B' C, or C' regions. In some embodiments, one or both of the ITRs are modified by a deletion, insertion, and/or substitution that results in the deletion of all or part of a stem-loop structure normally formed by the B and B' regions. In some embodiments, one or both of the ITRs are modified by a deletion, insertion, and/or substitution that results in the deletion of all or part of a stem-loop structure normally formed by the C and C' regions. In some embodiments, one or both of the ITRs are modified by a deletion, insertion, and/or substitution that results in the deletion of part of a stem-loop structure normally formed by the B and B' regions and/or part of a stem-loop structure normally formed by the C and C' regions. In some embodiments, one or both of the ITRs comprise a single stem-loop structure in the region that normally comprises a first stem-loop structure formed by the B and B' regions and a second stem-loop structure formed by the C and C' regions. In some embodiments, one or both of the ITRs comprise a single stem and two loops in the region that normally comprises a first stem-loop structure formed by the B and B' regions and a second stem-loop structure formed by the C and C' regions. In some embodiments, one or both of the ITRs comprise a single stem and a single loop in the region that normally comprises a first stem-loop structure formed by the B and B' regions and a second stem-loop structure formed by the C and C' regions. In some embodiments, both ITRs are altered in a manner that results in an overall three-dimensional symmetry when the ITRs are inverted relative to each other. In some embodiments, one or both of the ITRs comprises a nucleic acid sequence selected from the sequences in **Tables 2, 4A, 4B**, and **5**.

[0025] In some embodiments of any of the above aspects or embodiments, the ceDNA vector comprises a nucleic acid sequence selected from a sequence having at least 85% identity, at least 90% identity, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%,

at least 98%, at least 99% or at least 100% identity with a sequence in **Table 18** (*e.g.*, any one of SEQ ID NOs: 1-70, 442-483, or 642-646).

[0026] In another aspect, the disclosure provides a method of expressing an FVIII protein in a cell comprising contacting the cell with the ceDNA vector of any one of the aspects or embodiments herein. In some embodiments, the cell is a photoreceptor or a RPE cell. In some embodiments, the cell in *in vitro* or *in vivo*. In some embodiments of any of the above aspects or embodiments, the at least one nucleic acid sequence is codon optimized for expression in the eukaryotic cell. In some embodiments of any of the above aspects or embodiments, the at least one nucleic acid sequence is a sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or least 99% identity to any sequence set forth in **Table 1A** (*e.g.,* any one of SEQ ID NOs: 71-183, 556 and 626-633).

[0027] In another aspect, the disclosure provides a method of treating a subject with hemophilia A, comprising administering to the subject a ceDNA vector of any one of the aspects or embodiments herein, wherein at least one nucleic acid sequence encodes at least one FVIII protein.

[0028] In another aspect, the disclosure provides a method of treating a subject with hemophilia A, comprising administering to the subject a nucleic acid sequence selected from a sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or least 99% identity with a sequence in **Table 18** (*e.g.*, any one of SEQ ID NOs: 1-70, 442-483, or 642-646). According to one embodiment, the nucleic acid sequence is at least 85% identical, at least 90% identical, at least 91% identical, at least 92% identical, at least 93% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 96% identical, at least 97% identical, at least 98% identical or at least 99% identical to SEQ ID NO: 5. In one embodiment, the nucleic acid sequence comprises SEQ ID NO: 5. In another embodiment, the nucleic acid sequence consists of SEQ ID NO: 5. In some embodiments of any of the above aspects or embodiments, the ceDNA vector comprises a nucleic acid sequence selected from a sequence having at least 85% identity, at least 90% identity, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identity with SEQ ID NO: 42. In some embodiments, the ceDNA comprises a nucleic acid sequence consisting of SEQ ID NO: 42.

[0029] In another aspect, the disclosure provides a method of treating a subject with hemophilia B, comprising administering to the subject a nucleic acid sequence selected from a sequence having at least 85% identity, at least 90% identity, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or at least 100% identity with a sequence in **Table 18** (*e.g.*, any one of SEQ ID NOs: 1-70, 442-483, or 642-646).

[0030] In some embodiments of any of the above aspects or embodiments, levels of FVIII in the serum of the subject are increased in subjects administered the ceDNA vector compared to a control. In some embodiments, the increase in levels of FVIII is greater than about 40% compared to the

control. In some embodiments, the at least one nucleic acid sequence is a sequence having at least 85% identity, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or least 99% to any sequence set forth in **Table 1A** (*e.g.,* any one of SEQ ID NOs: 71-183, 556 and 626-633) or **Table 18** (*e.g.,* any one of SEQ ID NOs: 1-70, 442-483, or 642-646). According to one embodiment, the nucleic acid sequence is at least 85% identical, at least 90% identical, at least 91% identical, at least 92% identical, at least 93% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 96% identical, at least 97% identical, at least 98% identical or at least 99% identical to SEQ ID NO: 5. According to one embodiment, the nucleic acid sequence comprises SEQ ID NO:5 or consists of SEQ ID NO: 5.

[0031] In some embodiments of the foregoing aspect or embodiments, a level of FVIII in the plasma of the subject is increased in the subject after administration. In some embodiments, the level of FVIII in the plasma of the subject is increased by at least about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, 5-fold, 10-fold, 15-fold, 20-fold, 25-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, or 100-fold after administration. In some embodiments, a level of FVIII in the serum of the subject is increased in the subject administered the ceDNA vector as compared to a control. In some embodiments, the increase in the level of FVIII in the serum of the subject is greater than about 40%, 50%, 60%, 70%, 80%, 90%, 100%, 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, 5-fold, 10-fold, 15-fold, 20-fold, 25-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, or 100-fold compared to the control. In some embodiments, the control is a level of FVIII in the serum of the subject prior to administration, or wherein the control is a level of FVIII in the serum of a subject having hemophilia A who did not receive the administration.

[0032] In some embodiments, the ceDNA vector is administered at a dose of about 0.1 mg/kg, 0.2 mg/kg, 0.3 mg/kg, 0.4 mg/kg, 0.5 mg/kg, 0.75 mg/kg, 1 mg/kg, 1.5 mg/kg, 2 mg/kg, 2.5 mg/kg, 3 mg/kg, 3.5 mg/kg, 4 mg/kg, 5 mg/kg, 6 mg/kg, 7 mg/kg, 8 mg/kg, 9 mg/kg, or 10 mg/kg. In some embodiments, the ceDNA vector is administered at a dose of about 0.1 mg/kg to about 20 mg/kg. In some embodiments, the ceDNA vector is administered at a dose of about 0.1 mg/kg to about 15 mg/kg. In some embodiments, the ceDNA vector is administered at a dose of about 0.1 mg/kg to about 10 mg/kg. In some embodiments, the ceDNA vector is administered at a dose of about 0.1 mg/kg to about 5 mg/kg. In some embodiments, the ceDNA vector is administered at a dose of about 0.1 mg/kg to about 0.5 mg/kg. In some embodiments, the ceDNA vector is administered at a dose of about 0.5 mg/kg to about 20 mg/kg. In some embodiments, the ceDNA vector is administered at a dose of about 0.5 mg/kg to about 15 mg/kg. In some embodiments, the ceDNA vector is administered at a dose of about 0.5 mg/kg to about 10 mg/kg. In some embodiments, the ceDNA vector is administered at a dose of about 0.5 mg/kg to about 5 mg/kg. In some embodiments, the ceDNA vector is administered at a dose of about 1 mg/kg to about 20 mg/kg. In some embodiments, the ceDNA vector is administered at a dose of about 1 mg/kg to about 15 mg/kg. In some embodiments, the ceDNA vector

is administered at a dose of about 1 mg/kg to about 10 mg/kg. In some embodiments, the ceDNA vector is administered at a dose of about 1 mg/kg to about 5 mg/kg. In some embodiments, the ceDNA vector is administered at a dose of about 5 mg/kg to about 20 mg/kg. In some embodiments, the ceDNA vector is administered at a dose of about 5 mg/kg to about 15 mg/kg. In some embodiments, the ceDNA vector is administered at a dose of about 5 mg/kg to about 10 mg/kg. In some embodiments, the ceDNA vector is administered at a dose of about 10 mg/kg to about 20 mg/kg. In some embodiments, the ceDNA vector is administered at a dose of about 10 mg/kg to about 15 mg/kg. In some embodiments, the ceDNA vector is administered at a dose of about 15 mg/kg to about 20 mg/kg. In some embodiments, the ceDNA vector is administered at a dose of about 0.5 mg/kg, 0.75 mg/kg, 1 mg/kg, 1.5 mg/kg, 2 mg/kg, 2.5 mg/kg, 3 mg/kg, 3.5 mg/kg, 4 mg/kg, or 5 mg/kg. In some embodiments, the ceDNA vector is administered at a dose of about 0.5 mg/kg, 1 mg/kg, 1.5 mg/kg, 2 mg/kg, 2.5 mg/kg, 3 mg/kg, 3.5 mg/kg, 4 mg/kg, or 5 mg/kg.

[0033] In some embodiments, the administration restores at least about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% of FVIII plasma levels of normal individuals not affected by hemophilia A. In some embodiments, the administration restores at least about 10% of FVIII plasma levels of normal individuals not affected by hemophilia A. In some embodiments, the administration restores at least about 15% of FVIII plasma levels of normal individuals not affected by hemophilia A. In some embodiments, the administration restores at least about 20% of FVIII plasma levels of normal individuals not affected by hemophilia A. In some embodiments, the administration restores at least about 25% of FVIII plasma levels of normal individuals not affected by hemophilia A. In some embodiments, the administration restores at least about 30% of FVIII plasma levels of normal individuals not affected by hemophilia A. In some embodiments, the administration restores at least about 35% of FVIII plasma levels of normal individuals not affected by hemophilia A. In some embodiments, the administration restores at least about 40% of FVIII plasma levels of normal individuals not affected by hemophilia A. In some embodiments, the administration restores at least about 45% of FVIII plasma levels of normal individuals not affected by hemophilia A. In some embodiments, the administration restores at least about 50% of FVIII plasma levels of normal individuals not affected by hemophilia A.

[0034] In some embodiments of any of the above aspects or embodiments, the ceDNA vector is administered to a photoreceptor cell, or an RPE cell, or both.

[0035] In some embodiments of any of the above aspects or embodiments, the ceDNA vector expresses the FVIII protein in a photoreceptor cell, or an RPE cell, or both.

[0036] In some embodiments of any of the above aspects or embodiments, the ceDNA vector is administered by any one or more of subretinal injection, suprachoroidal injection or intravitreal injection.

[0037] In another aspect, the disclosure provides a pharmaceutical composition comprising the ceDNA vector of any one of the aspects or embodiments herein.

[0038] In another aspect, the disclosure provides a cell containing a ceDNA vector of any of the aspects or embodiments herein. In some embodiments, the cell is a photoreceptor cell, or a RPE cell, or both.

[0039] In another aspect, the disclosure provides a composition comprising a ceDNA vector of any of the aspects or embodiments herein, and a lipid. In some embodiments, the lipid is a lipid nanoparticle (LNP). In another aspect, the disclosure provides a composition comprising a ceDNA vector, wherein the ceDNA vector comprises a nucleic acid sequence at least 85% identical, at least 90% identical, at least 91% identical, at least 92% identical, at least 93% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 96% identical, at least 97% identical, at least 98% identical or at least 99% identical to, comprises, or consists of SEQ ID NO: 5, and a lipid. In another aspect, the disclosure provides a composition comprising a ceDNA vector, wherein the ceDNA vector comprises a nucleic acid sequence at least 85% identical, at least 90% identical, at least 91% identical, at least 92% identical, at least 93% identical, at least 94% identical, at least 95% identical, at least 96% identical, at least 96% identical, at least 97% identical, at least 98% identical or at least 99% identical to, comprises, or consists of SEQ ID NO: 42, and a lipid. In some embodiments, the lipid is an LNP.

[0040] In another aspect, the disclosure provides a kit comprising the ceDNA vector of any of the aspects or embodiments herein, the pharmaceutical composition of any of the aspects or embodiments herein, the cell of any of the aspects or embodiments herein, or the composition of any of the aspects or embodiments herein.

[0041] In another aspect, the disclosure provides capsid-free close-ended DNA (ceDNA) vector comprising at least one nucleic acid sequence between flanking inverted terminal repeats (ITRs), wherein at least one nucleic acid sequence encodes at least one protein, wherein the ceDNA vector comprises a promoter or promoter set operatively linked to the least one nucleic acid sequence that encodes the at least one protein, and wherein the promoter is selected from the group consisting of human a1 antitrypsin (hAAT) promoter, minimal transthyretin promoter (TTRm), hAAT_core_C06, hAAT_core_C07, hAAT_core_08, hAAT_core_C09, hAAT_core_C10, and hAAT_core_truncated. In some embodiments, the promoter is selected from a nucleic acid sequence having at least 85% identity to any one of SEQ ID NOs: 210-217. In some embodiments, the promoter set comprises a synthetic liver specific promoter set including enhancers and core promoter, without 5pUTR. In some embodiments, the promoter set is selected from a nucleic acid sequence having at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 100% identity to, comprises, or consists of any one of SEQ ID NOs: 184-197, 400, 401, 484, and 617-624.

[0042] In some embodiments of any of the aspects or embodiments herein, the ceDNA vector comprises an enhancer. In some embodiments, the enhancer is selected from the group consisting of: a Serpin enhancer (SerpEnh), the transthyretin (TTRe) gene enhancer (TTRe), the Hepatic Nuclear Factor 1 binding site (HNF1), Hepatic Nuclear Factor 4 binding site (HNF4), Human apolipoprotein E/C-I liver specific enhancer (ApoE_Enh), the enhancer region from Pro-albumin gene (ProEnh), a CpG minimized version of the ApoE_Enh (Human apolipoprotein E/C-I liver specific enhancer) (ApoE_Enh_C03, ApoE_Enh_C04, ApoE_Enh_C09, and ApoE_Enh_C10), and Hepatic nuclear factor enhancer array embedded in GE-856 (Embedded_enhancer_HNF_array). In some embodiments, the Serpin enhancer comprises a nucleic acid sequence at least 85% identical to SEQ ID NO: 198. In some embodiments, the enhancer is selected from a nucleic acid sequence having at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 100% identity to, comprises, or consists of any one of SEQ ID NOs: 198-209, 485 and 557-616.

[0043] In another aspect, the disclosure provides a method of expressing a protein in a cell comprising contacting the cell with the ceDNA vector of any of the aspects or embodiments herein. In some embodiments, the cell is a photoreceptor or a RPE cell. In some embodiments, the cell in *in vitro* or *in vivo*. In some embodiments of any of the aspects or embodiments herein, the at least one nucleic acid sequence is codon optimized for expression in the eukaryotic cell.

[0044] In some embodiments of any of the aspects or embodiments herein, the at least one nucleic acid sequence that encodes at least one FVIII protein is selected from a nucleic acid sequence having at least 85% identity to any one of SEQ ID NOs: 556 and 626-633, and wherein the ceDNA vector comprises an enhancer, wherein the enhancer is selected from a nucleic acid sequence having at least 85 % identity to any one of SEQ ID NOs: 557-616.

[0045] In another aspect, the disclosure provides a DNA vector comprising a nucleic acid sequence at least 85% identical to SEQ ID NOs: 71-183, 556 and 626-633. In some embodiments, the DNA vector comprises an enhancer sequence having at least 95% identity to any one of SEQ ID NOs: 198-209, 485, 557-616. In some embodiments, the DNA vector comprises a SerpEnh sequence having at least 95% identity to any one of SEQ ID NOs: 198 and 557-616. In some embodiments, the DNA vector comprises a SerpEnh sequence having at least 95% identity to any one of SEQ ID NOs: 557-616. In some embodiments, the DNA vector comprises a SerpEnh sequence having at least 95% identity to any one of SEQ ID NOs: 557-568. In some embodiments, the DNA vector comprises a SerpEnh sequence having at least 95% identity to any one of SEQ ID NOs: 569 and 570.

[0046] In some embodiments, wherein the DNA vector comprises a SerpEnh sequence having at least 95% identity to any one of SEQ ID NO: 571. In some embodiments, the DNA vector comprises a SerpEnh sequence having at least 95% identity to any one of SEQ ID NO: 572. In some embodiments, the DNA vector comprises a SerpEnh sequence having at least 95% identity to any one

of SEQ ID NO: 611. In some embodiments, the DNA vector comprises a SerpEnh sequence having at least 95% identity to any one of SEQ ID NO: 603.

[0047] In some embodiments of the aspects and embodiments herein, the DNA vector comprises a TTRe sequence. In some embodiments, the TTRe sequence is set forth in SEQ ID NO: 199 or a sequence having at least 95% identity thereof. In some embodiments, the DNA vector comprises a TTR promoter. In some embodiments, the TTR promoter is set forth in SEQ ID NO: 211 or a sequence having 95% identity thereof. In some embodiments, the DNA vector comprises a 5' untranslated region (5' UTR) sequence selected from the group consisting of SEQ ID NO: 411, SEQ ID NO: 412, SEQ ID NO: 413, SEQ ID NO: 414, SEQ ID NO: 415 , SEQ ID NO: 416 , SEQ ID NO: 417, SEQ ID NO: 418, SEQ ID NO: 419, SEQ ID NO: 420, SEQ ID NO: 421, SEQ ID NO: 422, SEQ ID NO: 423, SEQ ID NO: 424, SEQ ID NO: 425, SEQ ID NO: 426, SEQ ID NO: 427, SEQ ID NO: 428, SEQ ID NO: 429, SEQ ID NO: 430, SEQ ID NO: 431, SEQ ID NO: 432, SEQ ID NO: 433, SEQ ID NO: 434, SEQ ID NO: 435, and SEQ ID NO: 436. In some embodiments, the DNA vector comprises an intron sequence selected from the group consisting of SEQ ID NO: 235, SEQ ID NO: 236, SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239 , SEQ ID NO: 240 , SEQ ID NO: 241, SEQ ID NO: 242, SEQ ID NO: 243, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, and SEQ ID NO: 248. In some embodiments, the DNA vector further comprises an intron sequence having at least 95% identity to SEQ ID NO: 235. In some embodiments, the DNA vector comprises a 3'UTR sequence. In some embodiments, the 3'UTR sequence comprises a WPRE element and/or bGH poly A signal sequence or a sequence having at least 95% identity to any one of SEQ ID NOs: 283-291 and 634. In some embodiments, the DNA vector comprises a mircroRNA (mir) sequence set forth in SEQ ID NO: 543 or a sequence having at least 95% identity thereof. In some embodiments, the DNA vector comprises a spacer sequence selected from a sequence having at least 85% identity to any sequence set forth in Table 15 (SEQ ID NOs:318-332 and 635-641). In some embodiments, the DNA vector comprises at least one ITR flanking 5' and/or 3' end of the nucleic acid sequence at least 95% identical to SEQ ID NO:556. In some embodiments, the at least one ITR flanking 5' and/or 3'is a wild-type AAV ITR(s). In some embodiments, the DNA vector is a closed-ended DNA (ceDNA). In some embodiments, the DNA vector is a plasmid. In some embodiments, the DNA vector comprises a nucleic acid sequence encoding a single chain (SC) FVIII. In some embodiments, the nucleic acid sequence is set forth in SEQ ID NO: 556 or a sequence having at least 99% identity thereto.

[0048] In another aspect, the disclosure provides a ceDNA vector comprising a nucleic acid sequence of SEQ ID NO: 42 or a nucleic acid sequence at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 42.

[0049] In another aspect, the disclosure provides a ceDNA vector comprising a nucleic acid sequence of SEQ ID NO: 642 or a nucleic acid sequence at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 642.

[0050] In another aspect, the disclosure provides a ceDNA vector comprising a nucleic acid sequence of SEQ ID NO: 643 or a nucleic acid sequence at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 643.

[0051] In another aspect, the disclosure provides a ceDNA vector comprising a nucleic acid sequence of SEQ ID NO: 644 or a nucleic acid sequence at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 644.

[0052] In another aspect, the disclosure provides a ceDNA vector comprising a nucleic acid sequence of SEQ ID NO: 645 or a nucleic acid sequence at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 645.

[0053] In another aspect, the disclosure provides a ceDNA vector comprising a nucleic acid sequence of SEQ ID NO: 646 or a nucleic acid sequence at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 646.

[0054] These and other aspects of the disclosure are described in further detail below.

DESCRIPTION OF DRAWINGS

[0055] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0056] Embodiments of the present disclosure, briefly summarized above and discussed in greater detail below, can be understood by reference to the illustrative embodiments of the disclosure depicted in the appended drawings. However, the appended drawings illustrate only typical embodiments of the disclosure and are therefore not to be considered limiting of scope, for the disclosure may admit to other equally effective embodiments.

[0057] **FIG. 1A** provides the T-shaped stem-loop structure of a wild-type left ITR of AAV2 (SEQ ID NO: 52) with identification of A-A' arm, B-B' arm, C-C' arm, two Rep binding sites (RBE and RBE') and also shows the terminal resolution site (*TRS*). The RBE contains a series of 4 duplex tetramers that are believed to interact with either Rep 78 or Rep 68. In addition, the RBE' is also believed to interact with Rep complex assembled on the wild-type ITR or mutated ITR in the construct. The D and D' regions contain transcription factor binding sites and other conserved structure. FIG. 1A discloses SEQ ID NO: 544. **FIG. 1B** shows proposed Rep-catalyzed nicking and ligating activities in a wild-type left ITR, including the T-shaped stem-loop structure of the wild-type left ITR of AAV2 with identification of A-A' arm, B-B' arm, C-C' arm, two Rep Binding sites (RBE and RBE') and also shows the terminal resolution site (*TRS*), and the D and D' region comprising several transcription factor binding sites and other conserved structure. FIG. 1B discloses SEQ ID NO: 545.

[0058] **FIG. 2A** provides the primary structure (polynucleotide sequence) (left) (SEQ ID NO: 547) and the secondary structure (right) (SEQ ID NO: 547) of the RBE-containing portions of the A-A' arm, and the C-C' and B-B' arm of the wild-type left AAV2 ITR. **FIG. 2B** shows an exemplary mutated ITR (also referred to as a modified ITR) sequence for the left ITR. Shown is the primary structure (left) (SEQ ID NO: 549) and the predicted secondary structure (right) (SEQ ID NO: 549) of the RBE portion of the A-A' arm, the C arm and B-B' arm of an exemplary mutated left ITR (ITR-1, left). **FIG. 2C** shows the primary structure (left) (SEQ ID NO: 550) and the secondary structure (right) (SEQ ID NO: 550) of the RBE-containing portion of the A-A' loop, and the B-B' and C-C' arms of wild-type right AAV2 ITR. **FIG. 2D** shows an exemplary right modified ITR. Shown is the primary structure (left) (SEQ ID NO: 551) and the predicted secondary structure (right) (SEQ ID NO: 551) of the RBE containing portion of the A-A' arm, the B-B' and the C arm of an exemplary mutant right ITR (ITR-1, right). Any combination of left and right ITR (*e.g.,* AAV2 ITRs or other viral serotype or synthetic ITRs) can be used as taught herein. Each of **FIGS. 2A-2D** polynucleotide sequences refer to the sequence used in the plasmid or bacmid/baculovirus genome used to produce the ceDNA as described herein. Also included in each of **FIGS. 2A-2D** are corresponding ceDNA secondary structures inferred from the ceDNA vector configurations in the plasmid or bacmid/baculovirus genome and the predicted Gibbs free energy values.

[0059] **FIG. 3A** is a schematic illustrating an upstream process for making baculovirus infected insect cells (BIICs) that are useful in the production of a ceDNA vector for expression of the FVIII as disclosed herein in the process described in the schematic in FIG. 4B. **FIG. 3B** is a schematic of an exemplary method of ceDNA production and **FIG. 3C** illustrates a biochemical method and process to confirm ceDNA vector production. **FIG. 3D** and **FIG. 3E** are schematic illustrations describing a process for identifying the presence of ceDNA in DNA harvested from cell pellets obtained during the ceDNA production processes in FIG. 3B. **FIG. 3D** shows schematic expected bands for an exemplary ceDNA either left uncut or digested with a restriction endonuclease and then subjected to electrophoresis on either a native gel or a denaturing gel. The leftmost schematic is a native gel, and shows multiple bands suggesting that in its duplex and uncut form ceDNA exists in at least monomeric and dimeric states, visible as a faster-migrating smaller monomer and a slower-migrating dimer that is twice the size of the monomer. The schematic second from the left shows that when ceDNA is cut with a restriction endonuclease, the original bands are gone and faster-migrating (*e.g.,* smaller) bands appear, corresponding to the expected fragment sizes remaining after the cleavage. Under denaturing conditions, the original duplex DNA is single-stranded and migrates as a species twice as large as observed on native gel because the complementary strands are covalently linked. Thus, in the second schematic from the right, the digested ceDNA shows a similar banding distribution to that observed on native gel, but the bands migrate as fragments twice the size of their native gel counterparts. The rightmost schematic shows that uncut ceDNA under denaturing conditions migrates as a single-

stranded open circle, and thus the observed bands are twice the size of those observed under native conditions where the circle is not open. In this figure "kb" is used to indicate relative size of nucleotide molecules based, depending on context, on either nucleotide chain length (*e.g.,* for the single stranded molecules observed in denaturing conditions) or number of base pairs (*e.g.,* for the double-stranded molecules observed in native conditions). **FIG. 3E** shows DNA having a non-continuous structure. The ceDNA can be cut by a restriction endonuclease, having a single recognition site on the ceDNA vector, and generate two DNA fragments with different sizes (1kb and 2kb) in both neutral and denaturing conditions. **FIG. 3E** also shows a ceDNA having a linear and continuous structure. The ceDNA vector can be cut by the restriction endonuclease and generate two DNA fragments that migrate as 1kb and 2kb in neutral conditions, but in denaturing conditions, the stands remain connected and produce single strands that migrate as 2kb and 4kb.

[0060] **FIG. 4** is an exemplary picture of a denaturing gel running examples of ceDNA vectors with (+) or without (-) digestion with endonucleases (EcoRI for ceDNA construct 1 and 2; BamH1 for ceDNA construct 3 and 4; SpeI for ceDNA construct 5 and 6; and XhoI for ceDNA construct 7 and 8) Constructs 1-8 are described in Example 1 of International Application PCT PCT/US18/49996, which is incorporated herein in its entirety by reference. Sizes of bands highlighted with an asterisk were determined and provided on the bottom of the picture.

[0061] **FIG. 5** is an annotated schematic of the ceDNA1368 construct (6007 bp). FIG. 5 discloses SEQ ID NOS: 8 and 552, respectively, in order of appearance.

[0062] **FIG. 6** is an annotated schematic of the ceDNA1652 construct (6250 bp). FIG. 6 discloses SEQ ID NOS: 43 and 552, respectively, in order of appearance.

[0063] **FIG. 7** is an annotated schematic of the ceDNA1923 construct (5996 bp). FIG. 7 discloses SEQ ID NO: 68.

[0064] **FIG. 8** is an annotated schematic of the ceDNA1373 having an intron inbetween Exon 1 and Exon 2 (*i.e.*, GE-857 "miniF8_500/500" which is a mini Factor VIII intron 1 chimera, 500 nucleotides from 5'-end of intron, 500 nucleotides from 3'-end of intron) and another intron located 5'-UTR between a promoter (TTRm) and the ATG start site (*i.e.*, GE-023 "MVM_intron"). FIG. 8 discloses SEQ ID NO: 51.

[0065] **FIG. 9** shows a schematic of FVIII and its domains, as processed to active FVIIIa.

[0066] **FIG. 10A** and **FIG. 10B** are schematics detailing insertion of an intron (miniF8_50/100 intron) into FVIII ORF of ceDNA1367. **FIG. 10A** depicts Chimeric FVIII intron with functional splice donor and acceptor sites is inserted at native position of intron 1 into codon optimized FVIII ORF. **FIG. 10B** depicts intron flanking regions (33bp) derived from FVIII Wt cDNA sequence were substituted for codon optimized sequence in FVIII CDS. FIG. 10B discloses SEQ ID NO: 553.

[0067] **FIG. 11A** and **FIG. 11B** are schematics detailing insertion of introns into a FVIII ORF. **FIG. 11A** depicts a chimeric FVIII intron (miniF8_200_5p and miniF8_200_3p) with functional splice donor and acceptor sites inserted at native position of intron 1 into a codon optimized FVIII ORF.

FIG. 11B depicts an enhancer element (Embedded_enhancer_HNF_array) inserted inbetween 5p and 3p regions of the chimeric intron. FIG. 11B discloses SEQ ID NO: 554.

[0068] **FIG. 12** is a schematic detailing substitution of heterologous secretion signal sequences (N-terminal sequences) for the native FVIII signal sequence. Substitution of the native FVIII signal sequence for a signal sequence from chymotrypsinogen (CHY-SSv1) ORF. FVIII mature peptide is shown at the top. The sequence of FVIII N-terminus signal sequence and mature peptide cleavage site are shown at the bottom. FIG. 12 discloses SEQ ID NOS: 487-490, respectively, in order of appearance.

[0069] **FIG. 13** shows a schematic of B-domain selection for the constructs described herein, ranging from a complete B domain deletion (commonly known as BDD-SQ); a B domain having V3 peptide only (known as BDD V3; McIntosh *et al.*, 2013, Blood, 121:3335-3344); a B domain having 226 amino acid with 6 N-linked glycosylation sites (266BD; 226a/N6; see Miao *et al.*, Blood (2004); and a complete B domain deletion in a single chain (SC) in which A2 domain is linked to A3 domain having a slight deletion (4 amino acid of "EITR" (SEQ ID NO: 486)) in its N-terminus of the native A3, known as "Afstyla" style (BDD-SC). FIG. 13 discloses SEQ ID NOS: 491 and 491, respectively, in order of appearance.

[0070] **FIG. 14** is a graph that shows a comparison between the chromogenic activity assay versus ELISA to validate the assay method to determine FVIII activity. Various constructs were tested for FVIII activity with the chromogenic assay and the FVIII protein quantity using ELISA. The constructs tested were ceDNA692 (BBD-SQ), ceDNA704 (BDD-V3), ceDNA1270 (226/F309S), ceDNA1368 (SC) and ceDNA1373 (SC/F309S)).

[0071] **FIG. 15** depicts FVIII activity *in vitro* ceDNA (ceDNA692 (BBD-SQ)); ceDNA693 (BBD-SQ); ceDNA694 (BBD-SQ); ceDNA1391 (226/F309S); ceDNA1270 (226/F309S); ceDNA1367 (SC/F309S); ceDNA1373 (SC/F309S); ceDNA1368 (SC); and ceDNA1374 (SC)) and *in vivo* hydrodynamic injection Study 1 and Study 2 at Day 3 (ceDNA692 (BBD-SQ); ceDNA694 (BBD-SQ); ceDNA933 (226BD/F309S); ceDNA1265; ceDNA1270 (226/F309S); ceDNA1270 repeat (rep); ceDNA1367 (SC/F309S); ceDNA1373 (SC/F309S); ceDNA1368 (SC); and ceDNA1374 (SC)).

[0072] **FIG. 16** depicts the results of an *in vivo* study on FVIII activity at day 11 using constructs ceDNA933 (226BD/F309S), ceDNA1270 (226/F309S), ceDNA1367 (SC/F309S), and ceDNA1368 (SC) formulated in LNP.

[0073] **FIG. 17** shows the results of codon optimization on FVIII activity. The FVIII activity was measure from *in vivo* and *in vitro* studies using various ceDNA of FVIII SC, codon optimized FVIII sequences (ceDNA1362; ceDNA1368; ceDNA1374; ceDNA1838; ceDNA1840; ceDNA1918; ceDNA1919; ceDNA1920; ceDNA1921; ceDNA1922; and ceDNA1923). Hydrodynamic (HD)

[0074] **FIG. 18** shows that codon optimized constructs without F309S mutation: *i.e.*, ceDNA1368 and its variants such as ceDNA1923, ceDNA1823, ceDNA1840 which shows improvements on plasma FVIII concentration (IU/ml). Hydrodynamic (HD)

[0075] **FIG. 19** depicts optimization of 3' untranslated regions (UTR) and their effect on FVIII activity and plasma FVIII.

[0076] **FIG. 20** depicts the effect of different promoters and enhancers on FVIII activity.

[0077] **FIG. 21** depicts results from *in vitro* studies showing the effect of different introns on expression of ceDNA FVIII as measured by chromogenic FVIII activity.

[0078] **FIG. 22** shows plasma FVIII chromogenic activity (IU/mL) at 11 days after administration of ceDNAFVIII formulated in LNPs *in vivo*, as measured by the chromogenic assays for FVIII activity (see, Example 12).

[0079] **FIG. 23** depicts the effect of different DNA nuclear targeting sequences (DTS) on FVIII activity *in vitro* and *in vivo*.

[0080] **FIG. 24** depicts the effects of leader sequences on FVIII activity *in vitro* and *in vivo*.

[0081] **FIG. 25** shows the results from *in vivo* studies in mice and non-human primates (NHP) using various ceDNA vectors to express FVIII protein, as described in Examples 10, 15 and 16. Results show plasma FVIII concentration (IU/ml). Mouse vehicle: Example 10, PBS, day 5, n=5; Mouse DP#1: Example 10, ceDNA1270 in LNP formulation 1 (Ionizable lipid: DSPC : Cholesterol : PEG-Lipid + DSPE-PEG-GalNAc4 (47.5 : 10.0 : 39.2 : 3.3), 1mpk, day 5, n=4; Mouse DP#2: Example 10, ceDNA1270, LNP formulation 2 (Ionizable lipid : DSPC : Cholesterol : PEG-Lipid + DSPE-PEG2000-GalNAc4 (47.3 : 10.0 : 40.5 : 2.3), 2mpk, day 5, n=5; NHP vehicle: Example 14, saline, day 5, n=2; NHP DP#1: Example 14, ceDNA1270 in LNP formulation 1 (Ionizable lipid: DSPC : Cholesterol : PEG-Lipid + DSPE-PEG-GalNAc4 (47.5 : 10.0 : 39.2 : 3.3), 1mpk, day 5, n=2; NHP DP#2: Example 15, ceDNA 1270 in LNP formulation 2 (Ionizable lipid : DSPC : Cholesterol : PEG-Lipid + DSPE-PEG2000-GalNAc4 (47.3 : 10.0 : 40.5 : 2.3), 2mpk, day 5, n=2.

[0082] **FIG. 26** shows the results from *in vivo* studies in FVIII knockout mice, as described in Example 11. Results show plasma FVIII concentration (IU/ml) at day 10. The following ceDNA constructs were tested at the indicated doses (mg/kg) ceDNA1270, ceDNA1368, ceDNA1923, ceDNA1651. As shown in FIG. 26, after 10 days, mice administered these ceDNA constructs at all of the doses tested showed increases in plasma FVIII concentration. Overall, the increase in FVIII plasma concentration was dose dependent. ceDNA1270 showed a dramatic increase in plasma FVIII concentration from the 0.5 mg/kg dose to the 2.0 mg/kg dose.

[0083] **FIG. 27** depicts a chart showing the result of FVIII expression using various spacer variants of 3x hSerpEnh (2-mer and 11-mer) and Serpin enhancer sequence variants (*e.g.*, bushbaby Serpin enhancer, Chinese tree shrew Serpin enhancer). One dose of 50ng plasmid containing FVIII ceDNA

sequence was hydrodynamically injected into the tail vein of Rag2 mice on day 0 with a single blood collection at day 3 (~72hr post dose) for FVIII activity.

[0084] **FIG. 28** depicts a chart showing the results from an *in vivo* study in which C57BL/6J mice were hydrodynamically injected with FVIII-ceDNA, and FVIII activity was measured at Day 3 from the serum of the treated mice. The ceDNA constructs were: (1) ceDNA construct 10 (wild-type left ITR : left ITR spacer : 3x hSerpEnh VD promoter set : Mouse TTR 5'UTR : MVM Intron: hFVIII-F309S_BD226seq124-BDD-F309 ORF which is identical to the ORF sequence of ceDNA 1651) : WPRE_3pUTR : bGH : Right ITR Spacer : wild-type right ITR; (2) ceDNA construct 60 which has the identical sequence to ceDNA construct 10 except it contains 3x_hSerpEnh-2mer spacer v17; (3) ceDNA construct 61 which has the identical sequence to ceDNA construct 10 except it contains 3x_SerpEnh_11-mer_spacers_v3; (4) ceDNA construct 62 which has the identical sequence to ceDNA construct 10 except it has 3x_Bushbaby SerpEnh with adenine (A) spacers ("Aspacers") located at 5' upstream of the TTR promoter); (5) and ceDNA construct 39 which has the similar sequence to ceDNA construct 10 except that it contains a truncated right ITR).

DETAILED DESCRIPTION

[0085] Provided herein is a method for treating hemophilia A using a ceDNA vector comprising one or more nucleic acids that encode an FVIII therapeutic protein or fragment thereof. Also provided herein are ceDNA vectors for expression of FVIII protein as described herein comprising one or more nucleic acids, *e.g.,* heterologous nucleic acids that encode for the FVIII protein. In some embodiments, the expression of FVIII protein can comprise secretion of the therapeutic protein out of the cell in which it is expressed. Alternatively, in some embodiments, the expressed FVIII protein can act or function (*e.g.,* exert its effect) within the cell in which it is expressed. In some embodiments, the ceDNA vector expresses FVIII protein in the liver, in a muscle (*e.g.,* a skeletal muscle) of a subject, or in another body part, which can act as a depot for FVIII therapeutic protein production and secretion to many systemic compartments.

I. Definitions

[0086] Unless otherwise defined herein, scientific and technical terms used in connection with the present application shall have the meanings that are commonly understood by those of ordinary skill in the art to which this disclosure belongs. It should be understood that this disclosure is not limited to the particular methodology, protocols, and reagents, *etc.*, described herein and as such can vary. The terminology used herein is for the purpose of describing particular embodiments only and is not intended to limit the scope of the present disclosure, which is defined solely by the claims. Definitions of common terms in immunology and molecular biology can be found in The Merck Manual of Diagnosis and Therapy, 19th Edition, published by Merck Sharp & Dohme Corp., 2011 (ISBN 978-0-911910-19-3); Robert S. Porter *et al.* (eds.), Fields Virology, 6th Edition, published by Lippincott Williams & Wilkins, Philadelphia, PA, USA (2013), Knipe, D.M. and Howley, P.M. (ed.), The

Encyclopedia of Molecular Cell Biology and Molecular Medicine, published by Blackwell Science Ltd., 1999-2012 (ISBN 9783527600908); and Robert A. Meyers (ed.), Molecular Biology and Biotechnology: a Comprehensive Desk Reference, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8); Immunology by Werner Luttmann, published by Elsevier, 2006; Janeway's Immunobiology, Kenneth Murphy, Allan Mowat, Casey Weaver (eds.), Taylor & Francis Limited, 2014 (ISBN 0815345305, 9780815345305); Lewin's Genes XI, published by Jones & Bartlett Publishers, 2014 (ISBN-1449659055); Michael Richard Green and Joseph Sambrook, Molecular Cloning: A Laboratory Manual, 4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., USA (2012) (ISBN 1936113414); Davis *et al.*, Basic Methods in Molecular Biology, Elsevier Science Publishing, Inc., New York, USA (2012) (ISBN 044460149X); Laboratory Methods in Enzymology: DNA, Jon Lorsch (ed.) Elsevier, 2013 (ISBN 0124199542); Current Protocols in Molecular Biology (CPMB), Frederick M. Ausubel (ed.), John Wiley and Sons, 2014 (ISBN047150338X, 9780471503385), Current Protocols in Protein Science (CPPS), John E. Coligan (ed.), John Wiley and Sons, Inc., 2005; and Current Protocols in Immunology (CPI) (John E. Coligan, ADA M Kruisbeek, David H Margulies, Ethan M Shevach, Warren Strobe, (eds.) John Wiley and Sons, Inc., 2003 (ISBN 0471142735, 9780471142737), the contents of which are all incorporated by reference herein in their entireties.

[0087] As used herein, the terms, "administration," "administering" and variants thereof refers to introducing a composition or agent (*e.g.,* a therapeutic nucleic acid or an immunosuppressant as described herein) into a subject and includes concurrent and sequential introduction of one or more compositions or agents. "Administration" can refer, *e.g.,* to therapeutic, pharmacokinetic, diagnostic, research, placebo, and experimental methods. "Administration" also encompasses *in vitro* and *ex vivo* treatments. The introduction of a composition or agent into a subject is by any suitable route, including orally, pulmonarily, intranasally, parenterally (intravenously, intramuscularly, intraperitoneally, or subcutaneously), rectally, intralymphatically, intratumorally, or topically. The introduction of a composition or agent into a subject is by electroporation. Administration includes self-administration and the administration by another. Administration can be carried out by any suitable route. A suitable route of administration allows the composition or the agent to perform its intended function. For example, if a suitable route is intravenous, the composition is administered by introducing the composition or agent into a vein of the subject.

[0088] As used herein, the phrases "nucleic acid therapeutic", "therapeutic nucleic acid" and "TNA" are used interchangeably and refer to any modality of therapeutic using nucleic acids as an active component of therapeutic agent to treat a disease or disorder. As used herein, these phrases refer to RNA-based therapeutics and DNA-based therapeutics. Non-limiting examples of RNA-based therapeutics include mRNA, antisense RNA and oligonucleotides, ribozymes, aptamers, interfering RNAs (RNAi), Dicer-substrate dsRNA, small hairpin RNA (shRNA), asymmetrical interfering RNA

(aiRNA), microRNA (miRNA). Non-limiting examples of DNA-based therapeutics include minicircle DNA, minigene, viral DNA (*e.g.,* Lentiviral or AAV genome) or non-viral synthetic DNA vectors, closed-ended linear duplex DNA (ceDNA / CELiD), plasmids, bacmids, doggybone (dbDNA™) DNA vectors, minimalistic immunological-defined gene expression (MIDGE)-vector, nonviral ministring DNA vector (linear-covalently closed DNA vector), or dumbbell-shaped DNA minimal vector ("dumbbell DNA").

[0089] As used herein, an "effective amount" or "therapeutically effective amount" of a therapeutic agent, such as a FVIII therapeutic protein or fragment thereof, is an amount sufficient to produce the desired effect, *e.g.,* treatment or prevention of hemophilia A. Suitable assays for measuring expression of a target gene or target sequence include, *e.g.,* examination of protein or RNA levels using techniques known to those of skill in the art such as dot blots, northern blots, *in situ* hybridization, ELISA, immunoprecipitation, enzyme function, as well as phenotypic assays known to those of skill in the art. However, dosage levels are based on a variety of factors, including the type of injury, the age, weight, sex, medical condition of the patient, the severity of the condition, the route of administration, and the particular active agent employed. Thus, the dosage regimen may vary widely, but can be determined routinely by a physician using standard methods. Additionally, the terms "therapeutic amount", "therapeutically effective amounts" and "pharmaceutically effective amounts" include prophylactic or preventative amounts of the compositions of the described disclosure. In prophylactic or preventative applications of the described disclosure, pharmaceutical compositions or medicaments are administered to a patient susceptible to, or otherwise at risk of, a disease, disorder or condition in an amount sufficient to eliminate or reduce the risk, lessen the severity, or delay the onset of the disease, disorder or condition, including biochemical, histologic and/or behavioral symptoms of the disease, disorder or condition, its complications, and intermediate pathological phenotypes presenting during development of the disease, disorder or condition. It is generally preferred that a maximum dose be used, that is, the highest safe dose according to some medical judgment. According to some embodiments, the disease, disorder or condition is hemophilia A. The terms "dose" and "dosage" are used interchangeably herein.

[0090] As used herein the term "therapeutic effect" refers to a consequence of treatment, the results of which are judged to be desirable and beneficial. A therapeutic effect can include, directly or indirectly, the arrest, reduction, or elimination of a disease manifestation. A therapeutic effect can also include, directly or indirectly, the arrest reduction or elimination of the progression of a disease manifestation.

[0091] For any therapeutic agent described herein therapeutically effective amount may be initially determined from preliminary *in vitro* studies and/or animal models. A therapeutically effective dose may also be determined from human data. The applied dose may be adjusted based on the relative bioavailability and potency of the administered compound. Adjusting the dose to achieve maximal

efficacy based on the methods described above and other well-known methods is within the capabilities of the ordinarily skilled artisan. General principles for determining therapeutic effectiveness, which may be found in Chapter 1 of Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10th Edition, McGraw-Hill (New York) (2001), incorporated herein by reference, are summarized below.

[0092] Pharmacokinetic principles provide a basis for modifying a dosage regimen to obtain a desired degree of therapeutic efficacy with a minimum of unacceptable adverse effects. In situations where the drug's plasma concentration can be measured and related to therapeutic window, additional guidance for dosage modification can be obtained.

[0093] As used herein, the terms "heterologous nucleic acid sequence" and "transgene" are used interchangeably and refer to a nucleic acid of interest (other than a nucleic acid encoding a capsid polypeptide) that is incorporated into and may be delivered and expressed by a ceDNA vector as disclosed herein. In one embodiment, a nucleic acid sequence may be a heterologous nucleic acid sequence. According to some embodiments, the term "heterologous nucleic acid" is meant to refer to a nucleic acid (or transgene) that is not present in, expressed by, or derived from the cell or subject to which it is contacted.

[0094] As used herein, the terms "expression cassette" and "transcription cassette" are used interchangeably and refer to a linear stretch of nucleic acids that includes a transgene that is operably linked to one or more promoters or other regulatory sequences sufficient to direct transcription of the transgene, but which does not comprise capsid-encoding sequences, other vector sequences or inverted terminal repeat regions. An expression cassette may additionally comprise one or more *cis*-acting sequences (*e.g.,* promoters, enhancers, or repressors), one or more introns, and one or more post-transcriptional regulatory elements.

[0095] The terms "polynucleotide" and "nucleic acid," used interchangeably herein, refer to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. Thus, this term includes single, double, or multi-stranded DNA or RNA, genomic DNA, cDNA, DNA-RNA hybrids, or a polymer including purine and pyrimidine bases or other natural, chemically or biochemically modified, non-natural, or derivatized nucleotide bases. "Oligonucleotide" generally refers to polynucleotides of between about 5 and about 100 nucleotides of single- or double-stranded DNA. However, for the purposes of this disclosure, there is no upper limit to the length of an oligonucleotide. Oligonucleotides are also known as "oligomers" or "oligos" and may be isolated from genes, or chemically synthesized by methods known in the art. The terms "polynucleotide" and "nucleic acid" should be understood to include, as applicable to the embodiments being described, single-stranded (such as sense or antisense) and double-stranded polynucleotides. DNA may be in the form of, *e.g.,* antisense molecules, plasmid DNA, DNA-DNA duplexes, pre-condensed DNA, PCR products, vectors (P1, PAC, BAC, YAC, artificial chromosomes), expression cassettes, chimeric

sequences, chromosomal DNA, or derivatives and combinations of these groups. DNA may be in the form of minicircle, plasmid, bacmid, minigene, ministring DNA (linear covalently closed DNA vector), closed-ended linear duplex DNA (CELiD or ceDNA), doggybone (dbDNA™) DNA, dumbbell shaped DNA, minimalistic immunological-defined gene expression (MIDGE)-vector, viral vector or nonviral vectors. RNA may be in the form of small interfering RNA (siRNA), Dicer-substrate dsRNA, small hairpin RNA (shRNA), asymmetrical interfering RNA (aiRNA), microRNA (miRNA), mRNA, rRNA, tRNA, viral RNA (vRNA), and combinations thereof. Nucleic acids include nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, and which have similar binding properties as the reference nucleic acid. Examples of such analogs and/or modified residues include, without limitation, phosphorothioates, phosphorodiamidate morpholino oligomer (morpholino), phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2'-O-methyl ribonucleotides, locked nucleic acid (LNA™), and peptide nucleic acids (PNAs). Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (*e.g.,* degenerate codon substitutions), alleles, orthologs, SNPs, and complementary sequences as well as the sequence explicitly indicated.

[0096] "Nucleotides" contain a sugar deoxyribose (DNA) or ribose (RNA), a base, and a phosphate group. Nucleotides are linked together through the phosphate groups.

[0097] "Bases" include purines and pyrimidines, which further include natural compounds adenine, thymine, guanine, cytosine, uracil, inosine, and natural analogs, and synthetic derivatives of purines and pyrimidines, which include, but are not limited to, modifications which place new reactive groups such as, but not limited to, amines, alcohols, thiols, carboxylates, and alkylhalides.

[0098] The term "nucleic acid construct" as used herein refers to a nucleic acid molecule, either single- or double-stranded, which is isolated from a naturally occurring gene or which is modified to contain segments of nucleic acids in a manner that would not otherwise exist in nature or which is synthetic. The term nucleic acid construct is synonymous with the term "expression cassette" when the nucleic acid construct contains the control sequences required for expression of a coding sequence of the present disclosure. An "expression cassette" includes a DNA coding sequence operably linked to a promoter.

[0099] By "hybridizable" or "complementary" or "substantially complementary" it is meant that a nucleic acid (*e.g.,* RNA) includes a sequence of nucleotides that enables it to non-covalently bind, *i.e.* form Watson-Crick base pairs and/or G/U base pairs, "anneal", or "hybridize," to another nucleic acid in a sequence-specific, antiparallel, manner (*i.e.,* a nucleic acid specifically binds to a complementary nucleic acid) under the appropriate *in vitro* and/or *in vivo* conditions of temperature and solution ionic

strength. As is known in the art, standard Watson-Crick base-pairing includes: adenine (A) pairing with thymidine (T), adenine (A) pairing with uracil (U), and guanine (G) pairing with cytosine (C). In addition, it is also known in the art that for hybridization between two RNA molecules (*e.g.,* dsRNA), guanine (G) base pairs with uracil (U). For example, G/U base-pairing is partially responsible for the degeneracy (*i.e.,* redundancy) of the genetic code in the context of tRNA anti-codon base-pairing with codons in mRNA. In the context of this disclosure, a guanine (G) of a protein-binding segment (dsRNA duplex) of a subject DNA-targeting RNA molecule is considered complementary to an uracil (U), and *vice versa*. As such, when a G/U base-pair can be made at a given nucleotide position a protein-binding segment (dsRNA duplex) of a subject DNA-targeting RNA molecule, the position is not considered to be non-complementary, but is instead considered to be complementary.

[00100] The terms "peptide," "polypeptide," and "protein" are used interchangeably herein, and refer to a polymeric form of amino acids of any length, which can include coded and non-coded amino acids, chemically or biochemically modified or derivatized amino acids, and polypeptides having modified peptide backbones.

[00101] A DNA sequence that "encodes" a particular FVIII protein is a DNA nucleic acid sequence that is transcribed into the particular RNA and/or protein. A DNA polynucleotide may encode an RNA (mRNA) that is translated into protein, or a DNA polynucleotide may encode an RNA that is not translated into protein (*e.g.,* tRNA, rRNA, or a DNA-targeting RNA; also called "non-coding" RNA or ncRNA").

[0001] As used herein, the term "fusion protein" as used herein refers to a polypeptide which comprises protein domains from at least two different proteins. For example, a fusion protein may comprise (i) FVIII or fragment thereof and (ii) at least one non-GOI protein. Fusion proteins encompassed herein include, but are not limited to, an antibody, or Fc or antigen-binding fragment of an antibody fused to a FVIII protein, *e.g.,* an extracellular domain of a receptor, ligand, enzyme or peptide. The FVIII protein or fragment thereof that is part of a fusion protein can be a monospecific antibody or a bispecific or multispecific antibody.

[00102] As used herein, the term "genomic safe harbor gene" or "safe harbor gene" refers to a gene or loci that a nucleic acid sequence can be inserted such that the sequence can integrate and function in a predictable manner (*e.g.,* express a protein of interest) without significant negative consequences to endogenous gene activity, or the promotion of cancer. In some embodiments, a safe harbor gene is also a loci or gene where an inserted nucleic acid sequence can be expressed efficiently and at higher levels than a non-safe harbor site.

[00103] As used herein, the term "gene delivery" means a process by which foreign DNA is transferred to host cells for applications of gene therapy.

[00104] As used herein, the term "terminal repeat" or "TR" includes any viral terminal repeat or synthetic sequence that comprises at least one minimal required origin of replication and a region

comprising a palindrome hairpin structure. A Rep-binding sequence ("RBS") (also referred to as RBE (Rep-binding element)) and a terminal resolution site ("TRS") together constitute a "minimal required origin of replication" and thus the TR comprises at least one RBS and at least one TRS. TRs that are the inverse complement of one another within a given stretch of polynucleotide sequence are typically each referred to as an "inverted terminal repeat" or "ITR". In the context of a virus, ITRs mediate replication, virus packaging, integration and provirus rescue. As was unexpectedly found in the disclosure herein, TRs that are not inverse complements across their full length can still perform the traditional functions of ITRs, and thus the term ITR is used herein to refer to a TR in a ceDNA genome or ceDNA vector that is capable of mediating replication of ceDNA vector. It will be understood by one of ordinary skill in the art that in complex ceDNA vector configurations more than two ITRs or asymmetric ITR pairs may be present. The ITR can be an AAV ITR or a non-AAV ITR, or can be derived from an AAV ITR or a non-AAV ITR. For example, the ITR can be derived from the family *Parvoviridae*, which encompasses parvoviruses and dependoviruses (*e.g.,* canine parvovirus, bovine parvovirus, mouse parvovirus, porcine parvovirus, human parvovirus B-19), or the SV40 hairpin that serves as the origin of SV40 replication can be used as an ITR, which can further be modified by truncation, substitution, deletion, insertion and/or addition. Parvoviridae family viruses consist of two subfamilies: *Parvovirinae*, which infect vertebrates, and *Densovirinae*, which infect invertebrates. Dependoparvoviruses include the viral family of the adeno-associated viruses (AAV) which are capable of replication in vertebrate hosts including, but not limited to, human, primate, bovine, canine, equine and ovine species. For convenience herein, an ITR located 5' to (upstream of) an expression cassette in a ceDNA vector is referred to as a "5' ITR" or a "left ITR", and an ITR located 3' to (downstream of) an expression cassette in a ceDNA vector is referred to as a "3' ITR" or a "right ITR".

[00105] A "wild-type ITR" or "WT-ITR" refers to the sequence of a naturally occurring ITR sequence in an AAV or other dependovirus that retains, *e.g.,* Rep binding activity and Rep nicking ability. The nucleic acid sequence of a WT-ITR from any AAV serotype may slightly vary from the canonical naturally occurring sequence due to degeneracy of the genetic code or drift, and therefore WT-ITR sequences encompassed for use herein include WT-ITR sequences as result of naturally occurring changes taking place during the production process (*e.g.,* a replication error).

[00106] As used herein, the term "substantially symmetrical WT-ITRs" or a "substantially symmetrical WT-ITR pair" refers to a pair of WT-ITRs within a single ceDNA genome or ceDNA vector that are both wild-type ITRs that have an inverse complement sequence across their entire length. For example, an ITR can be considered to be a wild-type sequence, even if it has one or more nucleotides that deviate from the canonical naturally occurring sequence, so long as the changes do not affect the properties and overall three-dimensional structure of the sequence. In some aspects, the deviating nucleotides represent conservative sequence changes. As one non-limiting example, a

sequence that has at least 95%, 96%, 97%, 98%, or 99% sequence identity to the canonical sequence (as measured, *e.g.,* using BLAST at default settings), and also has a symmetrical three-dimensional spatial organization to the other WT-ITR such that their 3D structures are the same shape in geometrical space. The substantially symmetrical WT-ITR has the same A, C-C' and B-B' loops in 3D space. A substantially symmetrical WT-ITR can be functionally confirmed as WT by determining that it has an operable Rep binding site (RBE or RBE') and terminal resolution site (TRS) that pairs with the appropriate Rep protein. One can optionally test other functions, including transgene expression under permissive conditions.

[00107] As used herein, the phrases of "modified ITR" or "mod-ITR" or "mutant ITR" are used interchangeably herein and refer to an ITR that has a mutation in at least one or more nucleotides as compared to the WT-ITR from the same serotype. The mutation can result in a change in one or more of A, C, C', B, B' regions in the ITR, and can result in a change in the three-dimensional spatial organization (*i.e.* its 3D structure in geometric space) as compared to the 3D spatial organization of a WT-ITR of the same serotype.

[00108] As used herein, the term "asymmetric ITRs" also referred to as "asymmetric ITR pairs" refers to a pair of ITRs within a single ceDNA genome or ceDNA vector that are not inverse complements across their full length. As one non-limiting example, an asymmetric ITR pair does not have a symmetrical three-dimensional spatial organization to their cognate ITR such that their 3D structures are different shapes in geometrical space. Stated differently, an asymmetrical ITR pair have the different overall geometric structure, *i.e.,* they have different organization of their A, C-C' and B-B' loops in 3D space (*e.g.,* one ITR may have a short C-C' arm and/or short B-B' arm as compared to the cognate ITR). The difference in sequence between the two ITRs may be due to one or more nucleotide addition, deletion, truncation, or point mutation. In one embodiment, one ITR of the asymmetric ITR pair may be a wild-type AAV ITR sequence and the other ITR a modified ITR as defined herein (*e.g.,* a non-wild-type or synthetic ITR sequence). In another embodiment, neither ITRs of the asymmetric ITR pair is a wild-type AAV sequence and the two ITRs are modified ITRs that have different shapes in geometrical space (*i.e.,* a different overall geometric structure). In some embodiments, one mod-ITRs of an asymmetric ITR pair can have a short C-C' arm and the other ITR can have a different modification (*e.g.,* a single arm, or a short B-B' arm etc.) such that they have different three-dimensional spatial organization as compared to the cognate asymmetric mod-ITR.

[00109] As used herein, the term "symmetric ITRs" refers to a pair of ITRs within a single ceDNA genome or ceDNA vector that are wild-type or mutated (*e.g.,* modified relative to wild-type) dependoviral ITR sequences and are inverse complements across their full length. In one non-limiting example, both ITRs are wild-type ITRs sequences from AAV2. In another example, neither ITRs are wild-type ITR AAV2 sequences (*i.e.,* they are a modified ITR, also referred to as a mutant ITR), and can have a difference in sequence from the wild-type ITR due to nucleotide addition, deletion,

substitution, truncation, or point mutation. For convenience herein, an ITR located 5' to (upstream of) an expression cassette in a ceDNA vector is referred to as a "5' ITR" or a "left ITR", and an ITR located 3' to (downstream of) an expression cassette in a ceDNA vector is referred to as a "3' ITR" or a "right ITR".

[00110] As used herein, the terms "substantially symmetrical modified-ITRs" or a "substantially symmetrical mod-ITR pair" refers to a pair of modified-ITRs within a single ceDNA genome or ceDNA vector that are both that have an inverse complement sequence across their entire length. For example, the modified ITR can be considered substantially symmetrical, even if it has some nucleic acid sequences that deviate from the inverse complement sequence so long as the changes do not affect the properties and overall shape. As one non-limiting example, a sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the canonical sequence (as measured using BLAST at default settings), and also has a symmetrical three-dimensional spatial organization to their cognate modified ITR such that their 3D structures are the same shape in geometrical space. Stated differently, a substantially symmetrical modified-ITR pair have the same A, C-C' and B-B' loops organized in 3D space. In some embodiments, the ITRs from a mod-ITR pair may have different reverse complement nucleic acid sequences but still have the same symmetrical three-dimensional spatial organization – that is both ITRs have mutations that result in the same overall 3D shape. For example, one ITR (*e.g.,* 5' ITR) in a mod-ITR pair can be from one serotype, and the other ITR (*e.g.,* 3' ITR) can be from a different serotype, however, both can have the same corresponding mutation (*e.g.,* if the 5'ITR has a deletion in the C region, the cognate modified 3'ITR from a different serotype has a deletion at the corresponding position in the C' region), such that the modified ITR pair has the same symmetrical three-dimensional spatial organization. In such embodiments, each ITR in a modified ITR pair can be from different serotypes (*e.g.,* AAV1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12) such as the combination of AAV2 and AAV6, with the modification in one ITR reflected in the corresponding position in the cognate ITR from a different serotype. In one embodiment, a substantially symmetrical modified ITR pair refers to a pair of modified ITRs (mod-ITRs) so long as the difference in nucleic acid sequences between the ITRs does not affect the properties or overall shape and they have substantially the same shape in 3D space. As a non-limiting example, a mod-ITR that has at least 95%, 96%, 97%, 98% or 99% sequence identity to the canonical mod-ITR as determined by standard means well known in the art such as BLAST (Basic Local Alignment Search Tool), or BLASTN at default settings, and also has a symmetrical three-dimensional spatial organization such that their 3D structure is the same shape in geometric space. A substantially symmetrical mod-ITR pair has the same A, C-C' and B-B' loops in 3D space, *e.g.,* if a modified ITR in a substantially symmetrical mod-ITR pair has a deletion of a C-C' arm, then the cognate mod-ITR has the corresponding deletion of the C-C' loop and also has a similar 3D structure of the remaining A and B-B' loops in the same shape in geometric space of its cognate mod-ITR.

[00111] The term "flanking" refers to a relative position of one nucleic acid sequence with respect to another nucleic acid sequence. Generally, in the sequence ABC, B is flanked by A and C. The same is true for the arrangement AxBxC. Thus, a flanking sequence precedes or follows a flanked sequence but need not be contiguous with, or immediately adjacent to the flanked sequence. In one embodiment, the term flanking refers to terminal repeats at each end of the linear duplex ceDNA vector.

[00112] As used herein, the terms "treat," "treating," and/or "treatment" include abrogating, substantially inhibiting, slowing or reversing the progression of a condition, substantially ameliorating clinical symptoms of a condition, or substantially preventing the appearance of clinical symptoms of a condition, obtaining beneficial or desired clinical results. According to some embodiments, the condition is hemophilia A. Treating further refers to accomplishing one or more of the following: (a) reducing the severity of the disorder; (b) limiting development of symptoms characteristic of the disorder(s) being treated; (c) limiting worsening of symptoms characteristic of the disorder(s) being treated; (d) limiting recurrence of the disorder(s) in patients that have previously had the disorder(s); and (e) limiting recurrence of symptoms in patients that were previously asymptomatic for the disorder(s). Beneficial or desired clinical results, such as pharmacologic and/or physiologic effects include, but are not limited to, preventing the disease, disorder or condition from occurring in a subject that may be predisposed to the disease, disorder or condition but does not yet experience or exhibit symptoms of the disease (prophylactic treatment), alleviation of symptoms of the disease, disorder or condition, diminishment of extent of the disease, disorder or condition, stabilization (*i.e.*, not worsening) of the disease, disorder or condition, preventing spread of the disease, disorder or condition, delaying or slowing of the disease, disorder or condition progression, amelioration or palliation of the disease, disorder or condition, and combinations thereof, as well as prolonging survival as compared to expected survival if not receiving treatment.

[00113] As used herein, the term "increase," "enhance," "raise" (and like terms) generally refers to the act of increasing, either directly or indirectly, a concentration, level, function, activity, or behavior relative to the natural, expected, or average, or relative to a control condition.

[00114] As used herein, the term "minimize", "reduce", "decrease," and/or "inhibit" (and like terms) generally refers to the act of reducing, either directly or indirectly, a concentration, level, function, activity, or behavior relative to the natural, expected, or average, or relative to a control condition.

[00115] As used herein, the term "ceDNA genome" refers to an expression cassette that further incorporates at least one inverted terminal repeat region. A ceDNA genome may further comprise one or more spacer regions. In some embodiments the ceDNA genome is incorporated as an intermolecular duplex polynucleotide of DNA into a plasmid or viral genome.

[00116] As used herein, the term "ceDNA spacer region" refers to an intervening sequence that separates functional elements in the ceDNA vector or ceDNA genome. In some embodiments, ceDNA spacer regions keep two functional elements at a desired distance for optimal functionality. In some

embodiments, ceDNA spacer regions provide or add to the genetic stability of the ceDNA genome within *e.g.,* a plasmid or baculovirus. In some embodiments, ceDNA spacer regions facilitate ready genetic manipulation of the ceDNA genome by providing a convenient location for cloning sites and the like. For example, in certain aspects, an oligonucleotide "polylinker" containing several restriction endonuclease sites, or a non-open reading frame sequence designed to have no known protein (*e.g.,* transcription factor) binding sites can be positioned in the ceDNA genome to separate the *cis* – acting factors, *e.g.,* inserting a 6mer, 12mer, 18mer, 24mer, 48mer, 86mer, 176mer, etc. between the terminal resolution site and the upstream transcriptional regulatory element. Similarly, the spacer may be incorporated between the polyadenylation signal sequence and the 3'-terminal resolution site.

[00117] As used herein, the terms "Rep binding site, "Rep binding element, "RBE" and "RBS" are used interchangeably and refer to a binding site for Rep protein (*e.g.,* AAV Rep 78 or AAV Rep 68) which upon binding by a Rep protein permits the Rep protein to perform its site-specific endonuclease activity on the sequence incorporating the RBS. An RBS sequence and its inverse complement together form a single RBS. RBS sequences are known in the art, and include, for example, 5'-GCGCGCTCGCTCGCTC-3′ (SEQ ID NO: 437), an RBS sequence identified in AAV2. Any known RBS sequence may be used in the embodiments of the disclosure, including other known AAV RBS sequences and other naturally known or synthetic RBS sequences. Without being bound by theory it is thought that he nuclease domain of a Rep protein binds to the duplex nucleic acid sequence GCTC, and thus the two known AAV Rep proteins bind directly to and stably assemble on the duplex oligonucleotide, 5'-(GCGC)(GCTC)(GCTC)(GCTC)-3' (SEQ ID NO: 437). In addition, soluble aggregated conformers (*i.e.,* undefined number of inter-associated Rep proteins) dissociate and bind to oligonucleotides that contain Rep binding sites. Each Rep protein interacts with both the nitrogenous bases and phosphodiester backbone on each strand. The interactions with the nitrogenous bases provide sequence specificity whereas the interactions with the phosphodiester backbone are non- or less- sequence specific and stabilize the protein-DNA complex.

[00118] As used herein, the terms "terminal resolution site" and "TRS" are used interchangeably herein and refer to a region at which Rep forms a tyrosine-phosphodiester bond with the 5' thymidine generating a 3' OH that serves as a substrate for DNA extension via a cellular DNA polymerase, *e.g.,* DNA pol delta or DNA pol epsilon. Alternatively, the Rep-thymidine complex may participate in a coordinated ligation reaction. In some embodiments, a TRS minimally encompasses a non-base-paired thymidine. In some embodiments, the nicking efficiency of the TRS can be controlled at least in part by its distance within the same molecule from the RBS. When the acceptor substrate is the complementary ITR, then the resulting product is an intramolecular duplex. TRS sequences are known in the art, and include, for example, 5'-GGTTGA-3', the hexanucleotide sequence identified in AAV2. Any known TRS sequence may be used in the embodiments of the disclosure, including other known

AAV TRS sequences and other naturally known or synthetic TRS sequences such as AGTT (SEQ ID NO: 438), GGTTGG, AGTTGG, AGTTGA, and other motifs such as RRTTRR.

[00119] As used herein, the term "ceDNA-plasmid" refers to a plasmid that comprises a ceDNA genome as an intermolecular duplex.

[00120] As used herein, the term "ceDNA-bacmid" refers to an infectious baculovirus genome comprising a ceDNA genome as an intermolecular duplex that is capable of propagating in *E. coli* as a plasmid, and so can operate as a shuttle vector for baculovirus.

[00121] As used herein, the term "ceDNA-baculovirus" refers to a baculovirus that comprises a ceDNA genome as an intermolecular duplex within the baculovirus genome.

[00122] As used herein, the terms "ceDNA-baculovirus infected insect cell" and "ceDNA-BIIC" are used interchangeably, and refer to an invertebrate host cell (including, but not limited to an insect cell (*e.g.,* an Sf9 cell)) infected with a ceDNA-baculovirus.

[00123] As used herein, the term "ceDNA" refers to capsid-free closed-ended linear double stranded (*ds*) duplex DNA for non-viral gene transfer, synthetic or otherwise. Detailed description of ceDNA is described in International application of PCT/US2017/020828, filed March 3, 2017, the entire contents of which are expressly incorporated herein by reference. Certain methods for the production of ceDNA comprising various inverted terminal repeat (ITR) sequences and configurations using cell-based methods are described in Example 1 of International applications PCT/US18/49996, filed September 7, 2018, and PCT/US2018/064242, filed December 6, 2018 each of which is incorporated herein in its entirety by reference. Certain methods for the production of synthetic ceDNA vectors comprising various ITR sequences and configurations are described, *e.g.,* in International application PCT/US2019/14122, filed January 18, 2019, the entire content of which is incorporated herein by reference.

[00124] As used herein, the term "closed-ended DNA vector" refers to a capsid-free DNA vector with at least one covalently closed end and where at least part of the vector has an intramolecular duplex structure.

[00125] As used herein, the terms "ceDNA vector" and "ceDNA" are used interchangeably and refer to a closed-ended DNA vector comprising at least one terminal palindrome. In some embodiments, the ceDNA comprises two covalently-closed ends.

[00126] As used herein, the term "neDNA" or "nicked ceDNA" refers to a closed-ended DNA having a nick or a gap of 1-100 base pairs in a stem region or spacer region 5' upstream of an open reading frame (*e.g.,* a promoter and transgene to be expressed).

[00127] As used herein, the terms "gap" refers to a discontinued portion of synthetic DNA vector of the present disclosure, creating a stretch of single stranded DNA portion in otherwise double stranded ceDNA. The gap can be 1 base-pair to 100 base-pair long in length in one strand of a duplex DNA. Typical gaps, designed and created by the methods described herein and synthetic vectors generated by

the methods can be, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59 or 60 bp long in length. Exemplified gaps in the present disclosure can be 1 bp to 10 bp long, 1 to 20 bp long, 1 to 30 bp long in length.

[00128] As defined herein, "reporters" refer to proteins that can be used to provide detectable read-outs. Reporters generally produce a measurable signal such as fluorescence, color, or luminescence. Reporter protein coding sequences encode proteins whose presence in the cell or organism is readily observed. For example, fluorescent proteins cause a cell to fluoresce when excited with light of a particular wavelength, luciferases cause a cell to catalyze a reaction that produces light, and enzymes such as β-galactosidase convert a substrate to a colored product. Exemplary reporter polypeptides useful for experimental or diagnostic purposes include, but are not limited to β-lactamase, β -galactosidase (LacZ), alkaline phosphatase (AP), thymidine kinase (TK), green fluorescent protein (GFP) and other fluorescent proteins, chloramphenicol acetyltransferase (CAT), luciferase, and others well known in the art.

[00129] As used herein, the terms "sense" and "antisense" refer to the orientation of the structural element on the polynucleotide. The sense and antisense versions of an element are the reverse complement of each other.

[00130] As used herein, the term "synthetic AAV vector" and "synthetic production of AAV vector" refers to an AAV vector and synthetic production methods thereof in an entirely cell-free environment.

[00131] As used herein, "reporters" refer to proteins that can be used to provide detectable read-outs. Reporters generally produce a measurable signal such as fluorescence, color, or luminescence. Reporter protein coding sequences encode proteins whose presence in the cell or organism is readily observed. For example, fluorescent proteins cause a cell to fluoresce when excited with light of a particular wavelength, luciferases cause a cell to catalyze a reaction that produces light, and enzymes such as β-galactosidase convert a substrate to a colored product. Exemplary reporter polypeptides useful for experimental or diagnostic purposes include, but are not limited to β-lactamase, β -galactosidase (LacZ), alkaline phosphatase (AP), thymidine kinase (TK), green fluorescent protein (GFP) and other fluorescent proteins, chloramphenicol acetyltransferase (CAT), luciferase, and others well known in the art.

[00132] As used herein, the term "effector protein" refers to a polypeptide that provides a detectable read-out, either as, for example, a reporter polypeptide, or more appropriately, as a polypeptide that kills a cell, *e.g.,* a toxin, or an agent that renders a cell susceptible to killing with a chosen agent or lack thereof. Effector proteins include any protein or peptide that directly targets or damages the host cell's DNA and/or RNA. For example, effector proteins can include, but are not limited to, a restriction endonuclease that targets a host cell DNA sequence (whether genomic or on an extrachromosomal element), a protease that degrades a polypeptide target necessary for cell survival, a

DNA gyrase inhibitor, and a ribonuclease-type toxin. In some embodiments, the expression of an effector protein controlled by a synthetic biological circuit as described herein can participate as a factor in another synthetic biological circuit to thereby expand the range and complexity of a biological circuit system's responsiveness.

[00133] Transcriptional regulators refer to transcriptional activators and repressors that either activate or repress transcription of a gene of interest, such as FVIII. Promoters are regions of nucleic acid that initiate transcription of a particular gene. Transcriptional activators typically bind nearby to transcriptional promoters and recruit RNA polymerase to directly initiate transcription. Repressors bind to transcriptional promoters and sterically hinder transcriptional initiation by RNA polymerase. Other transcriptional regulators may serve as either an activator or a repressor depending on where they bind and cellular and environmental conditions. Non-limiting examples of transcriptional regulator classes include, but are not limited to homeodomain proteins, zinc-finger proteins, winged-helix (forkhead) proteins, and leucine-zipper proteins.

[00134] As used herein, a "repressor protein" or "inducer protein" is a protein that binds to a regulatory sequence element and represses or activates, respectively, the transcription of sequences operatively linked to the regulatory sequence element. Preferred repressor and inducer proteins as described herein are sensitive to the presence or absence of at least one input agent or environmental input. Preferred proteins as described herein are modular in form, comprising, for example, separable DNA-binding and input agent-binding or responsive elements or domains.

[00135] As used herein, "carrier" includes any and all solvents, dispersion media, vehicles, coatings, diluents, antibacterial and antifungal agents, isotonic and absorption delaying agents, buffers, carrier solutions, suspensions, colloids, and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Supplementary active ingredients can also be incorporated into the compositions. The phrase "pharmaceutically-acceptable" refers to molecular entities and compositions that do not produce a toxic, an allergic, or similar untoward reaction when administered to a host.

[00136] As used herein, an "input agent responsive domain" is a domain of a transcription factor that binds to or otherwise responds to a condition or input agent in a manner that renders a linked DNA binding fusion domain responsive to the presence of that condition or input. In one embodiment, the presence of the condition or input results in a conformational change in the input agent responsive domain, or in a protein to which it is fused, that modifies the transcription-modulating activity of the transcription factor.

[00137] The term "*in vivo*" refers to assays or processes that occur in or within an organism, such as a multicellular animal. In some of the aspects described herein, a method or use can be said to occur "*in vivo*" when a unicellular organism, such as a bacterium, is used. The term "*ex vivo*" refers to methods and uses that are performed using a living cell with an intact membrane that is outside of the

body of a multicellular animal or plant, *e.g.,* explants, cultured cells, including primary cells and cell lines, transformed cell lines, and extracted tissue or cells, including blood cells, among others. The term "*in vitro*" refers to assays and methods that do not require the presence of a cell with an intact membrane, such as cellular extracts, and can refer to the introducing of a programmable synthetic biological circuit in a non-cellular system, such as a medium not comprising cells or cellular systems, such as cellular extracts.

[00138] The term "promoter," as used herein, refers to any nucleic acid sequence that regulates the expression of another nucleic acid sequence by driving transcription of the nucleic acid sequence, which can be a target gene, *e.g.,* heterologous target gene, encoding a protein or an RNA. Promoters can be constitutive, inducible, repressible, tissue-specific, or any combination thereof. A promoter is a control region of a nucleic acid sequence at which initiation and rate of transcription of the remainder of a nucleic acid sequence are controlled. A promoter can also contain genetic elements at which regulatory proteins and molecules can bind, such as RNA polymerase and other transcription factors. In some embodiments of the aspects described herein, a promoter can drive the expression of a transcription factor that regulates the expression of the promoter itself. Within the promoter sequence will be found a transcription initiation site, as well as protein binding domains responsible for the binding of RNA polymerase. Eukaryotic promoters will often, but not always, contain "TATA" boxes and "CAT" boxes. Various promoters, including inducible promoters, may be used to drive the expression of transgenes in the ceDNA vectors disclosed herein. A promoter sequence may be bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background.

[00139] The term "enhancer" as used herein refers to a cis-acting regulatory sequence (*e.g.,* 50-1,500 base pairs) that binds one or more proteins (*e.g.,* activator proteins, or transcription factor) to increase transcriptional activation of a nucleic acid sequence. Enhancers can be positioned up to 1,000,000 base pars upstream of the gene start site or downstream of the gene start site that they regulate. An enhancer can be positioned within an intronic region, or in the exonic region of an unrelated gene. An enhancer can be one naturally associated with a promoter, a gene or a sequence.

[00140] A promoter can be said to drive expression or drive transcription of the nucleic acid sequence that it regulates. The phrases "operably linked," "operatively positioned," "operatively linked," "under control," and "under transcriptional control" indicate that a promoter is in a correct functional location and/or orientation in relation to a nucleic acid sequence it regulates to control transcriptional initiation and/or expression of that sequence. An "inverted promoter," as used herein, refers to a promoter in which the nucleic acid sequence is in the reverse orientation, such that what was the coding strand is now the non-coding strand, and vice versa. Inverted promoter sequences can be

used in various embodiments to regulate the state of a switch. In addition, in various embodiments, a promoter can be used in conjunction with an enhancer.

[00141] A promoter can be one naturally associated with a gene or sequence, as can be obtained by isolating the 5' non-coding sequences located upstream of the coding segment and/or exon of a given gene or sequence. Such a promoter can be referred to as "endogenous." Similarly, in some embodiments, an enhancer can be one naturally associated with a nucleic acid sequence, located either downstream or upstream of that sequence.

[00142] In some embodiments, a coding nucleic acid segment is positioned under the control of a "recombinant promoter" or "heterologous promoter," both of which refer to a promoter that is not normally associated with the encoded nucleic acid sequence it is operably linked to in its natural environment. A recombinant or heterologous enhancer refers to an enhancer not normally associated with a given nucleic acid sequence in its natural environment. Such promoters or enhancers can include promoters or enhancers of other genes; promoters or enhancers isolated from any other prokaryotic, viral, or eukaryotic cell; and synthetic promoters or enhancers that are not "naturally occurring," *i.e.,* comprise different elements of different transcriptional regulatory regions, and/or mutations that alter expression through methods of genetic engineering that are known in the art. In addition to producing nucleic acid sequences of promoters and enhancers synthetically, promoter sequences can be produced using recombinant cloning and/or nucleic acid amplification technology, including PCR, in connection with the synthetic biological circuits and modules disclosed herein (see, *e.g.,* U.S. Pat. No. 4,683,202, U.S. Pat. No. 5,928,906, each incorporated herein by reference). Furthermore, it is contemplated that control sequences that direct transcription and/or expression of sequences within non-nuclear organelles such as mitochondria, chloroplasts, and the like, can be employed as well.

[00143] As described herein, an "inducible promoter" is one that is characterized by initiating or enhancing transcriptional activity when in the presence of, influenced by, or contacted by an inducer or inducing agent. An "inducer" or "inducing agent," as defined herein, can be endogenous, or a normally exogenous compound or protein that is administered in such a way as to be active in inducing transcriptional activity from the inducible promoter. In some embodiments, the inducer or inducing agent, *i.e.,* a chemical, a compound or a protein, can itself be the result of transcription or expression of a nucleic acid sequence (*i.e.,* an inducer can be an inducer protein expressed by another component or module), which itself can be under the control or an inducible promoter. In some embodiments, an inducible promoter is induced in the absence of certain agents, such as a repressor. Examples of inducible promoters include but are not limited to, tetracycline, metallothionine, ecdysone, mammalian viruses (*e.g.,* the adenovirus late promoter; and the mouse mammary tumor virus long terminal repeat (MMTV-LTR)) and other steroid-responsive promoters, rapamycin responsive promoters and the like.

[00144] The terms "DNA regulatory sequences," "control elements," and "regulatory elements," used interchangeably herein, refer to transcriptional and translational control sequences, such as promoters, enhancers, polyadenylation signals, terminators, protein degradation signals, and the like, that provide for and/or regulate transcription of a non-coding sequence (*e.g.,* DNA-targeting RNA) or a coding sequence (*e.g.,* site-directed modifying polypeptide, or Cas9/Csn1 polypeptide) and/or regulate translation of an encoded polypeptide.

[00145] "Operably linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. For instance, a promoter is operably linked to a coding sequence if the promoter affects its transcription or expression. An "expression cassette" includes a DNA sequence, *e.g.,* heterologous DNA sequence, that is operably linked to a promoter or other regulatory sequence sufficient to direct transcription of the transgene in the ceDNA vector. Suitable promoters include, for example, tissue specific promoters or promoters of AAV origin.

[00146] The term "subject" as used herein refers to a human or animal, to whom treatment, including prophylactic treatment, with the ceDNA vector according to the present disclosure, is provided. Usually, the animal is a vertebrate such as, but not limited to a primate, rodent, domestic animal or game animal. Primates include but are not limited to, chimpanzees, cynomologous monkeys, spider monkeys, and macaques, *e.g.,* Rhesus. Rodents include mice, rats, woodchucks, ferrets, rabbits and hamsters. Domestic and game animals include, but are not limited to, cows, horses, pigs, deer, bison, buffalo, feline species, *e.g.,* domestic cat, canine species, *e.g.,* dog, fox, wolf, avian species, *e.g.,* chicken, emu, ostrich, and fish, *e.g.,* trout, catfish and salmon. In certain embodiments of the aspects described herein, the subject is a mammal, *e.g.,* a primate or a human. A subject can be male or female. Additionally, a subject can be an infant or a child. In some embodiments, the subject can be a neonate or an unborn subject, *e.g.,* the subject is *in utero.* Preferably, the subject is a mammal. The mammal can be a human, non-human primate, mouse, rat, dog, cat, horse, or cow, but is not limited to these examples. Mammals other than humans can be advantageously used as subjects that represent animal models of diseases and disorders. In addition, the methods and compositions described herein can be used for domesticated animals and/or pets. A human subject can be of any age, gender, race or ethnic group, *e.g.,* Caucasian (white), Asian, African, black, African American, African European, Hispanic, Mideastern, etc. In some embodiments, the subject can be a patient or other subject in a clinical setting. In some embodiments, the subject is already undergoing treatment. In some embodiments, the subject is an embryo, a fetus, neonate, infant, child, adolescent, or adult. In some embodiments, the subject is a human fetus, human neonate, human infant, human child, human adolescent, or human adult. In some embodiments, the subject is an animal embryo, or non-human embryo or non-human primate embryo. In some embodiments, the subject is a human embryo.

[00147] As used herein, the term "host cell", includes any cell type that is susceptible to transformation, transfection, transduction, and the like with a nucleic acid construct or ceDNA

expression vector of the present disclosure. As non-limiting examples, a host cell can be an isolated primary cell, pluripotent stem cells, $CD34^+$ cells), induced pluripotent stem cells, or any of a number of immortalized cell lines (*e.g.,* HepG2 cells). Alternatively, a host cell can be an *in situ* or *in vivo* cell in a tissue, organ or organism.

[00148] The term "exogenous" refers to a substance present in a cell other than its native source. The term "exogenous" when used herein can refer to a nucleic acid (*e.g.,* a nucleic acid encoding a polypeptide) or a polypeptide that has been introduced by a process involving the hand of man into a biological system such as a cell or organism in which it is not normally found and one wishes to introduce the nucleic acid or polypeptide into such a cell or organism. Alternatively, "exogenous" can refer to a nucleic acid or a polypeptide that has been introduced by a process involving the hand of man into a biological system such as a cell or organism in which it is found in relatively low amounts and one wishes to increase the amount of the nucleic acid or polypeptide in the cell or organism, *e.g.,* to create ectopic expression or levels. In contrast, the term "endogenous" refers to a substance that is native to the biological system or cell.

[00149] The term "sequence identity" refers to the relatedness between two nucleic acid sequences. For purposes of the present disclosure, the degree of sequence identity between two deoxyribonucleotide sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, supra) as implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice *et al.*, 2000, supra), preferably version 3.0.0 or later. The optional parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EDNAFULL (EMBOSS version of NCBI NUC4.4) substitution matrix. The output of Needle labeled "longest identity" (obtained using the -nobrief option) is used as the percent identity and is calculated as follows: (Identical Deoxyribonucleotides.times.100)/(Length of Alignment-Total Number of Gaps in Alignment). The length of the alignment is preferably at least 10 nucleotides, preferably at least 25 nucleotides more preferred at least 50 nucleotides and most preferred at least 100 nucleotides.

[00150] The term "homology" or "homologous" as used herein is defined as the percentage of nucleotide residues that are identical to the nucleotide residues in the corresponding sequence on the target chromosome, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent nucleotide sequence homology can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, ClustalW2 or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. In some embodiments, a nucleic acid sequence (*e.g.,* DNA sequence), for example of a homology arm, is considered "homologous" when the sequence is at least

70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or more, identical to the corresponding native or unedited nucleic acid sequence (*e.g.,* genomic sequence) of the host cell.

[00151] The term "heterologous," as used herein, means a nucleotide or polypeptide sequence that is not found in the native nucleic acid or protein, respectively. A heterologous nucleic acid sequence may be linked to a naturally-occurring nucleic acid sequence (or a variant thereof) (*e.g.,* by genetic engineering) to generate a chimeric nucleotide sequence encoding a chimeric polypeptide. A heterologous nucleic acid sequence may be linked to a variant polypeptide (*e.g.,* by genetic engineering) to generate a nucleotide sequence encoding a fusion variant polypeptide.

[00152] A "vector" or "expression vector" is a replicon, such as plasmid, bacmid, phage, virus, virion, or cosmid, to which another DNA segment, *i.e.,* an "insert", may be attached so as to bring about the replication of the attached segment in a cell. A vector can be a nucleic acid construct designed for delivery to a host cell or for transfer between different host cells. As used herein, a vector can be viral or non-viral in origin and/or in final form, however for the purpose of the present disclosure, a "vector" generally refers to a ceDNA vector, as that term is used herein. The term "vector" encompasses any genetic element that is capable of replication when associated with the proper control elements and that can transfer gene sequences to cells. In some embodiments, a vector can be an expression vector or recombinant vector.

[00153] As used herein, the term "expression vector" refers to a vector that directs expression of an RNA or polypeptide from sequences linked to transcriptional regulatory sequences on the vector. The sequences expressed will often, but not necessarily, be heterologous to the cell. An expression vector may comprise additional elements, for example, the expression vector may have two replication systems, thus allowing it to be maintained in two organisms, for example in human cells for expression and in a prokaryotic host for cloning and amplification. The term "expression" refers to the cellular processes involved in producing RNA and proteins and as appropriate, secreting proteins, including where applicable, but not limited to, for example, transcription, transcript processing, translation and protein folding, modification and processing. "Expression products" include RNA transcribed from a gene, and polypeptides obtained by translation of mRNA transcribed from a gene. The term "gene" means the nucleic acid sequence which is transcribed (DNA) to RNA *in vitro* or *in vivo* when operably linked to appropriate regulatory sequences. The gene may or may not include regions preceding and following the coding region, *e.g.,* 5' untranslated (5'UTR) or "leader" sequences and 3' UTR or "trailer" sequences, as well as intervening sequences (introns) between individual coding segments (exons).

[00154] By "recombinant vector" is meant a vector that includes a nucleic acid sequence, *e.g.,* heterologous nucleic acid sequence, or "transgene" that is capable of expression *in vivo*. It should be understood that the vectors described herein can, in some embodiments, be combined with other

suitable compositions and therapies. In some embodiments, the vector is episomal. The use of a suitable episomal vector provides a means of maintaining the nucleotide of interest in the subject in high copy number extra chromosomal DNA thereby eliminating potential effects of chromosomal integration.

[00155] The phrase "genetic disease" as used herein refers to a disease, partially or completely, directly or indirectly, caused by one or more abnormalities in the genome, especially a condition that is present from birth. The abnormality may be a mutation, an insertion or a deletion. The abnormality may affect the coding sequence of the gene or its regulatory sequence. The genetic disease may be, but not limited to DMD, hemophilia, cystic fibrosis, Huntington's chorea, familial hypercholesterolemia (LDL receptor defect), hepatoblastoma, Wilson's disease, congenital hepatic porphyria, inherited disorders of hepatic metabolism, Lesch Nyhan syndrome, sickle cell anemia, thalassemia, xeroderma pigmentosum, Fanconi's anemia, retinitis pigmentosa, ataxia telangiectasia, Bloom's syndrome, retinoblastoma, and Tay-Sachs disease.

[00156] As used herein the term "comprising" or "comprises" is used in reference to compositions, methods, and respective component(s) thereof, that are essential to the method or composition, yet open to the inclusion of unspecified elements, whether essential or not.

[00157] As used herein the term "consisting essentially of" refers to those elements required for a given embodiment. The term permits the presence of elements that do not materially affect the basic and novel or functional characteristic(s) of that embodiment. The use of "comprising" indicates inclusion rather than limitation.

[00158] The term "consisting of" refers to compositions, methods, and respective components thereof as described herein, which are exclusive of any element not recited in that description of the embodiment.

[00159] As used herein the term "consisting essentially of" refers to those elements required for a given embodiment. The term permits the presence of additional elements that do not materially affect the basic and novel or functional characteristic(s) of that embodiment of the disclosure.

[00160] As used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural references unless the context clearly dictates otherwise. Thus, for example, references to "the method" includes one or more methods, and/or steps of the type described herein and/or which will become apparent to those persons skilled in the art upon reading this disclosure and so forth. Similarly, the word "or" is intended to include "and" unless the context clearly indicates otherwise. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of this disclosure, suitable methods and materials are described below. The abbreviation, "*e.g.*" is derived from the Latin exempli gratia and is used herein to indicate a non-limiting example. Thus, the abbreviation "*e.g.*" is synonymous with the term "for example."

[00161] Groupings of alternative elements or embodiments of the disclosure disclosed herein are not to be construed as limitations. Each group member can be referred to and claimed individually or in any combination with other members of the group or other elements found herein. One or more members of a group can be included in, or deleted from, a group for reasons of convenience and/or patentability. When any such inclusion or deletion occurs, the specification is herein deemed to contain the group as modified thus fulfilling the written description of all Markush groups used in the appended claims.

[00162] In some embodiments of any of the aspects, the disclosure described herein does not concern a process for cloning human beings, processes for modifying the germ line genetic identity of human beings, uses of human embryos for industrial or commercial purposes or processes for modifying the genetic identity of animals which are likely to cause them suffering without any substantial medical benefit to man or animal, and also animals resulting from such processes.

[00163] Other terms are defined herein within the description of the various aspects of the disclosure.

[00164] All patents and other publications; including literature references, issued patents, published patent applications, and co-pending patent applications; cited throughout this application are expressly incorporated herein by reference for the purpose of describing and disclosing, for example, the methodologies described in such publications that might be used in connection with the technology described herein. These publications are provided solely for their disclosure prior to the filing date of the present application. Nothing in this regard should be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior disclosure or for any other reason. All statements as to the date or representation as to the contents of these documents is based on the information available to the applicants and does not constitute any admission as to the correctness of the dates or contents of these documents.

[00165] The description of embodiments of the disclosure is not intended to be exhaustive or to limit the disclosure to the precise form disclosed. While specific embodiments of, and examples for, the disclosure are described herein for illustrative purposes, various equivalent modifications are possible within the scope of the disclosure, as those skilled in the relevant art will recognize. For example, while method steps or functions are presented in a given order, alternative embodiments may perform functions in a different order, or functions may be performed substantially concurrently. The teachings of the disclosure provided herein can be applied to other procedures or methods as appropriate. The various embodiments described herein can be combined to provide further embodiments. Aspects of the disclosure can be modified, if necessary, to employ the compositions, functions and concepts of the above references and application to provide yet further embodiments of the disclosure. Moreover, due to biological functional equivalency considerations, some changes can be made in protein structure without affecting the biological or chemical action in kind or amount.

These and other changes can be made to the disclosure in light of the detailed description. All such modifications are intended to be included within the scope of the appended claims.

[00166] Specific elements of any of the foregoing embodiments can be combined or substituted for elements in other embodiments. Furthermore, while advantages associated with certain embodiments of the disclosure have been described in the context of these embodiments, other embodiments may also exhibit such advantages, and not all embodiments need necessarily exhibit such advantages to fall within the scope of the disclosure.

II. Expression of a FVIII Protein from a ceDNA vector

[00167] The technology described herein is directed in general to the expression and/or production of FVIII protein in a cell from a non-viral DNA vector, *e.g.,* a ceDNA vector as described herein. ceDNA vectors for expression of FVIII protein are described in the section entitled "ceDNA vectors in general". In particular, ceDNA vectors for expression of FVIII protein comprise a pair of ITRs (*e.g.,* symmetric or asymmetric as described herein) and between the ITR pair, a nucleic acid encoding an FVIII protein operatively linked to a promoter or regulatory sequence. A distinct advantage of ceDNA vectors for expression of FVIII protein over traditional AAV vectors, and even lentiviral vectors, is that there is no size constraint for the nucleic acid sequences, *e.g.,* heterologous nucleic acid sequences, encoding a desired protein. Even a full length 6.8kb FVIII protein can be expressed from a single ceDNA vector. Thus, the ceDNA vectors described herein can be used to express a therapeutic FVIII protein in a subject in need thereof, *e.g.,* a subject with hemophilia A.

[00168] As one will appreciate, the ceDNA vector technologies can be adapted to any level of complexity or can be used in a modular fashion, where expression of different components of a FVIII protein can be controlled in an independent manner. For example, it is specifically contemplated that the ceDNA vector technologies described here can be as simple as using a single ceDNA vector to express a single gene sequence (*e.g.,* a FVIII protein) or can be as complex as using multiple ceDNA vectors, where each vector expresses multiple FVIII proteins or associated co-factors or accessory proteins that are each independently controlled by different promoters. The following embodiments are specifically contemplated and can adapted by one of skill in the art as desired.

[00169] In one embodiment, a single ceDNA vector can be used to express a single component of an FVIII protein. Alternatively, a single ceDNA vector can be used to express multiple components (*e.g.,* at least 2) of a FVIII protein under the control of a single promoter (*e.g.,* a strong promoter), optionally using an IRES sequence(s) to ensure appropriate expression of each of the components, *e.g.,* co-factors or accessory proteins.

[00170] As one of skill in the art will appreciate, it is often desirable to express components of a FVIII protein at different expression levels, thus controlling the stoichiometry of the individual components expressed to ensure efficient FVIII protein folding and combination in the cell. Additional

variations of ceDNA vector technologies can be envisioned by one of skill in the art or can be adapted from protein production methods using conventional vectors.

A. Nucleic Acids

[00171] The characterization and development of nucleic acid molecules for potential therapeutic use are provided herein. According to some embodiments, the nucleic acids for therapeutic use encode a FVIII protein. In some embodiments, chemical modification of oligonucleotides for the purpose of altered and improved *in vivo* properties (delivery, stability, lifetime, folding, target specificity), as well as their biological function and mechanism that directly correlate with therapeutic application, are described where appropriate.

[00172] The therapeutic nucleic acid described herein is a closed ended double stranded DNA, *e.g.*, ceDNA. A distinct advantage of ceDNA vectors for expression of a therapeutic protein over traditional AAV vectors, and even lentiviral vectors, is that there is no size constraint for the nucleic acid sequences, *e.g.*, heterologous nucleic acid sequences, encoding a desired protein. Thus, ceDNA vectors can be used to express a FVIII protein in a subject in need thereof.

[00173] In general, a ceDNA vector for expression of a FVIII as disclosed herein, comprises in the 5' to 3' direction: a first adeno-associated virus (AAV) inverted terminal repeat (ITR), a nucleic acid sequence of interest (for example an expression cassette as described herein) and a second AAV ITR. The ITR sequences selected from any of: (i) at least one WT ITR and at least one modified AAV inverted terminal repeat (mod-ITR) (*e.g.*, asymmetric modified ITRs); (ii) two modified ITRs where the mod-ITR pair have a different three-dimensional spatial organization with respect to each other (*e.g.*, asymmetric modified ITRs), or (iii) symmetrical or substantially symmetrical WT-WT ITR pair, where each WT-ITR has the same three-dimensional spatial organization, or (iv) symmetrical or substantially symmetrical modified ITR pair, where each mod-ITR has the same three-dimensional spatial organization.

[00174] In some embodiments, a transgene encoding the FVIII protein can also encode a secretory sequence so that the FVIII protein is directed to the Golgi Apparatus and Endoplasmic Reticulum where the FVIII protein is folded into the correct conformation by chaperone molecules as it passes through the ER and out of the cell. Exemplary secretory sequences include, but are not limited to VH-02 (SEQ ID NO: 88) and VK-A26 (SEQ ID NO: 89) and Igκ Kβ signal sequence (SEQ ID NO: 548), as well as a Gluc secretory signal that allows the tagged protein to be secreted out of the cytosol, TMD-ST secretory sequence, that directs the tagged protein to the Golgi.

[00175] Regulatory switches can also be used to fine tune the expression of the FVIII protein so that the FVII protein is expressed as desired, including but not limited to, expression of the FVIII protein at a desired expression level or amount, or alternatively, when there is the presence or absence of particular signal, including a cellular signaling event. For instance, as described herein, expression of

the FVIII protein from the ceDNA vector can be turned on or turned off when a particular condition occurs, as described herein in the section entitled Regulatory Switches.

[00176] For example, and for illustration purposes only, FVIII proteins can be used to turn off undesired reaction, such as too high a level of production of the FVIII protein. The FVIII gene can contain a signal peptide marker to bring the FVIII protein to the desired cell. However, in either situation it can be desirable to regulate the expression of the FVIII protein. ceDNA vectors readily accommodate the use of regulatory switches.

[00177] A distinct advantage of ceDNA vectors over traditional AAV vectors, and even lentiviral vectors, is that there is no size constraint for the nucleic acid sequence encoding the FVIII protein. Thus, even a full-length FVIII, as well as optionally any co-factors or assessor proteins can be expressed from a single ceDNA vector. In addition, depending on the necessary stiochemistry one can express multiple segments of the same FVIII protein, and can use same or different promoters, and can also use regulatory switches to fine tune expression of each region. For example, a ceDNA vector that comprises a dual promoter system can be used, so that a different promoter is used for each domain of the FVIII protein. Use of a ceDNA plasmid to produce the FVIII protein can include a unique combination of promoters for expression of the domains of the FVIII protein that results in the proper ratios of each domain for the formation of functional FVIII protein. Accordingly, in some embodiments, a ceDNA vector can be used to express different regions of FVIII protein separately (*e.g.,* under control of a different promoter).

[00178] In another embodiment, the FVIII protein expressed from the ceDNA vectors further comprises an additional functionality, such as fluorescence, enzyme activity, secretion signal or immune cell activator.

[00179] In some embodiments, the ceDNA encoding the FVIII protein can further comprise a linker domain, for example. As used herein "linker domain" refers to an oligo- or polypeptide region from about 2 to 100 amino acids in length, which links together any of the domains/regions of the FVIII protein as described herein. In some embodiment, linkers can include or be composed of flexible residues such as glycine and serine so that the adjacent protein domains are free to move relative to one another. Longer linkers may be used when it is desirable to ensure that two adjacent domains do not sterically interfere with one another. Linkers may be cleavable or non-cleavable. Examples of cleavable linkers include 2A linkers (for example T2A), 2A-like linkers or functional equivalents thereof and combinations thereof. The linker can be a linker region is T2A derived from *Thosea asigna* virus.

[00180] In some embodiments, a transgene encoding the FVIII protein can also include a signal sequence. In some embodiments, a transgene encoding the FVIII protein can have

It is well within the abilities of one of skill in the art to take a known and/or publically available protein sequence of FVIII, and reverse engineer a cDNA sequence to encode such a protein. The

cDNA can then be codon optimized to match the intended host cell and inserted into a ceDNA vector as described herein.

B. ceDNA vectors expressing FVIII Protein

[00181] A ceDNA vector for expression of FVIII protein having one or more sequences encoding a desired FVIII can comprise regulatory sequences such as promoters, secretion signals, polyA regions, and enhancers. At a minimum, a ceDNA vector comprises one or more nucleic acid sequences, *e.g.,* heterologous nucleic acid sequences, encoding a FVIII protein.

[00182] In order to achieve highly efficient and accurate FVIII protein assembly, it is specifically contemplated in some embodiments that the FVIII protein comprise an endoplasmic reticulum ER leader sequence to direct it to the ER, where protein folding occurs. For example, a sequence that directs the expressed protein(s) to the ER for folding.

[00183] In some embodiments, a cellular or extracellular localization signal (*e.g.,* secretory signal, nuclear localization signal, mitochondrial localization signal, *etc.*) is comprised in the ceDNA vector to direct the secretion or desired subcellular localization of FVIII such that the FVIII protein can bind to intracellular target(s) (*e.g.,* an intrabody) or extracellular target(s). In some embodiments, a FVIII sequence may contain a mutation that enhances FVIII secretion out of the ER. For example, FVIII secretion requires high levels of intracellular ATP, consistent with an ATP-dependent release from BiP. Mutation of Phe at position 309 to Ser or Ala (F309S) enhances the secretion of functional FVIII and reduced its ATP dependence. (Swaroop *et al.*, *J. Biol. Chem* (1997) 272:27428-34).

[00184] In some embodiments, a ceDNA vector for expression of FVIII protein as described herein permits the assembly and expression of any desired FVIII protein in a modular fashion. As used herein, the term "modular" refers to elements in a ceDNA expressing plasmid that can be readily removed from the construct. For example, modular elements in a ceDNA-generating plasmid comprise unique pairs of restriction sites flanking each element within the construct, enabling the exclusive manipulation of individual elements. Thus, the ceDNA vector platform can permit the expression and assembly of any desired FVIII ORF with any desired *cis*-acting elements such as enhancer(s), promoters, introns, 5'-UTR, 3'-UTR, poly-A, *etc.* Provided herein in various embodiments are ceDNA plasmid vectors that can reduce and/or minimize the amount of manipulation required to assemble a desired ceDNA vector encoding FVIII protein.

C. Exemplary FVIII Proteins expressed by ceDNA vectors

[00185] In particular, a ceDNA vector for expression of FVIII protein as disclosed herein can encode, for example, but is not limited to, FVIII proteins, as well as variants, and/or active fragments thereof, for use in the treatment, prophylaxis, and/or amelioration of one or more symptoms of hemophilia A. In one aspect, the hemophilia A is a human hemophilia A.

(i) FVIII therapeutic proteins and fragments thereof

[00186] Essentially any version of the FVIII therapeutic protein or fragment thereof (*e.g.*, functional fragment) can be encoded by and expressed in and from a ceDNA vector as described herein. One of skill in the art will understand that FVIII therapeutic protein includes all splice variants and orthologs of the FVIII protein. FVIII therapeutic protein includes intact molecules as well as fragments (*e.g.*, functional) thereof. According to embodiments of the present disclosure, nucleic acids encoding particular FVIII proteins are set forth in **Table 1A**.

Factor VIII

[00187] Factor VIII is the nonenzymatic cofactor to the activated clotting factor IX (FIXa), which, when proteolytically activated, interacts with FIXa to form a tight noncovalent complex that binds to and activates factor X (FX).

[00188] The Factor VIII gene or protein can also be referred to as F8, Coagulation Factor VIII, Procoagulant Component, Antihemophilic Factor, F8C, AHF, DXS1253E, FVIII, HEMA, or F8B. Expression of the Factor VIII gene is tissue-specific and is mostly observed in liver cells. The highest level of the mRNA and Factor VIII proteins has been detected in liver sinusoidal cells; significant amounts of Factor VIII are also present in hepatocytes and in Kupffer cells (resident macrophages of liver sinusoids). Moderate levels of Factor VIII protein are detectable in the serum and plasma. Low to moderate levels of Factor VIII protein are expressed in fetal brain, retina, kidney and testis.

[00189] Factor VIII mRNA is expressed throughout many tissues of the body, including bone marrow, whole blood, white blood cells, lymph nodes, thymus, brain, cerebral cortex, cerebellum, retina, spinal cord, tibial nerve, heart, artery, smooth muscle, skeletal muscle, small intestine, colon, adipocytes, kidney, liver, lung, spleen, stomach, esophagus, bladder, pancreas, thyroid, salivary gland, adrenal gland, pituitary gland, breast, skin, ovary, uterus, placenta, prostate, and testis. The FVIII gene localized on the long arm of the X chromosome occupies a region approximately 186 kbp long and consists of 26 exons (69–3,106 bp) and introns (from 207 bp to 32.4 kbp). The total length of the coding sequence of this gene is 9 kbp.

[00190] The mature factor VIII polypeptide comprises the A1–A2–B–A3–C1-C2 structural domains. Three acidic subdomains, which are denoted as a1–a3 – A1(a1)–A2(a2)–B–(a3)A3–C1–C2, localize at the boundaries of A domains and play a significant role in the interaction between FVIII and other proteins (in particular, with thrombin). Mutations in these subdomains reduce the level of factor VIII activation by thrombin (*see* **FIG. 9** for FVIII processing steps).

[00191] The factor VIII protein (Coagulation factor VIII isoform) is a preproprotein [Homo sapiens]; Accession number: NP_000123.1 (2351 aa) and has the sequence as set forth in SEQ ID NO: 492. According to some embodiments, an FVIII protein contemplated herein can be a modified FVIII protein. According to further embodimnts, the FVIII protein can have the B-domain deleted and comprise the amino acid sequence set forth in SEQ ID NO: 555).

[00192] According to some embodiments, FVIII expressed by some of the FVIII-ceDNA vectors disclosed herein is AFSTYLA®; recombinant, single chain coagulation factor VIII (rVIII-SingleChain); lonoctocog alfa; CAS Registry Number: 1388129-63-2.

[00193] AFSTYLA® is a single chain recombinant factor VIII (FVIII) that most of the B-domain occurring in wild-type, full-length FVIII and 4 amino acids of the adjacent acidic A3 domain are removed (*e.g.*, amino acids 765 to 1652 of full-length FVIII).

[00194] It is to be understood that the amino acid D (aspartic acid) at position 56 in SEQ ID NO: 555 above can be freely substituted with V (valine) as a wild-type variant and that any nucleotide sequence disclosed herein for FVIII-ceDNA ORF is to be contemplated to include corresponding nucleic acid sequence(s) for the valine variant at position 56.

[00195] Expression of FVIII therapeutic protein or fragment thereof from a ceDNA vector can be achieved both spatially and temporally using one or more inducible or repressible promoters, or tissue specific promoters (*e.g.*, synthetic liver specific promoters like TTR promoters (TTRm), CpG minimized hAAT promoters described herein), as known in the art or described herein, including regulatory switches as described herein.

[00196] In one embodiment, FVIII therapeutic protein can be an "therapeutic protein variant," which refers to the FVIII therapeutic protein having an altered amino acid sequence, composition or structure as compared to its corresponding native FVIII therapeutic protein. In one embodiment, FVIII is a functional version (*e.g.,* wild-type FVIII protein for D56V variant described above). It may also be useful to express a mutant version of FVIII protein such as a point mutation (F309 mutation) or deletion mutation (*e.g.*, B domain deleted and/or single chain recombinant FVIII) as described in many examples herein. FVIII therapeutic protein expressed from the ceDNA vectors may further comprise a sequence/moiety that confers an additional functionality, such as fluorescence, enzyme activity, or secretion signal. In one embodiment, an FVIII therapeutic protein variant comprises a non-native tag sequence for identification (*e.g.,* an immunotag) to allow it to be distinguished from endogenous FVIII therapeutic protein in a recipient host cell.

[00197] According to some embodiments, open reading frames (ORF) of the FVIII ceDNA vectors disclosed herein are codon optimized.

[00198] According to some other embodiments, the FVIII ceDNA vector is CpG minimizded. For example, enhancers, promoters, 5'UTR, spacers, introns, 3'UTR, and WPRE sequences in the FVIII ceDNA vectors can be modified to have minimized level of CpG to ensure the robust expression of the vector.

[00199] In one embodiment, the FVIII therapeutic protein encoding sequence can be derived from an existing host cell or cell line, for example, by reverse transcribing mRNA obtained from the host and amplifying the sequence using PCR.

(ii) ceDNA vectors expressing FVIII therapeutic protein

[00200] A ceDNA vector having one or more sequences encoding a desired FVIII therapeutic protein can comprise regulatory sequences such as promoters, secretion signals, introns, polyA regions, and enhancers to maximize expression of the FVIII therapeutic protein when delivered to a desired cell or tissue. At a minimum, a ceDNA vector comprises one or more nucleic acid sequences encoding the FVIII therapeutic protein or functional fragment thereof. In one embodiment, the ceDNA vector comprises an FVIII sequence set forth in any one of SEQ ID NOs: 71-183, 556 and 626-633.

[00201] According to some aspects, the disclosure provides a ceDNA vector comprising at least one nucleic acid sequence between flanking inverted terminal repeats (ITRs), wherein at least one nucleic acid sequence encodes at least one FVIII protein, wherein the at least one nucleic acid sequence that encodes at least one FVIII protein is selected from a sequence having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or least 99% identity to any sequence in **Table 1A** (SEQ ID NOs: 71-183, 556 and 626-633). According to some embodiments, the at least one nucleic acid sequence that encodes at least one FVIII protein is at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or least 99% identical to SEQ ID NO: 556. According to some embodiments, the at least one nucleic acid sequence that encodes at least one FVIII protein consists of SEQ ID NO: 556. According to some embodiments, the at least one nucleic acid that encodes at least one FVIII protein comprises SEQ ID NO: 556, wherein SEQ ID NO: 556 further comprises one or more modifications. According to some embodiments, the at least one nucleic acid comprising SEQ ID NO: 556, further comprising one or more modifications comprises or consists of a sequence selected from any one of SEQ ID NOs: 627- 633.

[00202] **Table 1A** provides sequence identifiers, descriptions of the codon optimized FVIII ORFs and the names used herein. **Table 1B** provides the corresponding GE numbers used herein for the names of FVIII ORFs.

Table 1A: Description of exemplary codon optimized FVIII ORF sequences, sequence identifiers and names used herein

SEQ ID NO	Description	Name
71	Codon optimized hFVIII with 226aa/N6 B-domain as found in ceDNA933	hFVIII-226variant-F309S_CpGmin-codop_ORF
72	Codon optimized hFVIII with 226aa/N6 B-domain as found in ceDNA1265	hFVIII-F309S-BD226-Codop-run4-seq102
73	Codon optimized hFVIII with 226aa/N6 B-domain as found in ceDNA1270	hFVIII-F309S-BD226seq124
74	Codon optimized hFVIII with SC B-domain as found in ceDNA1368	hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
75	Codon optimized hFVIII with SC B-domain and F309S encoding mutation as found in ceDNA1367	hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD
76	Codon optimized hFVIII with SC B-domain as found in ceDNA1374	hFVIII-F309S-BD226seq124 Afstyla-BDD-F309

77	Codon optimized hFVIII with SC B-domain and F309S encoding mutation as found in ceDNA1373	hFVIII-F309S-BD226seq 124-Afstyla-BDD
78	Codon optimized hFVIII with SC B-domain as found in ceDNA1918	FVIII-SC_0CpG_1_ORF
79	Codon optimized hFVIII with SC B-domain as found in ceDNA1919	FVIII-SC_0CpG_6_ORF
80	Codon optimized hFVIII with SC B-domain as found in ceDNA1920	FVIII-SC_0CpG_8_ORF
81	Codon optimized hFVIII with SC B-domain as found in ceDNA1921	FVIII-SC_5_ORF
82	Codon optimized hFVIII with SC B-domain as found in ceDNA1922	FVIII-SC_5k_wt3_3_ORF
83	Codon optimized hFVIII with SC B-domain as found in ceDNA1923	FVIII-SC_5k_wt3_5_ORF
84	Codon optimized hFVIII with SC B-domain and F309S encoding mutation as found in ceDNA1927	FVIII-SC_0CpG_1_F309S
85	Codon optimized hFVIII with SC B-domain and F309S encoding mutation as found in ceDNA1928	FVIII-SC_0CpG_6_F309S
86	Codon optimized hFVIII with SC B-domain and F309S encoding mutation as found in ceDNA1929	FVIII-SC_0CpG_8_F309S
87	Codon optimized hFVIII with SC B-domain and F309S encoding mutation as found in ceDNA1930	FVIII-SC_5_F309S
88	Codon optimized hFVIII with SC B-domain and F309S encoding mutation as found in ceDNA1931	FVIII-SC_5k_wt3_3_F309S
89	Codon optimized hFVIII with SC B-domain and F309S encoding mutation as found in ceDNA1932	FVIII-SC_5k_wt3_5_F309S
90	Codon optimized hFVIII with SC B-domain and F309S encoding mutation as found in ceDNA1933	FVIII-SC_5k_wt3_5-0CpG_6_ F309S hybrid
91	Sequence of Factor VIII ORF (GE-707) with signal seqeunce removed	FVIII_1368_ORF_ mat_peptide
92	Sequence of Factor VIII ORF (GE-715) with signal seqeunce removed	FVIII_1374_ ORF_mat_peptide
93	hFVIII ORF with SC B-domain	hFVIII-Wt-Afstyla BDD
94	Codon optimized hFVIII with SC B-domain and F309S encoding mutation. Intron engineered into between Exon1 and Exon2	FVIII-SC_1367_miniF8_ 50/100
95	Codon optimized hFVIII with SC B-domain and F309S encoding mutation. Intron engineered into between Exon1 and Exon2	FVIII-SC_1367_miniF8_ 50/200
96	Codon optimized hFVIII with SC B-domain and F309S encoding mutation. Intron engineered into between Exon1 and Exon2	FVIII-SC_1367_miniF8_ 200/200
97	Codon optimized hFVIII with SC B-domain and F309S encoding mutation. Intron engineered into between Exon1 and Exon2	FVIII-SC_1367_miniF8_ 500/500
98	Codon optimized hFVIII with SC B-domain and F309S encoding mutation. Intron engineered into between Exon1 and Exon2	FVIII-SC_1367_HBB_ intron1
99	Codon optimized hFVIII with SC B-domain and F309S encoding mutation. Intron engineered into between Exon1 and Exon2	FVIII-SC_1367_ Embedded_HCR1_ footprint123

100	Codon optimized hFVIII with SC B-domain and F309S encoding mutation. Intron engineered into between Exon1 and Exon2	FVIII-SC_1367_ Embedded_ProEnh
101	Codon optimized hFVIII with SC B-domain and F309S encoding mutation. Intron engineered into between Exon1 and Exon2	FVIII-SC_1367_ Embedded_enhancer_ HNF_array
102	Codon optimized hFVIII with SC B-domain and F309S encoding mutation. Intron engineered into between Exon1 and Exon2	FVIII-SC_1367_F8_intron8
103	Codon optimized hFVIII with SC B-domain and F309S encoding mutation. Intron engineered into between Exon1 and Exon2	FVIII-SC_1367_F8_intron16
104	Codon optimized hFVIII with SC B-domain and F309S encoding mutation. Intron engineered into between Exon1 and Exon2	FVIII-SC_1367_ MVM_intron
105	Codon optimized hFVIII with SC B-domain and F309S encoding mutation. Intron engineered into between Exon1 and Exon2	FVIII-SC_1367::33bpFlanks_miniF 8_50/100
106	Codon optimized hFVIII with SC B-domain and F309S encoding mutation. Intron engineered into between Exon1 and Exon2	FVIII-SC_1367::33bpFlanks_miniF 8_200/200
107	Codon optimized hFVIII with SC B-domain and F309S encoding mutation. Intron engineered into between Exon1 and Exon2	FVIII-SC_1367::33bpFlanks_HBB_ intron1
108	Codon optimized hFVIII with SC B-domain and F309S encoding mutation. Intron engineered into between Exon1 and Exon2	FVIII-SC_1367::33bpFlanks_F8_in ron8
109	Codon optimized hFVIII with SC B-domain and F309S encoding mutation. Intron engineered into between Exon1 and Exon2	FVIII-SC_1367::33bpFlanks_no intron
110	Codon optimized hFVIII with SC B-domain and F309S encoding mutation. Intron engineered into between Exon1 and Exon2	FVIII-SC_1373_miniF8_ 50/100
111	Codon optimized hFVIII with SC B-domain and F309S encoding mutation. Intron engineered into between Exon1 and Exon2	FVIII-SC_1373_miniF8_ 50/200
112	Codon optimized hFVIII with SC B-domain and F309S encoding mutation. Intron engineered into between Exon1 and Exon2	FVIII-SC_1373_miniF8_ 200/200
113	Codon optimized hFVIII with SC B-domain and F309S encoding mutation. Intron engineered into between Exon1 and Exon2	FVIII-SC_1373_miniF8_ 500/500
114	Codon optimized hFVIII with SC B-domain and F309S encoding mutation. Intron engineered into between Exon1 and Exon2	FVIII-SC_1373_ HBB_intron1
115	Codon optimized hFVIII with SC B-domain and F309S encoding mutation. Intron engineered into between Exon1 and Exon2	FVIII-SC_1373_ Embedded_HCR1_ footprint123
116	Codon optimized hFVIII with SC B-domain and F309S encoding mutation. Intron engineered into between Exon1 and Exon2	FVIII-SC_1373_ Embedded_ProEnh

117	Codon optimized hFVIII with SC B-domain and F309S encoding mutation. Intron engineered into between Exon1 and Exon2	FVIII-SC_1373_ Embedded_enhancer_ HNF_array
118	Codon optimized hFVIII with SC B-domain and F309S encoding mutation. Intron engineered into between Exon1 and Exon2	FVIII-SC_1373_ F8_intron8
119	Codon optimized hFVIII with SC B-domain and F309S encoding mutation. Intron engineered into between Exon1 and Exon2	FVIII-SC_1373_ F8_intron16
120	Codon optimized hFVIII with SC B-domain and F309S encoding mutation. Intron engineered into between Exon1 and Exon2	FVIII-SC_1373_ MVM_intron
121	Codon optimized hFVIII with SC B-domain and F309S encoding mutation. Intron engineered into between Exon1 and Exon2	FVIII-SC_1373::33bpFlanks_miniF 8_50/100
122	Codon optimized hFVIII with SC B-domain and F309S encoding mutation. Intron engineered into between Exon1 and Exon2	FVIII-SC_1373::33bpFlanks_miniF 8_200/200
123	Codon optimized hFVIII with SC B-domain and F309S encoding mutation. Intron engineered into between Exon1 and Exon2	FVIII-SC_1373::33bpFlanks_HBB_ intron1
124	Codon optimized hFVIII with SC B-domain and F309S encoding mutation. Intron engineered into between Exon1 and Exon2	FVIII-SC_1373::33bpFlanks_F8_in ron8
125	Codon optimized hFVIII with SC B-domain and F309S encoding mutation. Intron engineered into between Exon1 and Exon2	FVIII-SC_1373::33bpFlanks_no intron
126	Codon optimized hFVIII with SC B-domain and F309S encoding mutation	FVIII_SC_F309S_ GeneD_v4
127	Codon optimized hFVIII with SC B-domain	FVIII_SC_F309_ GeneD_v4
128	Codon optimized hFVIII with SC B-domain and F309S encoding mutation	FVIII_SC_F309S_ codop_3b
129	Codon optimized hFVIII with SC B-domain	FVIII_SC_F309_ codop_3b
130	Codon optimized hFVIII with SC B-domain and F309S encoding mutation	FVIII_SC_F309S_ GeneD_v3
131	Codon optimized hFVIII with SC B-domain and F309S encoding mutation	FVIII_SC_F309S_ GeneD_v2
132	Codon optimized hFVIII with SC B-domain and F309S encoding mutation	FVIII_SC_F309S_ codop_7b
133	Codon optimized hFVIII with SC B-domain and F309S encoding mutation	FVIII_SC_F309S_ codop_6b
134	Codon optimized hFVIII with SC B-domain and F309S encoding mutation	FVIII_SC_F309S_ codop_1b
135	Codon optimized hFVIII with SC B-domain	FVIII_SC_F309_ GeneD_v3
136	Codon optimized hFVIII with SC B-domain	FVIII_SC_F309_ GeneD_v2
137	Codon optimized hFVIII with SC B-domain	FVIII_SC_F309_ codop_7b
138	Codon optimized hFVIII with SC B-domain	FVIII_SC_F309_ codop_6b

139	Codon optimized hFVIII with SC B-domain	FVIII_SC_F309_ codop_1b
140	FVIII ORF from 1368 (Afstyla BDD) with heterologous A1AT leader sequence	FVIII_1368ORF_ A1AT-SSv3
141	FVIII ORF from 1374 (Afstyla BDD) with heterologous typsinogen leader sequence	FVIII_1374ORF_ TRYP-SSv2
142	FVIII ORF from 1374 (Afstyla BDD) with heterologous trans-Plasminogen Activator leader sequence	FVIII_1374ORF_ tPA-SSv1
143	FVIII ORF from 1374 (Afstyla BDD) with synthetic leader sequence	FVIII_1374ORF_ Secrecon-SSv2
144	FVIII ORF from 1374 (Afstyla BDD) with synthetic leader sequence	FVIII_1374ORF_ Secrecon-SSv1
145	FVIII ORF from 1374 (Afstyla BDD) with heterologous Fibroin-L leader sequence	FVIII_1374ORF_ Lonz-SSv2
146	FVIII ORF from 1374 (Afstyla BDD) with heterologous IL2 leader sequence	FVIII_1374ORF_ IL2-SSv1
147	FVIII ORF from 1374 (Afstyla BDD) with heterologous Gaussia leader sequence	FVIII_1374ORF_ Gaus-SSv1
148	FVIII ORF from 1374 (Afstyla BDD) with heterologous Chymotypsinogen leader sequence	FVIII_1374ORF_ CHY-SSv1
149	FVIII ORF from 1374 (Afstyla BDD) with heterologous Albumin leader sequence	FVIII_1374ORF_ ALB-SSv1
150	FVIII ORF from 1374 (Afstyla BDD) with heterologous A1AT leader sequence	FVIII_1374ORF_ A1AT-SSv3
151	FVIII ORF from 1368 (Afstyla BDD) with heterologous Trypsinogen leader sequence	FVIII_1368ORF_ TRYP-NS-struct-v2
152	FVIII ORF from 1368 (Afstyla BDD) with heterologous Trypsinogen leader sequence	FVIII_1368ORF_ TRYP-NS-CAI-v2
153	FVIII ORF from 1368 (Afstyla BDD) with heterologous trans-Plasminogen Activator leader sequence	FVIII_1368ORF_tPA-NS-struct
154	FVIII ORF from 1368 (Afstyla BDD) with heterologous trans-Plasminogen Activator leader sequence	FVIII_1368ORF_ tPA-NS-CAI-v2
155	FVIII ORF from 1368 (Afstyla BDD) with synthetic leader sequence	FVIII_1368ORF_ Secrecon-v1-NS-struct-v1
156	FVIII ORF from 1368 (Afstyla BDD) with synthetic leader sequence	FVIII_1368ORF_ Secrecon-v1-NS-CAI-v2
157	FVIII ORF from 1368 (Afstyla BDD) with heterologous Fibroin-L leader sequence	FVIII_1368ORF_ Lonz-SSv2
158	FVIII ORF from 1368 (Afstyla BDD) with heterologous Fibroin-L leader sequence	FVIII_1368ORF_ Lonz-NS-struct-v3
159	FVIII ORF from 1368 (Afstyla BDD) with heterologous Fibroin-L leader sequence	FVIII_1368ORF_ Lonz-NS-CAI-v2
160	FVIII ORF from 1368 (Afstyla BDD) with heterologous IL2 leader sequence	FVIII_1368ORF_ IL2-NS-struct-v2
161	FVIII ORF from 1368 (Afstyla BDD) with heterologous IL2 leader sequence	FVIII_1368ORF_ IL2-NS-CAI
162	FVIII ORF from 1368 (Afstyla BDD) with heterologous Gaussia leader sequence	FVIII_1368ORF_ Gaus-NS-struct-v2
163	FVIII ORF from 1368 (Afstyla BDD) with heterologous Gaussia leader sequence	FVIII_1368ORF_ Gaus-NS-CAI-v2
164	FVIII ORF from 1368 (Afstyla BDD) with heterologous Chymotrypsinogen leader sequence	FVIII_1368ORF_ CHY-NS-struct

165	FVIII ORF from 1368 (Afstyla BDD) with heterologous Chymotrypsinogen leader sequence	FVIII_1368ORF_ CHY-NS-CAI-v2
166	FVIII ORF from 1368 (Afstyla BDD) with heterologous A1AT leader sequence	FVIII_1368ORF_ A1AT-NS-struct
167	FVIII ORF from 1368 (Afstyla BDD) with heterologous A1AT leader sequence	FVIII_1368ORF_ A1AT-NS-CAI
168	FVIII ORF from 1368 (Afstyla BDD) with heterologous hCD33 leader sequence	FVIII-1368ORF_ CD33-NS-struct-v2
169	FVIII ORF from 1368 (Afstyla BDD) with heterologous hCD33 leader sequence.	FVIII-1368ORF_ CD33-NS-CAI-v2
170	FVIII ORF from 1368 (Afstyla BDD) with heterologous Albumin leader sequence	FVIII-1368ORF_ ALB-NS-struct
171	FVIII ORF from 1368 (Afstyla BDD) with heterologous Albumin leader sequence.	FVIII-1368ORF_ ALB-NS-CAI-v2
172	FVIII ORF from 1368 (Afstyla BDD) with heterologous CD33 leader sequence	FVIII_1368ORF_ CD33-SSv1
173	FVIII ORF from 1374 (Afstyla BDD) with heterologous A1AT leader sequence v2	FVIII_1374ORF_ A1AT-SSv2
174	FVIII ORF from 1368 (Afstyla BDD) with heterologous Trypsinogen leader sequence	FVIII_1368ORF_ TRYP-SSv1
175	FVIII ORF from 1368 (Afstyla BDD) with heterologous trans plasminogen activator leader sequence	FVIII_1368ORF_ tPA-SSv1
176	FVIII ORF from 1368 (Afstyla BDD) with heterologous Secrecon leader sequence	FVIII_1368ORF_ Secrecon-SSv2
177	FVIII ORF from 1368 (Afstyla BDD) with heterologous Secrecon leader sequence	FVIII_1368ORF_ Secrecon-SSv1
178	FVIII ORF from 1368 (Afstyla BDD) with heterologous Fibroin-L leader sequence.	FVIII_1368ORF_ Lonz-SSv1
179	FVIII ORF from 1368 (Afstyla BDD) with heterologous IL-2 leader sequence	FVIII_1368ORF_ IL2-SSv1
180	FVIII ORF from 1368 (Afstyla BDD) with heterologous Gaussia leader sequence	FVIII_1368ORF_ Gaus-SSv1
181	FVIII ORF from 1368 (Afstyla BDD) with heterologous Chymotrypsin leader sequence	FVIII_1368ORF_ CHY-SSv1
182	FVIII ORF from 1368 (Afstyla BDD) with heterologous A1AT leader sequence	FVIII_1368ORF_ A1AT-SSv2
183	FVIII ORF from 1368 (Afstyla BDD) with heterologous Albumin leader sequence	FVIII-1368ORF_ ALB-SSv1
556	FVIII ORF from 1651 (Afstyla BDD); reverts F309S mutation back to F309; expresss the equivalent amino acid sequence of SEQ ID NO: 555;	hFVIII-F309S-BD226seq124 BDD-F309; also referred to as hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
626	Modification of FVIII ORF from ceDNA-1651 ORF (SEQ ID NO: 556). Ablation of 1st cryptic ATG start codon introduced by codon optimization	hFVIII-1651-ORF-dATG1
627	Modification of FVIII ORF from SEQ ID NO: 556. Ablation o 1st and 2nd cryptic ATG start codon introduced by codon optimization	hFVIII-1651-ORF-dATG2
628	Modification of FVIII ORF from SEQ ID NO: 556. Ablation o all three cryptic ATG start codons introduced by codon optimization	hFVIII-1651-ORF-dATG3

629	Modification of FVIII ORF from SEQ ID NO: 556. Ablation o 2nd and 3rd ATG cryptic start codons introduced by codon optimization	hFVIII-1651ORF-dATG2-3
630	Modification of FVIII ORF from SEQ ID NO: 556. Ablation o all three ATG cryptic start codons introduced by codon optimization, first three CTG cryptic start codons and first TTC cryptic start codon	hFVIII-1651ORF-dATG3_dCTG3_dTTG1
631	Modification of FVIII ORF from SEQ ID NO: 556. Ablation o first ATG cryptic start codons introduced by codon optimization, first three CTG cryptic start codons and first TTC cryptic start codon	hFVIII-1651-ORF-dATG1_dCTG3_dTTG1
632	Modification of FVIII ORF from SEQ ID NO: 556. Ablation o 2nd and 3rd ATG cryptic start codons, as well as first three CTG cryptic start codons and first TTG cryptic start codon	hFVIII-1651-ORF-dATG2-3_dCTG3_dTTG1
633	Modification of FVIII ORF from SEQ ID NO: 556. Ablation o 2nd and 3rd ATG cryptic start codons, as well as first three CTG cryptic start codons and first TTG cryptic start codon. Ablation of three CpGs	hFVIII-1651-ORF-dATG2-3_dCTG3_dTTG1_dCpG3

Table 1B. General Element Numbers

GE #	ORF Name		GE #	ORF Name
GE-364	hFVIII-226variant-F309S_CpGmin-codop_ORF		GE-1201	FVIII-SC_1373::33bpFlanks_miniF8_50/100
GE-721	hFVIII-F309S-BD226-Codop-run4-seq102		GE-1202	FVIII-SC_1373::33bpFlanks_miniF8_200/200
GE-776	hFVIII-F309S-BD226seq124		GE-1203	FVIII-SC_1373::33bpFlanks_HBB_intron1
GE-707	hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309		GE-1204	FVIII-SC_1373::33bpFlanks_F8_intron8
GE-706	hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD		GE-1205	FVIII-SC_1373::33bpFlanks_no intron
GE-715	hFVIII-F309S-BD226seq124-Afstyla-BDD-F309		GE-968	FVIII_SC_F309S_GeneD_v4
GE-714	hFVIII-F309S-BD226seq124-Afstyla-BDD		GE-967	FVIII_SC_F309_GeneD_v4
GE-1025	FVIII-SC_0CpG_1_ORF		GE-966	FVIII_SC_F309S_codop_3b
GE-1026	FVIII-SC_0CpG_6_ORF		GE-965	FVIII_SC_F309_codop_3b
GE-1027	FVIII-SC_0CpG_8_ORF		GE-964	FVIII_SC_F309S_GeneD_v3
GE-1028	FVIII-SC_5_ORF		GE-963	FVIII_SC_F309S_GeneD_v2
GE-1029	FVIII-SC_5k_wt3_3_ORF		GE-962	FVIII_SC_F309S_codop_7b
GE-1030	FVIII-SC_5k_wt3_5_ORF		GE-961	FVIII_SC_F309S_codop_6b
GE-1032	FVIII-SC_0CpG_1_F309S		GE-960	FVIII_SC_F309S_codop_1b
GE-1033	FVIII-SC_0CpG_6_F309S		GE-959	FVIII_SC_F309_GeneD_v3
GE-1034	FVIII-SC_0CpG_8_F309S		GE-958	FVIII_SC_F309_GeneD_v2
GE-1035	FVIII-SC_5_F309S		GE-957	FVIII_SC_F309_codop_7b
GE-1036	FVIII-SC_5k_wt3_3_F309S		GE-956	FVIII_SC_F309_codop_6b
GE-1037	FVIII-SC_5k_wt3_5_F309S		GE-955	FVIII_SC_F309_codop_1b
GE-1038	FVIII-SC_5k_wt3_5-0CpG_6_F309S hybrid		GE-848	FVIII_1368ORF_A1AT-SSv3
GE-1168	FVIII_1368_ORF_mat_peptide		GE-847	FVIII_1374ORF_TRYP-SSv2
GE-1169	FVIII_1374_ORF_mat_peptide		GE-846	FVIII_1374ORF_tPA-SSv1
GE-712	hFVIII-Wt-Afstyla BDD		GE-845	FVIII_1374ORF_Secrecon-SSv2
GE-1174	FVIII-SC_1367_miniF8_50/100		GE-844	FVIII_1374ORF_Secrecon-SSv1
GE-1175	FVIII-SC_1367_miniF8_50/200		GE-843	FVIII_1374ORF_Lonz-SSv2

GE-1176	FVIII-SC_1367_miniF8_200/200		GE-842	FVIII_1374ORF_IL2-SSv1
GE-1177	FVIII-SC_1367_miniF8_500/500		GE-841	FVIII_1374ORF_Gaus-SSv1
GE-1178	FVIII-SC_1367_HBB_intron1		GE-840	FVIII_1374ORF_CHY-SSv1
GE-1179	FVIII-SC_1367_Embedded_HCR1_footprint123		GE-839	FVIII_1374ORF_ALB-SSv1
GE-1180	FVIII-SC_1367_Embedded_ProEnh		GE-838	FVIII_1374ORF_A1AT-SSv3
GE-1181	FVIII-SC_1367_Embedded_enhancer_HNF_array		GE-837	FVIII_1368ORF_TRYP-NS-struct-v2
GE-1182	FVIII-SC_1367_F8_intron8		GE-836	FVIII_1368ORF_TRYP-NS-CAI-v2
GE-1183	FVIII-SC_1367_F8_intron16		GE-835	FVIII_1368ORF_tPA-NS-struct
GE-1184	FVIII-SC_1367_MVM_intron		GE-834	FVIII_1368ORF_tPA-NS-CAI-v2
GE-1185	FVIII-SC_1367::33bpFlanks_miniF8_50/100		GE-833	FVIII_1368ORF_Secrecon-v1-NS-struct-v1
GE-1186	FVIII-SC_1367::33bpFlanks_miniF8_200/200		GE-832	FVIII_1368ORF_Secrecon-v1-NS-CAI-v2
GE-1187	FVIII-SC_1367::33bpFlanks_HBB_intron1		GE-831	FVIII_1368ORF_Lonz-SSv2
GE-1188	FVIII-SC_1367::33bpFlanks_F8_intron8		GE-830	FVIII_1368ORF_Lonz-NS-struct-v3
GE-1189	FVIII-SC_1367::33bpFlanks_no intron		GE-829	FVIII_1368ORF_Lonz-NS-CAI-v2
GE-1190	FVIII-SC_1373_miniF8_50/100		GE-828	FVIII_1368ORF_IL2-NS-struct-v2
GE-1191	FVIII-SC_1373_miniF8_50/200		GE-827	FVIII_1368ORF_IL2-NS-CAI
GE-1192	FVIII-SC_1373_miniF8_200/200		GE-826	FVIII_1368ORF_Gaus-NS-struct-v2
GE-1193	FVIII-SC_1373_miniF8_500/500		GE-825	FVIII_1368ORF_Gaus-NS-CAI-v2
GE-1194	FVIII-SC_1373_HBB_intron1		GE-824	FVIII_1368ORF_CHY-NS-struct
GE-1195	FVIII-SC_1373_Embedded_HCR1_footprint123		GE-823	FVIII_1368ORF_CHY-NS-CAI-v2
GE-1196	FVIII-SC_1373_Embedded_ProEnh		GE-822	FVIII_1368ORF_A1AT-NS-struct
GE-1197	FVIII-SC_1373_Embedded_enhancer_HNF_array		GE-821	FVIII_1368ORF_A1AT-NS-CAI
GE-1198	FVIII-SC_1373_F8_intron8		GE-820	FVIII-1368ORF_CD33-NS-struct-v2
GE-1199	FVIII-SC_1373_F8_intron16		GE-819	FVIII-1368ORF_CD33-NS-CAI-v2
GE-1200	FVIII-SC_1373_MVM_intron		GE-818	FVIII-1368ORF_ALB-NS-struct
GE-715	hFVIII-F309S-BD226seq124-BDD-F309*		GE-817	FVIII-1368ORF_ALB-NS-CAI-v2
GE-1667	hFVIII-1651-ORF-dATG1		GE-816	FVIII_1368ORF_CD33-SSv1

GE-1669	hFVIII-1651-ORF-dATG2		GE-815	FVIII_1374ORF_A1AT-SSv2
GE-1670	hFVIII-1651-ORF-dATG3		GE-814	FVIII_1368ORF_TRYP-SSv1
GE-1674	hFVIII-1651ORF-dATG2-3		GE-813	FVIII_1368ORF_tPA-SSv1
GE-1666	hFVIII-1651ORF-dATG3_dCTG3_dTTG1		GE-812	FVIII_1368ORF_Secrecon-SSv2
GE-1668	hFVIII-1651-ORF-dATG1_dCTG3_dTTG1		GE-811	FVIII_1368ORF_Secrecon-SSv1
GE-1675	hFVIII-1651-ORF-dATG2-3_dCTG3_dTTG1		GE-810	FVIII_1368ORF_Lonz-SSv1
GE-1698	hFVIII-1651-ORF-dATG2-3_dCTG3_dTTG1_dCpG3		GE-809	FVIII_1368ORF_IL2-SSv1
			GE-808	FVIII_1368ORF_Gaus-SSv1
			GE-807	FVIII_1368ORF_CHY-SSv1
			GE-806	FVIII_1368ORF_A1AT-SSv2
			GE-805	FVIII-1368ORF_ALB-SSv1

*The two names, hFVIII-F309S-BD226seq124-Afstyla-BDD-F309 and hFVIII-F309S-BD226seq124-BDD-F309 refer to the same sequence GE-715 (SEQ ID NOs: 76 and 556).

[00203] According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 71. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NOs: 71-183. In some embodiments, ceDNA vector having a nucleic acid sequence encoding FVIII (*e.g.*, Table 1A) encodes Val (V) instead of Asp (D) at the amino acid position 75 of SEQ ID NO: 492.

[00204] According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 71. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 71. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 72. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 72. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 73. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 73. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 74. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO:

74. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 75. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 75. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 76. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 76. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 77. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 77. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 78. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 78. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 79. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 79. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 80. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 80. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 81. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 81. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 82. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 82. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 83. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 83. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 84. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 84. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 85. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 85. According to some

embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 86. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 86. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 87. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 87. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 88. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 88. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 89. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 89. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 90. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 90. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 91. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 91. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 92. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 92. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 93. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 93. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 94. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 94. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 95. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 95. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 96. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 96. According to some embodiments, nucleic acid

sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 97. According to some embodiments, the enhancer consists of SEQ ID NO: 97. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 98. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 98. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 99. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 99. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 100. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 100. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 101. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 101. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 102. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 102. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 103. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 103. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 104. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 104. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 105. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 105. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 106. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 106. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 107. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 107. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid

sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 108. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 108. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 109. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 109. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 110. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 110. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 111. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 111. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 112. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 112. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 113. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 113. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 114. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 114. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 115. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 115. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 116. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 116. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 117. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 117. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 118. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 118. According to some embodiments, nucleic acid sequence encoding a FVIII

protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 119. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 119. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 120. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 120. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 121. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 121. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 122. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 122. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 123. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 123. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 124. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 124. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 125. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 125. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 126. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 126. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 127. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 127. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 128. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 128. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 129. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 129. According to some embodiments, nucleic

acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 130. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 130. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 131. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 131. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 132. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 132. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 133. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 133. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 134. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 134. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 135. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 135. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 136. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 136. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 137. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 137. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 138. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 138. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 139. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 139. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 140. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 140. According

to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 141. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 141. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 142. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 142. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 143. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 143. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 144. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 144. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 145. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 145. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 146. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 146. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 147. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 147. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 148. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 148. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 149. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 149. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 150. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 150. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 151. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or

consists of, SEQ ID NO: 151. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 152. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 152. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 153. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 153. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 154. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 154. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 155. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 155. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 156. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 156. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 157. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 157. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 158. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 158. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 159. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 159. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 160. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 160. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 161. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 161. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 162. According to some embodiments, the nucleic acid sequence encoding a

FVIII protein comprises, or consists of, SEQ ID NO: 162. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 163. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 163. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 164. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 164. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 165. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 165. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 166. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 166. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 167. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 167. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 168. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 168. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 169. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 169. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 170. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 170. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 171. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 171. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 172. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 172. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 173. According to some embodiments, the nucleic

acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 173. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 174. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 174. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 175. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 175. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 176. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 176. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 177. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 177. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 178. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 178. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 179. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 179. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 180. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 180. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 181. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 181. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 182. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 182. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 183. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 183. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 556.

According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 556. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 556. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 556. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 556. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 556. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 556.

[00205] According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 626. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 626. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 626. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 626. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 626. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 626. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 626.

[00206] According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 627. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 627. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 627. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 627. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 627. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 627. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 627.

[00207] According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID

NO: 628. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 628. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 628. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 628. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 628. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 628. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 628.

[00208] According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 629. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 629. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 629. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 629. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 629. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 629. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 629.

[00209] According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 630. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 630. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 630. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 630. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 630. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 630. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 630.

[00210] According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID

NO: 631. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 631. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 631. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 631. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 631. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 631. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 631.

[00211] According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 632. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 632. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 632. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 632. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 632. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 632. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 632.

[00212] According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 633. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 633. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 633. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 633. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 633. According to some embodiments, nucleic acid sequence encoding a FVIII protein comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 633. According to some embodiments, the nucleic acid sequence encoding a FVIII protein comprises, or consists of, SEQ ID NO: 633.

[00213] According to some embodiments, the ceDNA construct is ceDNA933, and comprises at least one nucleic acid sequence between flanking inverted terminal repeats (ITRs), wherein the at least one nucleic acid sequence comprises SEQ ID NO: 71.

[00214] According to some embodiments, the ceDNA construct is ceDNA1265, and comprises at least one nucleic acid sequence between flanking inverted terminal repeats (ITRs), wherein the at least one nucleic acid sequence comprises SEQ ID NO: 72.

[00215] According to some embodiments, the ceDNA construct is ceDNA1270, and comprises at least one nucleic acid sequence between flanking ITRs, wherein the at least one nucleic acid sequence comprises SEQ ID NO: 73.

[00216] According to some embodiments, the ceDNA construct is ceDNA1368, and comprises at least one nucleic acid sequence between flanking ITRs, wherein the at least one nucleic acid sequence comprises SEQ ID NO: 74.

[00217] According to some embodiments, the ceDNA construct is ceDNA1367, and comprises at least one nucleic acid sequence between flanking ITRs, wherein the at least one nucleic acid sequence comprises SEQ ID NO: 75.

[00218] According to some embodiments, the ceDNA construct is ceDNA1374, and comprises at least one nucleic acid sequence between flanking ITRs, wherein the at least one nucleic acid sequence comprises SEQ ID NO: 76.

[00219] According to some embodiments, the ceDNA construct is ceDNA1373, and comprises at least one nucleic acid sequence between flanking ITRs, wherein the at least one nucleic acid sequence comprises SEQ ID NO: 77.

[00220] According to some embodiments, the ceDNA construct is ceDNA1918, and comprises at least one nucleic acid sequence between flanking ITRs, wherein the at least one nucleic acid sequence comprises SEQ ID NO: 78.

[00221] According to some embodiments, the ceDNA construct is ceDNA1919, and comprises at least one nucleic acid sequence between flanking ITRs, wherein the at least one nucleic acid sequence comprises SEQ ID NO: 79.

[00222] According to some embodiments, the ceDNA construct is ceDNA1920, and comprises at least one nucleic acid sequence between flanking ITRs, wherein the at least one nucleic acid sequence comprises SEQ ID NO: 80.

[00223] According to some embodiments, the ceDNA construct is ceDNA1921, and comprises at least one nucleic acid sequence between flanking ITRs, wherein the at least one nucleic acid sequence comprises SEQ ID NO: 81.

[00224] According to some embodiments, the ceDNA construct is ceDNA1922, and comprises at least one nucleic acid sequence between flanking ITRs, wherein the at least one nucleic acid sequence comprises SEQ ID NO: 82.

[00225] According to some embodiments, the ceDNA construct is ceDNA1923, and comprises at least one nucleic acid sequence between flanking ITRs, wherein the at least one nucleic acid sequence comprises SEQ ID NO: 83.

[00226] According to some embodiments, the ceDNA construct is ceDNA1927, and comprises at least one nucleic acid sequence between flanking ITRs, wherein the at least one nucleic acid sequence comprises SEQ ID NO: 84.

[00227] According to some embodiments, the ceDNA construct is ceDNA1928, and comprises at least one nucleic acid sequence between flanking ITRs, wherein the at least one nucleic acid sequence comprises SEQ ID NO: 85.

[00228] According to some embodiments, the ceDNA construct is ceDNA1929, and comprises at least one nucleic acid sequence between flanking ITRs, wherein the at least one nucleic acid sequence comprises SEQ ID NO: 86.

[00229] According to some embodiments, the ceDNA construct is ceDNA1930, and comprises at least one nucleic acid sequence between flanking ITRs, wherein the at least one nucleic acid sequence comprises SEQ ID NO: 87.

[00230] According to some embodiments, the ceDNA construct is ceDNA1931, and comprises at least one nucleic acid sequence between flanking ITRs, wherein the at least one nucleic acid sequence comprises SEQ ID NO: 88.

[00231] According to some embodiments, the ceDNA construct is ceDNA1932, and comprises at least one nucleic acid sequence between flanking ITRs, wherein the at least one nucleic acid sequence comprises SEQ ID NO: 89.

[00232] According to some embodiments, the ceDNA construct is ceDNA1933, and comprises at least one nucleic acid sequence between flanking ITRs, wherein the at least one nucleic acid sequence comprises SEQ ID NO: 90.

[00233] According to some embodiments, the ceDNA construct is ceDNA1651, and comprises at least one nucleic acid sequence between flanking ITRs, wherein the at least one nucleic acid sequence comprises SEQ ID NO: 556. According to some embodiments, the ceDNA construct is ceDNA1651, and comprises or essentially consists of SEQ ID NO:42.

[00234] In any of the above embodiments, the at least one nucleic acid sequence can be a heterologous nucleic acid sequence.

(iii) FVIII therapeutic proteins and uses thereof for the treatment of hemophilia A

[00235] The ceDNA vectors described herein can be used to deliver therapeutic FVIII proteins for treatment of hemophilia A associated with inappropriate expression of the FVIII protein and/or mutations within the FVIII protein.

[00236] ceDNA vectors as described herein can be used to express any desired FVIII therapeutic protein. Exemplary therapeutic FVIII therapeutic proteins include but are not limited to any FVIII

protein, or portion thereof, expressed by the sequences (*e.g.*, any one of SEQ ID NOs: 71-183, 556 and 626-633) as set forth in **Table 1A and Table 1B** herein.

[00237] In one embodiment, the expressed FVIII therapeutic protein is functional for the treatment of a hemophilia A. In some embodiments, FVIII therapeutic protein does not cause an immune system reaction.

[00238] In another embodiment, the ceDNA vectors encoding FVIII therapeutic protein or fragment thereof (*e.g.,* functional fragment) can be used to generate a chimeric protein. Thus, it is specifically contemplated herein that a ceDNA vector expressing a chimeric protein can be administered to *e.g.,* to any one or more tissues selected from: liver, kidneys, gallbladder, prostate, adrenal gland. In some embodiments, when a ceDNA vector that has been engineered to express FVIII is administered to an infant, or administered to a subject in utero, one can administer the ceDNA vector to any one or more tissues selected from: liver, adrenal gland, heart, intestine, lung, and stomach, or to a liver stem cell precursor thereof for the *in vivo* or *ex vivo* treatment of hemophilia A.

[00239] *Hemophilia*

[00240] Hemophilia A is a genetic deficiency in clotting factor VIII, which causes increased bleeding and usually affects males. In the majority of cases it is inherited as an X-linked recessive trait, though there are cases which arise from spontaneous mutations. In terms of the symptoms of hemophilia A, there are internal or external bleeding episodes. Individuals with more severe hemophilia suffer more severe and more frequent bleeding, while others with mild hemophilia typically suffer more minor symptoms except after surgery or serious trauma. Moderate hemophiliacs have variable symptoms which manifest along a spectrum between severe and mild forms.

[00241] Current treatments to prevent bleeding in people with hemophilia A involve Factor VIII medication. Most individuals with severe hemophilia require regular supplementation with intravenous recombinant or plasma concentrate Factor VIII. Recombinant blood clotting factor VIII is one of the most complex proteins for industrial manufacturing due to the low efficiency of its gene transcription, massive intracellular loss of its proprotein during post-translational processing, and the instability of the secreted protein. Mild hemophiliacs can manage their condition with desmopressin, a drug which releases stored factor VIII from blood vessel walls.

[00242] There are many complications related to treatment of hemophilia A. In children, an easily accessible intravenous port can be inserted to minimize frequent traumatic intravenous cannulation. However, these ports are associated with high infection rate and a risk of clots forming at the tip of the catheter, rendering it useless. Viral infections can be common in hemophiliacs due to frequent blood transfusions which put patients at risk of acquiring blood borne infections, such as HIV, hepatitis B and hepatitis C. Prion infections can also be transmitted by blood transfusions. Another therapeutic complication of hemophilia A is the development of inhibitor antibodies against factor VIII due to frequent infusions. These develop as the body recognizes the infused factor VIII as foreign, as the

body does not produce its own copy. In these individuals, activated factor VII, a precursor to factor VIII in the coagulation cascade, can be infused as a treatment for hemorrhage in individuals with hemophilia and antibodies against replacement factor VIII.

[00243] *Coagulation Cascade*

[00244] Coagulation, also known as clotting, is the process by which blood changes from a liquid to a gel, forming a blood clot. It potentially results in hemostasis, the cessation of blood loss from a damaged vessel, followed by repair. The mechanism of coagulation involves activation, adhesion and aggregation of platelets along with deposition and maturation of fibrin. Disorders of coagulation are disease states which can result in bleeding (hemorrhage or bruising) or obstructive clotting (thrombosis).

[00245] Coagulation begins almost instantly after an injury to the blood vessel has damaged the endothelium lining the blood vessel. Exposure of blood to the subendothelial space initiates two processes: changes in platelets, and the exposure of subendothelial tissue factor to plasma Factor VII, which ultimately leads to fibrin formation. Platelets immediately form a plug at the site of injury; this is called primary hemostasis. Secondary hemostasis occurs simultaneously: additional coagulation factors or clotting factors beyond Factor VII (including Factor VIII) respond in a complex cascade to form fibrin strands, which strengthen the platelet plug.

[00246] The coagulation cascade of secondary hemostasis has two initial pathways which lead to fibrin formation. These are the contact activation pathway (also known as the intrinsic pathway), and the tissue factor pathway (also known as the extrinsic pathway), which both lead to the same fundamental reactions that produce fibrin. The primary pathway for the initiation of blood coagulation is the tissue factor (extrinsic) pathway. The pathways are a series of reactions, in which a zymogen (inactive enzyme precursor) of a serine protease and its glycoprotein co-factor are activated to become active components that then catalyze the next reaction in the cascade, ultimately resulting in cross-linked fibrin. Coagulation factors are generally indicated by Roman numerals, with a lowercase a appended to indicate an active form.

[00247] The coagulation factors are generally serine proteases (enzymes), which act by cleaving downstream proteins. The exceptions are tissue factor, FV, FVIII, FXIII. Tissue factor, FV and FVIII are glycoproteins, and Factor XIII is a transglutaminase. The coagulation factors circulate as inactive zymogens. The coagulation cascade is therefore classically divided into three pathways. The tissue factor and contact activation pathways both activate the "final common pathway" of factor X, thrombin and fibrin.

[00248] The main role of the tissue factor (extrinsic) pathway is to generate a "thrombin burst", a process by which thrombin, the most important constituent of the coagulation cascade in terms of its feedback activation roles, is released very rapidly. FVIIa circulates in a higher amount than any other activated coagulation factor. The process includes the following steps:

[00249] Step 1: Following damage to the blood vessel, FVII leaves the circulation and comes into contact with tissue factor (TF) expressed on tissue-factor-bearing cells (stromal fibroblasts and leukocytes), forming an activated complex (TF-FVIIa).

[00250] Step 2: TF-FVIIa activates FIX and FX.

[00251] Step 3: FVII is itself activated by thrombin, FXIa, FXII and FXa.

[00252] Step 4: The activation of FX (to form FXa) by TF-FVIIa is almost immediately inhibited by tissue factor pathway inhibitor (TFPI).

[00253] Step 5: FXa and its co-factor FVa form the prothrombinase complex, which activates prothrombin to thrombin.

[00254] Step 6: Thrombin then activates other components of the coagulation cascade, including FV and FVIII (which forms a complex with FIX), and activates and releases FVIII from being bound to von Willebrand factor (vWF).

[00255] Step 7: FVIIIa is the co-factor of FIXa, and together they form the "tenase" complex, which activates FX; and so the cycle continues.

[00256] The contact activation (intrinsic) pathway begins with formation of the primary complex on collagen by high-molecular-weight kininogen (HMWK), prekallikrein, and FXII (Hageman factor). Prekallikrein is converted to kallikrein and FXII becomes FXIIa. FXIIa converts FXI into FXIa. Factor XIa activates FIX, which with its co-factor FVIIIa form the tenase complex, which activates FX to FXa. The minor role that the contact activation pathway has in initiating clot formation can be illustrated by the fact that patients with severe deficiencies of FXII, HMWK, and prekallikrein do not have a bleeding disorder. Instead, contact activation system is more involved in inflammation, and innate immunity.

[00257] The final common pathway shared by the intrinsic and extrinsic coagulation pathways involves the conversion of prothrombin into thrombin and fibrinogen into fibrin. Thrombin has a large array of functions, not only the conversion of fibrinogen to fibrin, the building block of a hemostatic plug. In addition, it is the most important platelet activator and on top of that it activates Factors VIII and V and their inhibitor protein C (in the presence of thrombomodulin), and it activates Factor XIII, which forms covalent bonds that crosslink the fibrin polymers that form from activated monomers.

[00258] Following activation by the contact factor or tissue factor pathways, the coagulation cascade is maintained in a prothrombotic state by the continued activation of FVIII and FIX to form the tenase complex, until it is down-regulated by the anticoagulant pathways.

[00259] In some embodiments, a ceDNA vector for expression of FVIII protein as disclosed herein can also encode co-factors or other polypeptides, sense or antisense oligonucleotides, or RNAs (coding or non-coding; *e.g.,* siRNAs, shRNAs, micro-RNAs, and their antisense counterparts (*e.g.,* antagoMiR)) that can be used in conjunction with the FVIII protein expressed from the ceDNA. Additionally, expression cassettes comprising sequence encoding an FVIII protein can also include an

exogenous sequence that encodes a reporter protein to be used for experimental or diagnostic purposes, such as β-lactamase, β -galactosidase (LacZ), alkaline phosphatase, thymidine kinase, green fluorescent protein (GFP), chloramphenicol acetyltransferase (CAT), luciferase, and others well known in the art.

[00260] In one embodiment, the ceDNA vector comprises a nucleic acid sequence to express the FVIII protein that is functional for the treatment of hemophilia A. In a preferred embodiment, the therapeutic FVIII protein does not cause an immune system reaction, unless so desired.

III. ceDNA vector in general for use in production of FVIII therapeutic proteins

[00261] Embodiments of the disclosure are based on methods and compositions comprising close ended linear duplexed (ceDNA) vectors that can express the FVIII transgene. In some embodiments, the transgene is a sequence encoding an FVIII protein. According to some embodiments, the transgene is a nucleic acid sequence as set forth in **Table 1A** (*e.g.,* any one of SEQ ID NOs: 71-183, 556 and 626-633). The ceDNA vectors for expression of FVIII protein as described herein are not limited by size, thereby permitting, for example, expression of all of the components necessary for expression of a transgene from a single vector. The ceDNA vector for expression of FVIII protein is preferably duplex, *e.g.,* self-complementary, over at least a portion of the molecule, such as the expression cassette (*e.g.,* ceDNA is not a double stranded circular molecule). The ceDNA vector has covalently closed ends, and thus is resistant to exonuclease digestion (*e.g.,* exonuclease I or exonuclease III), *e.g.,* for over an hour at 37°C.

[00262] In general, a ceDNA vector for expression of FVIII protein as disclosed herein, comprises in the 5' to 3' direction: a first adeno-associated virus (AAV) inverted terminal repeat (ITR)(wild-type or modified), a nucleic acid sequence of interest (for example an expression cassette as described herein) and a second AAV ITR (wild-type or modified). According to some embodiments, the ITR sequences are selected from any of: (i) at least one WT ITR and at least one modified AAV inverted terminal repeat (mod-ITR) (*e.g.,* asymmetric modified ITRs); (ii) two modified ITRs where the mod-ITR pair have a different three-dimensional spatial organization with respect to each other (*e.g.,* asymmetric modified ITRs), or (iii) symmetrical or substantially symmetrical WT-WT ITR pair, where each WT-ITR has the same three-dimensional spatial organization, or (iv) symmetrical or substantially symmetrical modified ITR pair, where each mod-ITR has the same three-dimensional spatial organization.

[00263] Encompassed herein are methods and compositions comprising the ceDNA vector for FVIII protein production, which may further include a delivery system, such as but not limited to, a liposome nanoparticle delivery system. Non-limiting exemplary liposome nanoparticle systems encompassed for use are disclosed herein. In some aspects, the disclosure provides for a lipid nanoparticle comprising ceDNA and an ionizable lipid. For example, a lipid nanoparticle formulation that is made and loaded with a ceDNA vector obtained by the process is disclosed in International Application

PCT/US2018/050042, filed on September 7, 2018, which is incorporated herein by reference in its entirety.

[00264] The ceDNA vectors for expression of FVIII protein as disclosed herein have no packaging constraints imposed by the limiting space within the viral capsid. ceDNA vectors represent a viable eukaryotically-produced alternative to prokaryote-produced plasmid DNA vectors, as opposed to encapsulated AAV genomes. This permits the insertion of control elements, *e.g.,* regulatory switches as disclosed herein, large transgenes, multiple transgenes etc.

[00265] ceDNA vectors for expression of FVIII protein are capsid-free and can be obtained from a plasmid encoding in this order: a first ITR, an expression cassette comprising a transgene and a second ITR. The expression cassette may include one or more regulatory sequences that allows and/or controls the expression of the transgene, *e.g.,* where the expression cassette can comprise one or more of, in this order: an enhancer/promoter set, an ORF (transgene, *e.g.*, FVIII), a post-transcription regulatory element (*e.g.,* WPRE 3'UTR), and a polyadenylation and termination signal (*e.g.,* BGH polyA).

[00266] The expression cassette can also comprise an internal ribosome entry site (IRES) and/or a 2A element. The cis-regulatory elements include, but are not limited to, a promoter, a riboswitch, an insulator, a mir-regulatable element, a post-transcriptional regulatory element, a tissue- and cell type-specific promoter and an enhancer. In some embodiments the ITR can act as the promoter for the transgene, *e.g.,* FVIII protein. In some embodiments, the ceDNA vector comprises additional components to regulate expression of the transgene, for example, a regulatory switch, which are described herein in the section entitled "Regulatory Switches" for controlling and regulating the expression of the FVIII protein, and can include if desired, a regulatory switch which is a kill switch to enable controlled cell death of a cell comprising a ceDNA vector.

[00267] The expression cassette can comprise more than 4000 nucleotides, 5000 nucleotides, 10,000 nucleotides or 20,000 nucleotides, or 30,000 nucleotides, or 40,000 nucleotides or 50,000 nucleotides, or any range between about 4000-10,000 nucleotides or 10,000-50,000 nucleotides, or more than 50,000 nucleotides. In some embodiments, the expression cassette can comprise a transgene in the range of 500 to 50,000 nucleotides in length. In some embodiments, the expression cassette can comprise a transgene in the range of 500 to 75,000 nucleotides in length. In some embodiments, the expression cassette can comprise a transgene which is in the range of 500 to 10,000 nucleotides in length. In some embodiments, the expression cassette can comprise a transgene which is in the range of 1000 to 10,000 nucleotides in length. In some embodiments, the expression cassette can comprise a transgene which is in the range of 500 to 5,000 nucleotides in length. The ceDNA vectors do not have the size limitations of encapsidated AAV vectors, thus enable delivery of a large-size expression cassette to provide efficient transgene expression. In some embodiments, the ceDNA vector is devoid of prokaryote-specific methylation.

[00268] ceDNA expression cassette can include, for example, an expressible exogenous sequence (*e.g.,* open reading frame) or transgene that encodes a protein (*e.g.*, FVIII) that is either absent, inactive, or insufficient activity in the recipient subject or a gene that encodes a protein having a desired biological or a therapeutic effect. The transgene can encode a gene product that can function to correct the expression of a defective gene or transcript. In principle, the expression cassette can include any gene that encodes a protein, polypeptide or RNA that is either reduced or absent due to a mutation or which conveys a therapeutic benefit when overexpressed is considered to be within the scope of the disclosure.

[00269] The expression cassette can comprise any transgene (*e.g.,* encoding FVIII protein), for example, FVIII protein useful for treating hemophilia A in a subject, *i.e.*, a therapeutic FVIII protein. A ceDNA vector can be used to deliver and express any FVIII protein of interest in the subject, alone or in combination with nucleic acids encoding polypeptides, or non-coding nucleic acids (*e.g.,* RNAi, miRs etc.), as well as exogenous genes and nucleic acid sequences, including virus sequences in a subjects' genome, *e.g.,* HIV virus sequences and the like. Preferably a ceDNA vector disclosed herein is used for therapeutic purposes (*e.g.,* for medical, diagnostic, or veterinary uses) or immunogenic polypeptides. In certain embodiments, a ceDNA vector is useful to express any gene of interest in the subject, which includes one or more polypeptides, peptides, ribozymes, peptide nucleic acids, siRNAs, RNAis, antisense oligonucleotides, antisense polynucleotides, or RNAs (coding or non-coding; *e.g.,* siRNAs, shRNAs, guide RNAs (gRNAs), micro-RNAs, and their antisense counterparts (*e.g.,* antagoMiR)), antibodies, fusion proteins, or any combination thereof.

[00270] The expression cassette can also encode polypeptides, sense or antisense oligonucleotides, or RNAs (coding or non-coding; *e.g.,* siRNAs, shRNAs, micro-RNAs, and their antisense counterparts (*e.g.,* antagoMiR)). Expression cassettes can include an exogenous sequence that encodes a reporter protein to be used for experimental or diagnostic purposes, such as β-lactamase, β -galactosidase (LacZ), alkaline phosphatase, thymidine kinase, green fluorescent protein (GFP), chloramphenicol acetyltransferase (CAT), luciferase, and others well known in the art.

[00271] Sequences provided in the expression cassette, expression construct of a ceDNA vector for expression of FVIII protein described herein can be codon optimized for the target host cell. According to some embodiments, the sequence provided in the expression cassette is a sequence from **Table 1A** that is codon modified (*e.g.*, a sequence selected from one or more of SEQ ID NOs: 71-183, 556 and 626-633). As used herein, the term "codon optimized" or "codon optimization" refers to the process of modifying a nucleic acid sequence for enhanced expression in the cells of the vertebrate of interest, *e.g.,* mouse or human, by replacing at least one, more than one, or a significant number of codons of the native sequence (*e.g.,* a prokaryotic sequence) with codons that are more frequently or most frequently used in the genes of that vertebrate. Various species exhibit particular bias for certain codons of a particular amino acid. Typically, codon optimization does not alter the amino acid

sequence of the original translated protein. Optimized codons can be determined using *e.g.,* Aptagen's GENEFORGE® codon optimization and custom gene synthesis platform (Aptagen, Inc., 2190 Fox Mill Rd. Suite 300, Herndon, Va. 20171) or another publicly available database. In some embodiments, the nucleic acid encoding the FVIII protein is optimized for human expression, and/or is a human FVIII, or functional fragment thereof, as known in the art.

[00272] A transgene expressed by the ceDNA vector for expression of FVIII protein as disclosed herein encodes FVIII protein. There are many structural features of ceDNA vectors for expression of FVIII protein that differ from plasmid-based expression vectors. ceDNA vectors may possess one or more of the following features: the lack of original (*i.e.* not inserted) bacterial DNA, the lack of a prokaryotic origin of replication, being self-containing, *i.e.,* they do not require any sequences other than the two ITRs, including the Rep binding and terminal resolution sites (RBS and TRS), and an exogenous sequence between the ITRs, the presence of ITR sequences that form hairpins, and the absence of bacterial-type DNA methylation or indeed any other methylation considered abnormal by a mammalian host. In general, it is preferred for the present vectors not to contain any prokaryotic DNA but it is contemplated that some prokaryotic DNA may be inserted as an exogenous sequence, as a non-limiting example in a promoter or enhancer region. Another important feature distinguishing ceDNA vectors from plasmid expression vectors is that ceDNA vectors are single-strand linear DNA having closed ends, while plasmids are always double-strand DNA.

[00273] ceDNA vectors for expression of FVIII protein produced by the methods provided herein preferably have a linear and continuous structure rather than a non-continuous structure, as determined by restriction enzyme digestion assay (**FIG. 3D**). The linear and continuous structure is believed to be more stable from attack by cellular endonucleases, as well as less likely to be recombined and cause mutagenesis. Thus, a ceDNA vector in the linear and continuous structure is a preferred embodiment. The continuous, linear, single strand intramolecular duplex ceDNA vector can have covalently bound terminal ends, without sequences encoding AAV capsid proteins. These ceDNA vectors are structurally distinct from plasmids (including ceDNA plasmids described herein), which are circular duplex nucleic acid molecules of bacterial origin. The complimentary strands of plasmids may be separated following denaturation to produce two nucleic acid molecules, whereas in contrast, ceDNA vectors, while having complimentary strands, are a single DNA molecule and therefore even if denatured, remain a single molecule. In some embodiments, ceDNA vectors as described herein can be produced without DNA base methylation of prokaryotic type, unlike plasmids. Therefore, the ceDNA vectors and ceDNA-plasmids are different both in term of structure (in particular, linear versus circular) and also in view of the methods used for producing and purifying these different objects (see below), and also in view of their DNA methylation which is of prokaryotic type for ceDNA-plasmids and of eukaryotic type for the ceDNA vector.

[00274] There are several advantages of using a ceDNA vector for expression of FVIII protein as described herein over plasmid-based expression vectors, such advantages include, but are not limited to: 1) plasmids contain bacterial DNA sequences and are subjected to prokaryotic-specific methylation, *e.g.,* 6-methyl adenosine and 5-methyl cytosine methylation, whereas capsid-free AAV vector sequences are of eukaryotic origin and do not undergo prokaryotic-specific methylation; as a result, capsid-free AAV vectors are less likely to induce inflammatory and immune responses compared to plasmids; 2) while plasmids require the presence of a resistance gene during the production process, ceDNA vectors do not; 3) while a circular plasmid is not delivered to the nucleus upon introduction into a cell and requires overloading to bypass degradation by cellular nucleases, ceDNA vectors contain viral cis-elements, *i.e.,* ITRs, that confer resistance to nucleases and can be designed to be targeted and delivered to the nucleus. It is hypothesized that the minimal defining elements indispensable for ITR function are a Rep-binding site (RBS; 5'-GCGCGCTCGCTCGCTC-3' (SEQ ID NO: 437) for AAV2) and a terminal resolution site (TRS; 5'-AGTTGG-3' for AAV2) plus a variable palindromic sequence allowing for hairpin formation; and 4) ceDNA vectors do not have the over-representation of CpG dinucleotides often found in prokaryote-derived plasmids that reportedly binds a member of the Toll-like family of receptors, eliciting a T cell-mediated immune response. In contrast, transductions with capsid-free AAV vectors disclosed herein can efficiently target cell and tissue-types that are difficult to transduce with conventional AAV virions using various delivery reagent.

IV. Inverted Terminal Repeats (ITRs)

[00275] As disclosed herein, ceDNA vectors for expression of FVIII protein contain a transgene or nucleic acid sequence, *e.g.,* heterologous nucleic acid sequence, positioned between two inverted terminal repeat (ITR) sequences, where the ITR sequences can be an asymmetrical ITR pair or a symmetrical- or substantially symmetrical ITR pair, as these terms are defined herein. A ceDNA vector as disclosed herein can comprise ITR sequences that are selected from any of: (i) at least one WT ITR and at least one modified AAV inverted terminal repeat (mod-ITR) (*e.g.,* asymmetric modified ITRs); (ii) two modified ITRs where the mod-ITR pair have a different three-dimensional spatial organization with respect to each other (*e.g.,* asymmetric modified ITRs), or (iii) symmetrical or substantially symmetrical WT-WT ITR pair, where each WT-ITR has the same three-dimensional spatial organization, or (iv) symmetrical or substantially symmetrical modified ITR pair, where each mod-ITR has the same three-dimensional spatial organization, where the methods of the present disclosure may further include a delivery system, such as but not limited to a liposome nanoparticle delivery system.

[00276] In some embodiments, the ITR sequence can be from viruses of the *Parvoviridae* family, which includes two subfamilies: *Parvovirinae*, which infect vertebrates, and *Densovirinae*, which infect insects. The subfamily *Parvovirinae* (referred to as the parvoviruses) includes the genus

Dependovirus, the members of which, under most conditions, require coinfection with a helper virus such as adenovirus or herpes virus for productive infection. The genus *Dependovirus* includes adeno-associated virus (AAV), which normally infects humans (*e.g.,* serotypes 2, 3A, 3B, 5, and 6) or primates (*e.g.,* serotypes 1 and 4), and related viruses that infect other warm-blooded animals (*e.g.,* bovine, canine, equine, and ovine adeno-associated viruses). The parvoviruses and other members of the *Parvoviridae* family are generally described in Kenneth I. Berns, "Parvoviridae: The Viruses and Their Replication," Chapter 69 in FIELDS VIROLOGY (3d Ed. 1996).

[00277] While ITRs exemplified in the specification and Examples herein are AAV2 WT-ITRs, one of ordinary skill in the art is aware that one can as stated above use ITRs from any known parvovirus, for example a dependovirus such as AAV (*e.g.,* AAV1, AAV2, AAV3, AAV4, AAV5, AAV 5, AAV7, AAV8, AAV9, AAV10, AAV 11, AAV12, AAVrh8, AAVrh10, AAV-DJ, and AAV-DJ8 genome. *E.g.,* NCBI: NC 002077; NC 001401; NC001729; NC001829; NC006152; NC 006260; NC 006261), chimeric ITRs, or ITRs from any synthetic AAV. In some embodiments, the AAV can infect warm-blooded animals, *e.g.,* avian (AAAV), bovine (BAAV), canine, equine, and ovine adeno-associated viruses. In some embodiments the ITR is from B19 parvovirus (GenBank Accession No: NC 000883), Minute Virus from Mouse (MVM) (GenBank Accession No. NC 001510); goose parvovirus (GenBank Accession No. NC 001701); snake parvovirus 1 (GenBank Accession No. NC 006148). In some embodiments, the 5' WT-ITR can be from one serotype and the 3' WT-ITR from a different serotype, as discussed herein.

[00278] An ordinarily skilled artisan is aware that ITR sequences have a common structure of a double-stranded Holliday junction, which typically is a T-shaped or Y-shaped hairpin structure, where each WT-ITR is formed by two palindromic arms or loops (B-B' and C-C') embedded in a larger palindromic arm (A-A'), and a single stranded D sequence, (where the order of these palindromic sequences defines the flip or flop orientation of the ITR). See, for example, structural analysis and sequence comparison of ITRs from different AAV serotypes (AAV1-AAV6) and described in Grimm *et al.*, J. Virology, 2006; 80(1); 426-439; Yan *et al.,* J. Virology, 2005; 364-379; Duan *et al.*, Virology 1999; 261; 8-14. One of ordinary skill in the art can readily determine WT-ITR sequences from any AAV serotype for use in a ceDNA vector or ceDNA-plasmid based on the exemplary AAV2 ITR sequences provided herein. See, for example, the sequence comparison of ITRs from different AAV serotypes (AAV1-AAV6, and avian AAV (AAAV) and bovine AAV (BAAV)) described in Grimm *et al.*, J. Virology, 2006; 80(1); 426-439; that show the % identity of the left ITR of AAV2 to the left ITR from other serotypes: AAV-1 (84%), AAV-3 (86%), AAV-4 (79%), AAV-5 (58%), AAV-6 (left ITR) (100%) and AAV-6 (right ITR) (82%).

A. Symmetrical ITR pairs

[00279] In some embodiments, a ceDNA vector for expression of FVIII protein as described herein comprises, in the 5' to 3' direction: a first adeno-associated virus (AAV) inverted terminal repeat

(ITR), a nucleic acid sequence of interest (for example an expression cassette as described herein) and a second AAV ITR, where the first ITR (5' ITR) and the second ITR (3' ITR) are symmetric, or substantially symmetrical with respect to each other – that is, a ceDNA vector can comprise ITR sequences that have a symmetrical three-dimensional spatial organization such that their structure is the same shape in geometrical space, or have the same A, C-C' and B-B' loops in 3D space. In such an embodiment, a symmetrical ITR pair, or substantially symmetrical ITR pair can be modified ITRs (*e.g.,* mod-ITRs) that are not wild-type ITRs. A mod-ITR pair can have the same sequence which has one or more modifications from wild-type ITR and are reverse complements (inverted) of each other. In alternative embodiments, a modified ITR pair are substantially symmetrical as defined herein, that is, the modified ITR pair can have a different sequence but have corresponding or the same symmetrical three-dimensional shape.

(i) Wildtype ITRs

[00280] In some embodiments, the symmetrical ITRs, or substantially symmetrical ITRs are wild-type (WT-ITRs) as described herein. In some embodiments, both ITRs have a wild-type sequence, but do not necessarily have to be WT-ITRs from the same AAV serotype. In some embodiments, one WT-ITR can be from one AAV serotype, and the other WT-ITR can be from a different AAV serotype. In such an embodiment, a WT-ITR pair are substantially symmetrical as defined herein, *e.g.*, they can have one or more conservative nucleotide modification while still retaining the symmetrical three-dimensional spatial organization.

[00281] Accordingly, as disclosed herein, ceDNA vectors contain a transgene or nucleic acid sequence, *e.g.,* heterologous nucleic acid sequence, positioned between two flanking wild-type inverted terminal repeat (WT-ITR) sequences, that are either the reverse complement (inverted) of each other, or alternatively, are substantially symmetrical relative to each other, *e.g.*, a WT-ITR pair having symmetrical three-dimensional spatial organization. In some embodiments, a wild-type ITR sequence (*e.g.,* AAV WT-ITR) comprises a functional Rep binding site (RBS; *e.g.,* 5′-GCGCGCTCGCTCGCTC-3′ for AAV2, SEQ ID NO: 437) and a functional terminal resolution site (TRS; *e.g.,* 5′-AGTT-3', SEQ ID NO: 438).

[00282] In one aspect, ceDNA vectors for expression of FVIII protein are obtainable from a vector polynucleotide that encodes a nucleic acid sequence, *e.g.,* heterologous nucleic acid sequence, operatively positioned between two WT inverted terminal repeat sequences (WT-ITRs) (*e.g.,* AAV WT-ITRs). In some embodiments, both ITRs have a wild-type sequence, but do not necessarily have to be WT-ITRs from the same AAV serotype. In some embodiments, one WT-ITR can be from one AAV serotype, and the other WT-ITR can be from a different AAV serotype. In such an embodiment, the WT-ITR pair are substantially symmetrical as defined herein, that is, they can have one or more conservative nucleotide modification while still retaining the symmetrical three-dimensional spatial organization. In some embodiments, the 5' WT-ITR is from one AAV serotype, and the 3' WT-ITR is

from the same or a different AAV serotype. In some embodiments, the 5' WT-ITR and the 3'WT-ITR are mirror images of each other, that is they are symmetrical. In some embodiments, the 5' WT-ITR and the 3' WT-ITR are from the same AAV serotype.

[00283] WT ITRs are well known. In one embodiment the two ITRs are from the same AAV2 serotype. In certain embodiments one can use WT from other serotypes. There are a number of serotypes that are homologous, *e.g.,* AAV2, AAV4, AAV6, AAV8. In one embodiment, closely homologous ITRs (*e.g.,* ITRs with a similar loop structure) can be used. In another embodiment, one can use AAV WT ITRs that are more diverse, *e.g.,* AAV2 and AAV5, and still another embodiment, one can use an ITR that is substantially WT - that is, it has the basic loop structure of the WT but some conservative nucleotide changes that do not alter or affect the properties. When using WT-ITRs from the same viral serotype, one or more regulatory sequences may further be used. In certain embodiments, the regulatory sequence is a regulatory switch that permits modulation of the activity of the ceDNA, *e.g.,* the expression of the encoded FVIII protein.

[00284] In some embodiments, one aspect of the technology described herein relates to a ceDNA vector for expression of FVIII protein, wherein the ceDNA vector comprises at least one nucleic acid sequence, *e.g.,* heterologous nucleic acid sequence, encoding the FVIII protein, operably positioned between two wild-type inverted terminal repeat sequences (WT-ITRs), wherein the WT-ITRs can be from the same serotype, different serotypes or substantially symmetrical with respect to each other (*i.e.,* have the symmetrical three-dimensional spatial organization such that their structure is the same shape in geometrical space, or have the same A, C-C' and B-B' loops in 3D space). In some embodiments, the symmetric WT-ITRs comprises a functional terminal resolution site and a Rep binding site. In some embodiments, the nucleic acid sequence, *e.g.,* heterologous nucleic acid sequence, encodes a transgene, and the vector is not in a viral capsid.

[00285] In some embodiments, the WT-ITRs are the same but the reverse complement of each other. For example, the sequence AACG in the 5' ITR may be CGTT (*i.e.,* the reverse complement) in the 3' ITR at the corresponding site. In one example, the 5' WT-ITR sense strand comprises the sequence of ATCGATCG and the corresponding 3' WT-ITR sense strand comprises CGATCGAT (*i.e.,* the reverse complement of ATCGATCG). In some embodiments, the WT-ITRs ceDNA further comprises a terminal resolution site and a replication protein binding site (RPS) (sometimes referred to as a replicative protein binding site), *e.g.,* a Rep binding site.

[00286] Exemplary WT-ITR sequences for use in the ceDNA vectors for expression of FVIII protein comprising WT-ITRs are shown in **Table 2** herein, which shows pairs of WT-ITRs (5' WT-ITR and the 3' WT-ITR).

[00287] As an exemplary example, the present disclosure provides a ceDNA vector for expression of FVIII protein comprising a promoter operably linked to a transgene (*e.g.,* heterologous nucleic acid sequence), with or without the regulatory switch, where the ceDNA is devoid of capsid proteins and is:

(a) produced from a ceDNA-plasmid that encodes WT-ITRs, where each WT-ITR has the same number of intramolecularly duplexed base pairs in its hairpin secondary configuration (preferably excluding deletion of any AAA or TTT terminal loop in this configuration compared to these reference sequences), and (b) is identified as ceDNA using the assay for the identification of ceDNA by agarose gel electrophoresis under native gel and denaturing conditions in Example 1.

[00288] In some embodiments, the flanking WT-ITRs are substantially symmetrical to each other. In this embodiment the 5' WT-ITR can be from one serotype of AAV, and the 3' WT-ITR from a different serotype of AAV, such that the WT-ITRs are not identical reverse complements. For example, the 5' WT-ITR can be from AAV2, and the 3' WT-ITR from a different serotype (*e.g.,* AAV1, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12. In some embodiments, WT-ITRs can be selected from two different parvoviruses selected from any to of: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, snake parvovirus (*e.g.,* royal python parvovirus), bovine parvovirus, goat parvovirus, avian parvovirus, canine parvovirus, equine parvovirus, shrimp parvovirus, porcine parvovirus, or insect AAV. In some embodiments, such a combination of WT ITRs is the combination of WT-ITRs from AAV2 and AAV6. In one embodiment, the substantially symmetrical WT-ITRs are when one is inverted relative to the other ITR at least 90% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or at least 99.5% identical and all points in between, and has the same symmetrical three-dimensional spatial organization. In some embodiments, a WT-ITR pair are substantially symmetrical as they have symmetrical three-dimensional spatial organization, *e.g.,* have the same 3D organization of the A, C-C', B-B' and D arms. In one embodiment, a substantially symmetrical WT-ITR pair are inverted relative to the other, and are at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or at least 99.5% identical and all points in between, to each other, and one WT-ITR retains the Rep-binding site (RBS) of 5´-GCGCGCTCGCTCGCTC-3´ (SEQ ID NO: 437) and a terminal resolution site (TRS). In some embodiments, a substantially symmetrical WT-ITR pair are inverted relative to each other, and are at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, or at least 99.5% identical and all points in between, to each other, and one WT-ITR retains the Rep-binding site (RBS) of 5´-GCGCGCTCGCTCGCTC-3´ (SEQ ID NO: 437) and a terminal resolution site (TRS) and in addition to a variable palindromic sequence allowing for hairpin secondary structure formation. Homology can be determined by standard means well known in the art such as BLAST (Basic Local Alignment Search Tool), BLASTN at default setting.

[00289] In some embodiments, the structural element of the ITR can be any structural element that is involved in the functional interaction of the ITR with a large Rep protein (*e.g.,* Rep 78 or Rep 68). In certain embodiments, the structural element provides selectivity to the interaction of an ITR with a large Rep protein, *i.e.,* determines at least in part which Rep protein functionally interacts with the

ITR. In other embodiments, the structural element physically interacts with a large Rep protein when the Rep protein is bound to the ITR. Each structural element can be, *e.g.,* a secondary structure of the ITR, a nucleic acid sequence of the ITR, a spacing between two or more elements, or a combination of any of the above. In one embodiment, the structural elements are selected from the group consisting of an A and an A' arm, a B and a B' arm, a C and a C' arm, a D arm, a Rep binding site (RBE) and an RBE' (*i.e.,* complementary RBE sequence), and a terminal resolution sire (TRS).

[00290] By way of example only, Table 6 of International Publication No. WO/2019/161059 (incorporated by reference in its entirety herein), indicates exemplary combinations of WT-ITRs.

[00291] By way of example only, **Table 2** sets forth the corresponding SEQ ID NOs: of the sequences of exemplary WT-ITRs from some different AAV serotypes.

Table 2

AAV serotype	5' WT-ITR (LEFT)	3' WT-ITR (RIGHT)
AAV1	SEQ ID NO: 493	SEQ ID NO: 494
AAV2	SEQ ID NO: 495	SEQ ID NO: 496
AAV3	SEQ ID NO: 497	SEQ ID NO: 498
AAV4	SEQ ID NO: 499	SEQ ID NO: 500
AAV5	SEQ ID NO: 501	SEQ ID NO: 502
AAV6	SEQ ID NO: 503	SEQ ID NO: 504

[00292] In some embodiments, the nucleic acid sequence of the WT-ITR sequence can be modified (*e.g.,* by modifying 1, 2, 3, 4 or 5, or more nucleotides or any range therein), whereby the modification is a substitution for a complementary nucleotide, *e.g.,* G for a C, and *vice versa*, and T for an A, and *vice versa*.

[00293] The ceDNA vector for expression of FVIII protein as described herein can include WT-ITR structures that retains an operable RBE, TRS and RBE' portion. **FIG. 1A** and **FIG. 1B**, using wild-type ITRs for exemplary purposes, show one possible mechanism for the operation of a TRS site within a wild-type ITR structure portion of a ceDNA vector. In some embodiments, the ceDNA vector for expression of FVIII protein contains one or more functional WT-ITR polynucleotide sequences that comprise a Rep-binding site (RBS; 5'-GCGCGCTCGCTCGCTC-3' (SEQ ID NO: 437) for AAV2) and a terminal resolution site (TRS; 5'-AGTT (SEQ ID NO: 438)). In some embodiments, at least one WT-ITR is functional. In alternative embodiments, where a ceDNA vector for expression of FVIII protein comprises two WT-ITRs that are substantially symmetrical to each other, at least one WT-ITR is functional and at least one WT-ITR is non-functional.

B. Modified ITRs (mod-ITRs) in general for ceDNA vectors comprising asymmetric ITR pairs or symmetric ITR pairs

[00294] As discussed herein, a ceDNA vector for expression of FVIII protein can comprise a symmetrical ITR pair or an asymmetrical ITR pair. In both instances, one or both of the ITRs can be modified ITRs – the difference being that in the first instance (*i.e.,* symmetric mod-ITRs), the mod-

ITRs have the same three-dimensional spatial organization (*i.e.,* have the same A-A', C-C' and B-B' arm configurations), whereas in the second instance (*i.e.,* asymmetric mod-ITRs), the mod-ITRs have a different three-dimensional spatial organization (*i.e.,* have a different configuration of A-A', C-C' and B-B' arms).

[00295] In some embodiments, a modified ITR is an ITRs that is modified by deletion, insertion, and/or substitution as compared to a wild-type ITR sequence (*e.g.,* AAV ITR). In some embodiments, at least one of the ITRs in the ceDNA vector comprises a functional Rep binding site (RBS; *e.g.,* 5′-GCGCGCTCGCTCGCTC-3′ for AAV2, SEQ ID NO: 437) and a functional terminal resolution site (TRS; *e.g.,* 5′-AGTT-3', SEQ ID NO: 438.) In one embodiment, at least one of the ITRs is a non-functional ITR. In one embodiment, the different or modified ITRs are not each wild-type ITRs from different serotypes.

[00296] Specific alterations and mutations in the ITRs are described in detail herein, but in the context of ITRs, "altered" or "mutated" or "modified", it indicates that nucleotides have been inserted, deleted, and/or substituted relative to the wild-type, reference, or original ITR sequence. The altered or mutated ITR can be an engineered ITR. As used herein, "engineered" refers to the aspect of having been manipulated by the hand of man. For example, a polypeptide is considered to be "engineered" when at least one aspect of the polypeptide, *e.g.,* its sequence, has been manipulated by the hand of man to differ from the aspect as it exists in nature.

[00297] In some embodiments, a mod-ITR may be synthetic. In one embodiment, a synthetic ITR is based on ITR sequences from more than one AAV serotype. In another embodiment, a synthetic ITR includes no AAV-based sequence. In yet another embodiment, a synthetic ITR preserves the ITR structure described above although having only some or no AAV-sourced sequence. In some aspects, a synthetic ITR may interact preferentially with a wild-type Rep or a Rep of a specific serotype, or in some instances will not be recognized by a wild-type Rep and be recognized only by a mutated Rep.

[00298] The skilled artisan can determine the corresponding sequence in other serotypes by known means. For example, determining if the change is in the A, A', B, B', C, C' or D region and determine the corresponding region in another serotype. One can use BLAST® (Basic Local Alignment Search Tool) or other homology alignment programs at default status to determine the corresponding sequence. The disclosure further provides populations and pluralities of ceDNA vectors comprising mod-ITRs from a combination of different AAV serotypes – that is, one mod-ITR can be from one AAV serotype and the other mod-ITR can be from a different serotype. Without wishing to be bound by theory, in one embodiment one ITR can be from or based on an AAV2 ITR sequence and the other ITR of the ceDNA vector can be from or be based on any one or more ITR sequence of AAV serotype 1 (AAV1), AAV serotype 4 (AAV4), AAV serotype 5 (AAV5), AAV serotype 6 (AAV6), AAV serotype 7 (AAV7), AAV serotype 8 (AAV8), AAV serotype 9 (AAV9), AAV serotype 10 (AAV10), AAV serotype 11 (AAV11), or AAV serotype 12 (AAV12).

[00299] Any parvovirus ITR can be used as an ITR or as a base ITR for modification. Preferably, the parvovirus is a dependovirus. More preferably AAV. The serotype chosen can be based upon the tissue tropism of the serotype. AAV2 has a broad tissue tropism, AAV1 preferentially targets to neuronal and skeletal muscle, and AAV5 preferentially targets neuronal, retinal pigmented epithelia, and photoreceptors. AAV6 preferentially targets skeletal muscle and lung. AAV8 preferentially targets liver, skeletal muscle, heart, and pancreatic tissues. AAV9 preferentially targets liver, skeletal and lung tissue. In one embodiment, the modified ITR is based on an AAV2 ITR.

[00300] More specifically, the ability of a structural element to functionally interact with a particular large Rep protein can be altered by modifying the structural element. For example, the nucleic acid sequence of the structural element can be modified as compared to the wild-type sequence of the ITR. In one embodiment, the structural element (*e.g.,* A arm, A' arm, B arm, B' arm, C arm, C' arm, D arm, RBE, RBE', and TRS) of an ITR can be removed and replaced with a wild-type structural element from a different parvovirus. For example, the replacement structure can be from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, snake parvovirus (*e.g.,* royal python parvovirus), bovine parvovirus, goat parvovirus, avian parvovirus, canine parvovirus, equine parvovirus, shrimp parvovirus, porcine parvovirus, or insect AAV. For example, the ITR can be an AAV2 ITR and the A or A' arm or RBE can be replaced with a structural element from AAV5. In another example, the ITR can be an AAV5 ITR and the C or C' arms, the RBE, and the TRS can be replaced with a structural element from AAV2. In another example, the AAV ITR can be an AAV5 ITR with the B and B' arms replaced with the AAV2 ITR B and B' arms.

[00301] By way of example only, **Table 3** indicates exemplary modifications of at least one nucleotide (*e.g.,* a deletion, insertion and/ or substitution) in regions of a modified ITR, where X is indicative of a modification of at least one nucleic acid (*e.g.,* a deletion, insertion and/ or substitution) in that section relative to the corresponding wild-type ITR. In some embodiments, any modification of at least one nucleotide (*e.g.,* a deletion, insertion and/ or substitution) in any of the regions of C and/or C' and/or B and/or B' retains three sequential T nucleotides (*i.e.,* TTT) in at least one terminal loop. For example, if the modification results in any of: a single arm ITR (*e.g.,* single C-C' arm, or a single B-B' arm), or a modified C-B' arm or C'-B arm, or a two arm ITR with at least one truncated arm (*e.g.,* a truncated C-C' arm and/or truncated B-B' arm), at least the single arm, or at least one of the arms of a two arm ITR (where one arm can be truncated) retains three sequential T nucleotides (*i.e.,* TTT) in at least one terminal loop. In some embodiments, a truncated C-C' arm and/or a truncated B-B' arm has three sequential T nucleotides (*i.e.,* TTT) in the terminal loop.

[00302] **Table 3:** Exemplary combinations of modifications of at least one nucleotide (*e.g.,* a deletion, insertion and/ or substitution) to different B-B' and C-C' regions or arms of ITRs (X indicates a nucleotide modification, *e.g.,* addition, deletion or substitution of at least one nucleotide in the region).

B region	B' region	C region	C' region
X			
	X		
X	X		
		X	
			X
		X	X
X		X	
X			X
	X	X	
	X		X
X	X	X	
X	X		X
X		X	X
	X	X	X
X	X	X	X

[00303] In some embodiments, mod-ITR for use in a ceDNA vector for expression of FVIII protein comprises an asymmetric ITR pair, or a symmetric mod-ITR pair as disclosed herein, can comprise any one of the combinations of modifications shown in **Table 3**, and also a modification of at least one nucleotide in any one or more of the regions selected from: between A' and C, between C and C', between C' and B, between B and B' and between B' and A. In some embodiments, any modification of at least one nucleotide (*e.g.,* a deletion, insertion and/ or substitution) in the C or C' or B or B' regions, still preserves the terminal loop of the stem-loop. In some embodiments, any modification of at least one nucleotide (*e.g.,* a deletion, insertion and/ or substitution) between C and C' and/or B and B' retains three sequential T nucleotides (*i.e.,* TTT) in at least one terminal loop. In alternative embodiments, any modification of at least one nucleotide (*e.g.,* a deletion, insertion and/ or substitution) between C and C' and/or B and B' retains three sequential A nucleotides (*i.e.,* AAA) in at least one terminal loop. In some embodiments, a modified ITR for use herein can comprise any one of the combinations of modifications shown in **Table 3,** and also a modification of at least one nucleotide (*e.g.,* a deletion, insertion and/ or substitution) in any one or more of the regions selected from: A', A and/or D. For example, in some embodiments, a modified ITR for use herein can comprise any one of the combinations of modifications shown in **Table 3**, and also a modification of at least one nucleotide (*e.g.,* a deletion, insertion and/ or substitution) in the A region. In some embodiments, a modified ITR for use herein can comprise any one of the combinations of modifications shown in **Table 3**, and also a modification of at least one nucleotide (*e.g.,* a deletion, insertion and/ or substitution) in the A' region. In some embodiments, a modified ITR for use herein can comprise any one of the combinations of modifications shown in **Table 3**, and also a modification of at least one nucleotide (*e.g.,* a deletion, insertion and/ or substitution) in the A and/or A' region. In some embodiments, a modified ITR for use herein can comprise any one of the combinations of modifications shown in **Table 3**, and also a modification of at least one nucleotide (*e.g.,* a deletion, insertion and/ or substitution) in the D region.

[00304] In one embodiment, the nucleic acid sequence of the structural element can be modified (*e.g.,* by modifying 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 or more nucleotides or any range therein) to produce a modified structural element. In one embodiment, the specific modifications to the ITRs are exemplified herein (*e.g.,* shown in FIG. 7A-7B of PCT/US2018/064242, filed on December 6, 2018 and incorporated by reference in its entirety herein (*e.g.,* SEQ ID NOs: 97-98, 101-103, 105-108, 111-112, 117-134, 545-54 in PCT/US2018/064242). In some embodiments, an ITR can be modified (*e.g.,* by modifying 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 or more nucleotides or any range therein). In other embodiments, the ITR can have at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, or more sequence identity with one of the modified ITRs or the RBE-containing section of the A-A' arm and C-C' and B-B' arms of SEQ ID NO: 3, 4, 15-47, 101-116 or 165-187, or shown in Tables 2-9 (*i.e.,* SEQ ID NO: 110-112, 115-190, 200-468) of International application PCT/US18/49996, which is incorporated herein in its entirety by reference.

[00305] In some embodiments, a modified ITR can for example, comprise removal or deletion of all of a particular arm, *e.g.,* all or part of the A-A' arm, or all or part of the B-B' arm or all or part of the C-C' arm, or alternatively, the removal of 1, 2, 3, 4, 5, 6, 7, 8, 9 or more base pairs forming the stem of the loop so long as the final loop capping the stem (*e.g.,* single arm) is still present (*e.g.,* see ITR-21 in FIG. 7A of PCT/US2018/064242, filed December 6, 2018, incorporated by reference in its entirety herein). In some embodiments, a modified ITR can comprise the removal of 1, 2, 3, 4, 5, 6, 7, 8, 9 or more base pairs from the B-B' arm. In some embodiments, a modified ITR can comprise the removal of 1, 2, 3, 4, 5, 6, 7, 8, 9 or more base pairs from the C-C' arm (see, *e.g.,* ITR-1 in FIG. 3B, or ITR-45 in FIG. 7A of PCT/US2018/064242, filed December 6, 2018, incorporated by reference in its entirety herein). In some embodiments, a modified ITR can comprise the removal of 1, 2, 3, 4, 5, 6, 7, 8, 9 or more base pairs from the C-C' arm and the removal of 1, 2, 3, 4, 5, 6, 7, 8, 9 or more base pairs from the B-B' arm. Any combination of removal of base pairs is envisioned, for example, 6 base pairs can be removed in the C-C' arm and 2 base pairs in the B-B' arm. As an illustrative example, **FIG. 2B** shows an exemplary modified ITR with at least 7 base pairs deleted from each of the C portion and the C' portion, a substitution of a nucleotide in the loop between C and C' region, and at least one base pair deletion from each of the B region and B' regions such that the modified ITR comprises two arms where at least one arm (*e.g.,* C-C') is truncated. In some embodiments, the modified ITR also comprises at least one base pair deletion from each of the B region and B' regions, such that the B-B' arm is also truncated relative to WT ITR.

[00306] In some embodiments, a modified ITR can have between 1 and 50 (*e.g.,* 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50) nucleotide deletions relative to a full-length wild-type ITR sequence. In some embodiments, a modified ITR can have between 1 and 30 nucleotide

deletions relative to a full-length WT ITR sequence. In some embodiments, a modified ITR has between 2 and 20 nucleotide deletions relative to a full-length wild-type ITR sequence.

[00307] In some embodiments, a modified ITR does not contain any nucleotide deletions in the RBE-containing portion of the A or A' regions, so as not to interfere with DNA replication (*e.g.,* binding to an RBE by Rep protein, or nicking at a terminal resolution site). In some embodiments, a modified ITR encompassed for use herein has one or more deletions in the B, B', C, and/or C region as described herein.

[00308] In another embodiment, the structure of the structural element can be modified. For example, the structural element a change in the height of the stem and/or the number of nucleotides in the loop. For example, the height of the stem can be about 2, 3, 4, 5, 6, 7, 8, or 9 nucleotides or more or any range therein. In one embodiment, the stem height can be about 5 nucleotides to about 9 nucleotides and functionally interacts with Rep. In another embodiment, the stem height can be about 7 nucleotides and functionally interacts with Rep. In another example, the loop can have 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides or more or any range therein.

[00309] In another embodiment, the number of GAGY binding sites or GAGY-related binding sites within the RBE or extended RBE can be increased or decreased. In one example, the RBE or extended RBE, can comprise 1, 2, 3, 4, 5, or 6 or more GAGY binding sites or any range therein. Each GAGY binding site can independently be an exact GAGY sequence or a sequence similar to GAGY as long as the sequence is sufficient to bind a Rep protein.

[00310] In another embodiment, the spacing between two elements (such as but not limited to the RBE and a hairpin) can be altered (*e.g.,* increased or decreased) to alter functional interaction with a large Rep protein. For example, the spacing can be about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 nucleotides or more or any range therein.

[00311] The ceDNA vector for expression of FVIII protein as described herein can include an ITR structure that is modified with respect to the wild-type AAV2 ITR structure disclosed herein, but still retains an operable RBE, TRS and RBE´ portion. **FIG. 1A** and **FIG. 1B** show one possible mechanism for the operation of a TRS site within a wild-type ITR structure portion of a ceDNA vector for expression of FVIII protein. In some embodiments, the ceDNA vector for expression of FVIII protein contains one or more functional ITR polynucleotide sequences that comprise a Rep-binding site (RBS; 5′-GCGCGCTCGCTCGCTC-3′ (SEQ ID NO: 437) for AAV2) and a terminal resolution site (TRS; 5′-AGTT (SEQ ID NO: 438)). In some embodiments, at least one ITR (wt or modified ITR) is functional. In alternative embodiments, where a ceDNA vector for expression of FVIII protein comprises two modified ITRs that are different or asymmetrical to each other, at least one modified ITR is functional and at least one modified ITR is non-functional.

[00312] In some embodiments, the modified ITR (*e.g.,* the left or right ITR) of a ceDNA vector for expression of FVIII protein as described herein has modifications within the loop arm, the truncated

arm, or the spacer. Exemplary sequences of ITRs having modifications within the loop arm, the truncated arm, or the spacer are listed in Table 2 (*i.e.,* SEQ ID NOS: 135-190, 200-233); Table 3 (*e.g.,* SEQ ID Nos: 234-263); Table 4 (*e.g.,* SEQ ID NOs: 264-293); Table 5 (*e.g.,* SEQ ID Nos: 294-318 herein); Table 6 (*e.g.,* SEQ ID NO: 319-468; and Tables 7-9 (*e.g.,* SEQ ID Nos: 101-110, 111-112, 115-134) or Table 10A or 10B (*e.g.,* SEQ ID Nos: 9, 100, 469-483, 484-499) of International application PCT/US18/49996, which is incorporated herein in its entirety by reference.

[00313] In some embodiments, the modified ITR for use in a ceDNA vector for expression of FVIII protein comprising an asymmetric ITR pair, or symmetric mod-ITR pair is selected from any or a combination of those shown in Tables 2, 3, 4, 5, 6, 7, 8, 9 and 10A-10B of International application PCT/US18/49996 which is incorporated herein in its entirety by reference.

[00314] Additional exemplary modified ITRs for use in a ceDNA vector for expression of FVIII protein comprising an asymmetric ITR pair, or symmetric mod-ITR pair in each of the above classes are provided in **Tables 4A** and **4B**. The predicted secondary structure of the Right modified ITRs in **Table 4A** are shown in FIG. 7A of International Application PCT/US2018/064242, filed December 6, 2018, and the predicted secondary structure of the Left modified ITRs in **Table 4B** are shown in FIG. 7B of International Application PCT/US2018/064242, filed December 6, 2018, each of which is incorporated herein in its entirety by reference.

[00315] **Table 4A** and **Table 4B** show exemplary right and left modified ITRs.

Table 4A: Exemplary modified right ITRs. These exemplary modified right ITRs can further comprise the RBE of GCGCGCTCGCTCGCTC-3′ (SEQ ID NO: 437), spacer of ACTGAGGC (SEQ ID NO: 439), the spacer complement GCCTCAGT (SEQ ID NO: 440) and RBE' (*i.e.,* complement to RBE) of GAGCGAGCGAGCGCGC (SEQ ID NO: 441).

ITR Construct	**SEQ ID NO:**		**ITR Construct**	**SEQ ID NO:**
ITR-18 Right	505		ITR-27 Right	514
ITR-19 Right	506		ITR-28 Right	515
ITR-20 Right	507		ITR-29 Right	516
ITR-21 Right	508		ITR-30 Right	517
ITR-22 Right	509		ITR-31 Right	518
ITR-23 Right	510		ITR-32 Right	519
ITR-24 Right	511		ITR-49 Right	520
ITR-25 Right	512		ITR-50 right	521
ITR-26 Right	513			

TABLE 4B: Exemplary modified left ITRs. These exemplary modified left ITRs can further comprise the RBE of GCGCGCTCGCTCGCTC-3′ (SEQ ID NO: 437), spacer of ACTGAGGC (SEQ ID NO: 439), the spacer complement GCCTCAGT (SEQ ID NO: 440) and RBE complement (RBE') of GAGCGAGCGAGCGCGC (SEQ ID NO: 441).

ITR Construct	**SEQ ID NO:**		**ITR Construct**	**SEQ ID NO:**

ITR-33 Left	522		ITR-42 Left	531
ITR-34 Left	523		ITR-43 Left	532
ITR-35 Left	524		ITR-44 Left	533
ITR-36 Left	525		ITR-45 Left	534
ITR-37 Left	526		ITR-46 Left	535
ITR-38 Left	527		ITR-47 Left	536
ITR-39 Left	528		ITR-48 Left	537
ITR-40 Left	529		ITR-41 Left	530

[00316] In one embodiment, a ceDNA vector for expression of FVIII protein comprises, in the 5' to 3' direction: a first adeno-associated virus (AAV) inverted terminal repeat (ITR), a nucleic acid sequence of interest (for example an expression cassette as described herein) and a second AAV ITR, where the first ITR (5' ITR) and the second ITR (3' ITR) are asymmetric with respect to each other – that is, they have a different 3D-spatial configuration from one another. As an exemplary embodiment, the first ITR can be a wild-type ITR and the second ITR can be a mutated or modified ITR, or vice versa, where the first ITR can be a mutated or modified ITR and the second ITR a wild-type ITR. In some embodiment, the first ITR and the second ITR are both mod-ITRs, but have different sequences, or have different modifications, and thus are not the same modified ITRs, and have different 3D spatial configurations. Stated differently, a ceDNA vector with asymmetric ITRs comprises ITRs where any changes in one ITR relative to the WT-ITR are not reflected in the other ITR; or alternatively, where the asymmetric ITRs have a modified asymmetric ITR pair can have a different sequence and different three-dimensional shape with respect to each other. Exemplary asymmetric ITRs in the ceDNA vector for expression of FVIII protein and for use to generate a ceDNA-plasmid are shown in **Table 4A and 4B.**

[00317] In an alternative embodiment, a ceDNA vector for expression of FVIII protein comprises two symmetrical mod-ITRs - that is, both ITRs have the same sequence, but are reverse complements (inverted) of each other. In some embodiments, a symmetrical mod-ITR pair comprises at least one or any combination of a deletion, insertion, or substitution relative to wild-type ITR sequence from the same AAV serotype. The additions, deletions, or substitutions in the symmetrical ITR are the same but the reverse complement of each other. For example, an insertion of 3 nucleotides in the C region of the 5' ITR would be reflected in the insertion of 3 reverse complement nucleotides in the corresponding section in the C' region of the 3' ITR. Solely for illustration purposes only, if the addition is AACG in the 5' ITR, the addition is CGTT in the 3' ITR at the corresponding site. For example, if the 5' ITR sense strand is ATC**GA**TCG with an addition of *AACG* between the G and A to result in the sequence ATC**G***AACG***A**TCG (SEQ ID NO: 538). The corresponding 3' ITR sense strand is CGA**TC**GAT (the reverse complement of ATC**GA**TCG) with an addition of *CGTT* (*i.e.* the reverse complement of AACG) between the T and C to result in the sequence CGA**T***CGTT***C**GAT (SEQ ID NO: 539) (the reverse complement of ATC**G***AACG***A**TCG) (SEQ ID NO: 538).

[00318] In alternative embodiments, the modified ITR pair are substantially symmetrical as defined herein - that is, the modified ITR pair can have a different sequence but have corresponding or the same symmetrical three-dimensional shape. For example, one modified ITR can be from one serotype and the other modified ITR be from a different serotype, but they have the same mutation (*e.g.,* nucleotide insertion, deletion or substitution) in the same region. Stated differently, for illustrative purposes only, a 5' mod-ITR can be from AAV2 and have a deletion in the C region, and the 3' mod-ITR can be from AAV5 and have the corresponding deletion in the C' region, and provided the 5'mod-ITR and the 3' mod-ITR have the same or symmetrical three-dimensional spatial organization, they are encompassed for use herein as a modified ITR pair.

[00319] In some embodiments, a substantially symmetrical mod-ITR pair has the same A, C-C' and B-B' loops in 3D space, *e.g.,* if a modified ITR in a substantially symmetrical mod-ITR pair has a deletion of a C-C' arm, then the cognate mod-ITR has the corresponding deletion of the C-C' loop and also has a similar 3D structure of the remaining A and B-B' loops in the same shape in geometric space of its cognate mod-ITR. By way of example only, substantially symmetrical ITRs can have a symmetrical spatial organization such that their structure is the same shape in geometrical space. This can occur, *e.g.,* when a G-C pair is modified, for example, to a C-G pair or vice versa, or A-T pair is modified to a T-A pair, or vice versa. Therefore, using the exemplary example above of modified 5' ITR as a ATC**G***AACG***A**TCG (SEQ ID NO: 538), and modified 3' ITR as CGA**T***CGTT***C**GAT (SEQ ID NO: 539) (*i.e.,* the reverse complement of ATC**G***AACG***A**TCG (SEQ ID NO: 538)), these modified ITRs would still be symmetrical if, for example, the 5' ITR had the sequence of ATC**G***AAC*<u>*C*</u>**A**TCG (SEQ ID NO: 540), where G in the addition is modified to C, and the substantially symmetrical 3' ITR has the sequence of CGA**T***CGTT***C**GAT (SEQ ID NO: 539), without the corresponding modification of the T in the addition to a. In some embodiments, such a modified ITR pair are substantially symmetrical as the modified ITR pair has symmetrical stereochemistry.

[00320] **Table 5** shows exemplary symmetric modified ITR pairs (*i.e.* a left modified ITRs and the symmetric right modified ITR) for use in a ceDNA vector for expression of FVIII protein. The bold (red) portion of the sequences identify partial ITR sequences (*i.e.,* sequences of A-A', C-C' and B-B' loops), also shown in FIGS 31A-46B. These exemplary modified ITRs can comprise the RBE of GCGCGCTCGCTCGCTC-3′ (SEQ ID NO: 437), spacer of ACTGAGGC (SEQ ID NO: 439), the spacer complement GCCTCAGT (SEQ ID NO: 440) and RBE' (*i.e.,* complement to RBE) of GAGCGAGCGAGCGCGC (SEQ ID NO: 441).

Table 5: Exemplary symmetric modified ITR pairs (and corresponding SEQ ID NOs) in a ceDNA vector for expression of FVIII protein

LEFT modified ITR (modified 5' ITR)		Symmetric RIGHT modified ITR (modified 3' ITR)	
ITR-33 left	SEQ ID NO: 522	ITR-18, right	SEQ ID NO: 505
ITR-34 left	SEQ ID NO: 523	ITR-51, right	SEQ ID NO: 520
ITR-35 left	SEQ ID NO: 524	ITR-19, right	SEQ ID NO: 506

ITR-36 left	SEQ ID NO: 525	ITR-20, right	SEQ ID NO: 507
ITR-37 left	SEQ ID NO: 526	ITR-21, right	SEQ ID NO: 508
ITR-38 left	SEQ ID NO: 527	ITR-22 right	SEQ ID NO: 509
ITR-39 left	SEQ ID NO: 528	ITR-23, right	SEQ ID NO: 510
ITR-40 left	SEQ ID NO: 529	ITR-24, right	SEQ ID NO: 511
ITR-41 left	SEQ ID NO: 530	ITR-25 right	SEQ ID NO: 512
ITR-42 left	SEQ ID NO: 531	ITR-26 right	SEQ ID NO: 513
ITR-43 left	SEQ ID NO: 532	ITR-27 right	SEQ ID NO: 514
ITR-44 left	SEQ ID NO: 533	ITR-28 right	SEQ ID NO: 515
ITR-45 left	SEQ ID NO: 534	ITR-29, right	SEQ ID NO: 516
ITR-46 left	SEQ ID NO: 535	ITR-30, right	SEQ ID NO: 517
ITR-47, left	SEQ ID NO: 536	ITR-31, right	SEQ ID NO: 518
ITR-48, left	SEQ ID NO: 537	ITR-32 right	SEQ ID NO: 519

[00321] In some embodiments, a ceDNA vector for expression of FVIII protein comprising an asymmetric ITR pair can comprise an ITR with a modification corresponding to any of the modifications in ITR sequences or ITR partial sequences shown in any one or more of **Tables 4A-4B** herein, or the sequences shown in FIG. 7A-7B of International Application PCT/US2018/064242, filed December 6, 2018, which is incorporated herein in its entirety, or disclosed in Tables 2, 3, 4, 5, 6, 7, 8, 9 or 10A-10B of International application PCT/US18/49996 filed September 7, 2018 which is incorporated herein in its entirety by reference.

V. Exemplary ceDNA vectors

[00322] As described above, the present disclosure relates to recombinant ceDNA expression vectors and ceDNA vectors that encode FVIII protein, comprising any one of: an asymmetrical ITR pair, a symmetrical ITR pair, or substantially symmetrical ITR pair as described above. In certain embodiments, the disclosure relates to recombinant ceDNA vectors for expression of FVIII protein having flanking ITR sequences and a transgene, where the ITR sequences are asymmetrical, symmetrical or substantially symmetrical relative to each other as defined herein, and the ceDNA further comprises a nucleic acid sequence of interest (for example an expression cassette comprising the nucleic acid of a transgene) located between the flanking ITRs, wherein said nucleic acid molecule is devoid of viral capsid protein coding sequences.

[00323] The ceDNA expression vector for expression of FVIII protein may be any ceDNA vector that can be conveniently subjected to recombinant DNA procedures including nucleic acid sequence(s) as described herein, provided at least one ITR is altered. The ceDNA vectors for expression of FVIII protein of the present disclosure are compatible with the host cell into which the ceDNA vector is to be introduced. In certain embodiments, the ceDNA vectors may be linear. In certain embodiments, the ceDNA vectors may exist as an extrachromosomal entity. In certain embodiments, the ceDNA vectors of the present disclosure may contain an element(s) that permits integration of a donor sequence into the host cell's genome. As used herein “transgene” and “heterologous nucleic acid sequence” are synonymous, and may encode a FVIII protein, as described herein.

[00324] ceDNA vectors are capsid-free and can be obtained from a plasmid encoding in this order: a first ITR, an expressible transgene cassette and a second ITR, where the first and second ITR sequences are asymmetrical, symmetrical or substantially symmetrical relative to each other as defined herein. ceDNA vectors for expression of FVIII protein are capsid-free and can be obtained from a plasmid encoding in this order: a first ITR, an expressible transgene (protein or nucleic acid) and a second ITR, where the first and second ITR sequences are asymmetrical, symmetrical or substantially symmetrical relative to each other as defined herein. In some embodiments, the expressible transgene cassette includes, as needed: an enhancer/promoter, one or more homology arms, a donor sequence, a post-transcription regulatory element (*e.g.,* WPRE, *e.g.,* SEQ ID NO: 67)), and a polyadenylation and termination signal (*e.g.,* BGH polyA, *e.g.,* SEQ ID NO: 68).

A. Regulatory elements

[00325] The ceDNA vectors for expression of FVIII protein as described herein comprising an asymmetric ITR pair or symmetric ITR pair as defined herein, can further comprise a specific combination of cis-regulatory elements. The cis-regulatory elements include, but are not limited to, a promoter, a riboswitch, an insulator, a mir-regulatable element, a post-transcriptional regulatory element, a tissue- and cell type-specific promoter and an enhancer. In some embodiments, the ITR can act as the promoter for the transgene, *e.g.,* FVIII protein. In some embodiments, the ceDNA vector for expression of FVIII protein as described herein comprises additional components to regulate expression of the transgene, for example, regulatory switches as described herein, to regulate the expression of the transgene, or a kill switch, which can kill a cell comprising the ceDNA vector encoding FVIII protein thereof. Regulatory elements, including Regulatory Switches that can be used in the present disclosure are more fully discussed in International application PCT/US18/49996, which is incorporated herein in its entirety by reference.

[00326] Described herein are ceDNA vectors that comprise a codon optimized FVII nucleic acid sequence and combined with particular *cis*-elements (*e.g.*, promoters, enhancers, specific promoter and enhancer combinations). According to some embodiments, particular codon optimized FVIII nucleic acid sequences perform better when combined with one or more specific promoter sequence and/or a specific enhancer sequence, compared to the same codon optimized FVIII nucleic acid sequence combined with another promoter sequence and/or a specific enhancer sequence.

(i) Promoters:

[00327] It will be appreciated by one of ordinary skill in the art that promoters used in the ceDNA vectors for expression of FVIII protein as disclosed herein are tailored as appropriate for the specific sequences they are promoting.

[00328] Expression cassettes of the ceDNA vector for expression of FVIII protein can contain tissue-specific eukaryotic promoters to limit transgene expression to specific cell types and reduce toxic

effects and immune responses resulting from unregulated, ectopic expression. The promoter region used may further include one or more additional regulatory sequences (*e.g.,* native), *e.g.,* enhancers.

[00329] In some embodiments, a promoter may also be a promoter from a human gene. The promoter may also be a tissue specific promoter, such as a liver specific promoter, such as human alpha 1-antitypsin (HAAT). According to some embodiments, the promoter may be synthetic.

[00330] Non-limiting examples of suitable promoters for use in accordance with the present disclosure include any of the promoters described herein, or any of the following:

[00331] According to some embodiments, the promoter is hAAT core, the human a1 antitrypsin (hAAT) promoter (Core promoter sequence from human A1AT gene). According to some embodiments, the hAAT promoter comprises the sequence set forth as SEQ ID NO: 210.

[00332] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 210. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 210. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 210. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 210. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 210. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 210. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 210. According to some embodiments, the promoter consists of the nucleic acid sequence of SEQ ID NO: 210.

[00333] According to some embodiments, the promoter is the minimal transthyretin promoter (TTRm). According to some embodiments, the TTRm promoter comprises the sequence set forth as SEQ ID NO: 211.

[00334] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 211. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 211. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 211. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 211. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 211. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 211. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 211. According to some embodiments, the promoter consists of the nucleic acid sequence of SEQ ID NO: 211.

[00335] According to some embodiments, the promoter is hAAT_core_C06, a CpG minimized version of the hAAT core promoter (A1AT gene promoter). According to some embodiments, the hAAT promoter comprises the sequence set forth as SEQ ID NO: 212.

[00336] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 212. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 212. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 212. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 212. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 212. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 212. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 212. According to some embodiments, the promoter consists of the nucleic acid sequence of SEQ ID NO: 212.

[00337] According to some embodiments, the promoter is hAAT_core_C07, a CpG minimized version of the hAAT core promoter (A1AT gene promoter). According to some embodiments, the hAAT promoter comprises the sequence set forth as SEQ ID NO: 213.

[00338] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 213. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 213. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 213. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 213. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 213. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 213. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 213. According to some embodiments, the promoter consists of the nucleic acid sequence of SEQ ID NO: 213.

[00339] According to some embodiments, the promoter is hAAT_core_C08, a CpG minimized version of the hAAT core promoter (A1AT gene promoter). According to some embodiments, the hAAT promoter comprises the sequence set forth as SEQ ID NO: 214.

[00340] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 214. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 214. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 214. According to some embodiments, the promoter comprises a nucleic acid sequence at least

about 96% identical to SEQ ID NO: 214. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 214. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 214. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 214. According to some embodiments, the promoter consists of the nucleic acid sequence of SEQ ID NO: 214.

[00341] According to some embodiments, the promoter is hAAT_core_C09, a CpG minimized version of the hAAT core promoter (A1AT gene promoter). According to some embodiments, the hAAT promoter comprises the sequence set forth as SEQ ID NO: 215.

[00342] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 215. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 215. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 215. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 215. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 215. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 215. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 215. According to some embodiments, the promoter consists of the nucleic acid sequence of SEQ ID NO: 215.

[00343] According to some embodiments, the promoter is hAAT_core_C10, a CpG minimized version of the hAAT core promoter (A1AT gene promoter). According to some embodiments, the hAAT promoter comprises the sequence set forth as SEQ ID NO: 216.

[00344] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 216. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 216. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 216. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 216. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 216. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 216. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 216. According to some embodiments, the promoter consists of the nucleic acid sequence of SEQ ID NO: 216.

[00345] According to some embodiments, the promoter is hAAT_core_truncated, 5p truncated hAAT core promoter derived from hAAT_core (SEQ ID NO: 210). According to some embodiments, the hAAT promoter comprises the sequence set forth as SEQ ID NO: 217.

[00346] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 217. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 217. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 217. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 217. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 217. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 217. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 217. According to some embodiments, the promoter consists of the nucleic acid sequence of SEQ ID NO: 217.

[00347] **Table6** lists core promoter sequences, and their corresponding SEQ ID NOs, that can be implemented in ceDNA FVIII therapeutics described herein.

Table 6. Core Promoters

	Name	**Description**	**SEQ ID NO.**
GE-015	hAAT_core	Core promoter sequence from human A1AT gene	210
GE-1121	TTRm	Core promoter sequence from mouse Transthyretin gene	211
GE-1133	hAAT_core_C06	CpG minimized version of the hAAT core promoter (A1AT gene promoter)	212
GE-1134	hAAT_core_C07	CpG minimized version of the hAAT core promoter (A1AT gene promoter)	213
GE-1135	hAAT_core_C08	CpG minimized version of the hAAT core promoter (A1AT gene promoter)	214
GE-1136	hAAT_core_C09	CpG minimized version of the hAAT core promoter (A1AT gene promoter)	215
GE-1137	hAAT_core_C10	CpG minimized version of the hAAT core promoter (A1AT gene promoter) (also referred to as hAAT(979))	216
GE-1170	hAAT_core_truncated	5p truncated hAAT core promoter derived from GE-015	217

[00348] According to particular embodiments, the promoter is selected from the group consisting of: the VandenDriessche (referred to as "VD" or "VanD") promoter, human alpha 1-antitrypsin (hAAT) promoter (including the CpG minimized hAAT(979) promoter (CpGmin hAAT_core_C10) and other CpGmin_hAAT promoters like hAAT_core_C06; hAAT_core_C07; hAAT_core_C08; and hAAT_core_C09) and the transthyretin (TTR) liver specific promoter.

[00349] In some embodiments, the VD promoter comprises the minute virus mouse (MVM) intron, the minimal transthyretin promoter (TTRm), the serpin enhancer (72 bp) and TTRm 5' UTR. According to some embodiments, the TTRm comprises SEQ ID NO: 211. According to some embodiments, the serpin enhancer comprises tSEQ ID NO: 19. According to some embodiments, the TTRm 5'UTR comprises SEQ ID NO: 426.

[00350] According to further embodiments, the VD promoter comprises SEQ ID NO: 541.

[00351] According to some embodiments, the CpGmin_hAAT promoter comprises a sequence selected from any one of SEQ ID NOs: 212, 213, 214, 215 or 216.

(ii) Enhancers

[00352] In some embodiments, a ceDNA expressing FVIII comprises one or more enhancers. In some embodiments, an enhancer sequence is located 5' of the promoter sequence. In some embodiments, the enhancer sequence is located 3' of the promoter sequence. According to some embodiments, the enhancer is the enhancer region for Serpin1 gene (SerpEnh) as described by Chuah, M., *et al.* ((2014). Liver-Specific Transcriptional Modules Identified by Genome-Wide In Silico Analysis Enable Efficient Gene Therapy in Mice and Non-Human Primates Molecular Therapy, 22(9), 1605-1613, incorporated by reference in its entirety herein).

[00353] According to some embodiments, the sequence of the serpin enhancer (SerpEnh) is set forth in SEQ ID NO: 198.

[00354] According to some embodiments, the enhancer comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 198.

[00355] According to some embodiments, the enhancer is the enhancer region for Transthyretin (TTRe) gene (TTRe). According to some embodiments, the sequence of the enhancer region for Transthyretin (TTRe) gene (TTRe) is set forth in SEQ ID NO: 199.

[00356] According to some embodiments, the enhancer comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 199. According to some embodiments, the enhancer consists of SEQ ID NO: 199. According to some embodiments, the enhancer is the Hepatic Nuclear Factor 1 binding site (HNF1). According to some embodiments, the sequence of theHepatic Nuclear Factor 1 binding site (HNF1) is set forth in SEQ ID NO: 200.

[00357] According to some embodiments, the enhancer comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 200. According to some embodiments, the enhancer consists of SEQ ID NO: 200.

[00358] According to some embodiments, the enhancer is the Hepatic Nuclear Factor 4 binding site (HNF4). According to some embodiments, the sequence of the Hepatic Nuclear Factor 4 binding site (HNF4) is set forth in SEQ ID NO: 201.

[00359] According to some embodiments, the enhancer comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 201. According to some embodiments, the enhancer consists of SEQ ID NO: 201.

[00360] According to some embodiments, the enhancer is the Human apolipoprotein E/C-I liver specific enhancer (ApoE_Enh). According to some embodiments, the sequence of the Human apolipoprotein E/C-I liver specific enhancer (ApoE_Enh) is set forth in SEQ ID NO: 202.

[00361] According to some embodiments, the enhancer comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 202. According to some embodiments, the enhancer consists of SEQ ID NO: 202.

[00362] According to some embodiments, the enhancer is the Enhancer region from Pro-albumin gene (ProEnh). According to some embodiments, the sequence of the Enhancer region from Pro-albumin gene (ProEnh) is set forth in SEQ ID NO: 203.

[00363] According to some embodiments, the enhancer comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 203. According to some embodiments, the enhancer consists of SEQ ID NO: 203.

[00364] According to some embodiments, the enhancer is a CpG minimized version of the ApoE_Enh (Human apolipoprotein E/C-I liver specific enhancer) (ApoE_Enh_C03, ApoE_Enh_C04, ApoE_Enh_C09, and ApoE_Enh_C10). According to some embodiments, the sequence of ApoE_Enh_C03, ApoE_Enh_C04, ApoE_Enh_C09 and ApoE_Enh_C10 are set forth in SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206 and SEQ ID NO: 207.

[00365] According to some embodiments, the enhancer comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 204. According to some embodiments, the enhancer comprises, or consists of SEQ ID NO: 204.

[00366] According to some embodiments, the enhancer comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 205. According to some embodiments, the enhancer comprises, or consists of SEQ ID NO: 205.

[00367] According to some embodiments, the enhancer comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 206. According to some embodiments, the enhancer comprises, or consists of SEQ ID NO: 206.

According to some embodiments, the enhancer comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 207. According to some embodiments, the enhancer comprises, or consists of SEQ ID NO: 207.

[00368] According to some embodiments, the enhancer is the HCR1 footprint123 embedded in GE-856 (Embedded_HCR1_footprint123). According to some embodiments, the sequence of the HCR1 footprint123 embedded in GE-856 is set forth in SEQ ID NO: 208.

[00369] According to some embodiments, the enhancer comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 208. According to some embodiments, the enhancer comprises, or consists of SEQ ID NO: 208.

[00370] According to some embodiments, the enhancer is the Hepatic nuclear factor enhancer array embedded in GE-856 (Embedded_enhancer_HNF_array). According to some embodiments, the sequence of the Hepatic nuclear factor enhancer array embedded in GE-856 is set forth in SEQ ID NO: 209.

[00371] According to some embodiments, the enhancer comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 209. According to some embodiments, the enhancer comprises, or consists of SEQ ID NO: 209.

[00372] According to some embodiments, the enhancer is a derivative of Human apolipoprotein E/C-I liver specific enhancer (ApoE_enhancer_v2). According to some embodiments, the sequence of the ApoE_enhancer_v2 is set forth in SEQ ID NO: 485.

[00373] According to some embodiments, the enhancer comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 485. According to some embodiments, the enhancer comprises, or consists of SEQ ID NO: 485.

[00374] According to some embodiments, the enhancer is a derivative of Serpin enhancer from bushbaby (Bushbaby SerpEnh). According to some embodiments, the bushbaby Serpin enhancer sequence is shown below as SEQ ID NO: 557: GGGGGAAGCTACTGGTGAATATTAACCAAGGTCACCCAGTTATCAGGGAGCAAACAGGA GCTAAGTCCAT and set forth in SEQ ID NO: 557.

[00375] According to some embodiments, the enhancer comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 557. According to some embodiments, the enhancer comprises, or consists of SEQ ID NO: 557. According to some other embodiments, the bushbaby Serpin enhancer comprises 2x, 3x, 4x, 5x, 6x, 7x, and up to 10x repeats of the nucleic acid sequence comprising SEQ ID NO: 557, with or without a spacer sequence between each iteration of the sequence.

[00376] According to some embodiments, the enhancer is a derivative of Serpin enhancer from Chinese tree shrew (Chinese tree shrew SerpEnh). According to some embodiments, the Chinese tree shrew Serpin enhancer sequence is as follows: GGAGGCTGTTGGTGAATATTAACCAAGGTCACCTCAGTTATCGGAGGAGCAAACAAGGG CTAAGTCCAC and set forth in SEQ ID NO: 617.

[00377] According to some embodiments, the enhancer comprises a nucleic acid sequence at least about 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 617. According to some embodiments, the enhancer comprises, or consists of SEQ ID NO: 617. According to some other embodiments, the

bushbaby Serpin enhancer comprises 2x, 3x, 4x, 5x, 6x, 7x, and up to 10x repeats of the nucleic acid sequence comprising SEQ ID NO: 617, with or without a spacer sequence between each iteration.

[00378] According to some embodiments, the enhancer is a derivative of Serpin enhancer from human SERPINA1 enhancer with FOXA & HNF4 consensus sites and internal CpG removed (HNF4_FOXA_v1). According to some embodiments, the HNF4_FOXA_v1 Serpin enhancer sequence is as follows:

[00379] GGGGGAGGCTGCTGGTAAACATTAACCAAGGTCACCCCAGTTATCAGAGGAGC AAACAGGGGCAAAGTCCAC and set forth in SEQ ID NO: 625.

[00380] According to some embodiments, the enhancer comprises a nucleic acid sequence at least about 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 625. According to some embodiments, the enhancer comprises, or consists of SEQ ID NO: 625. According to some other embodiments, the HNF4_FOXA_v1 Serpin enhancer comprises 2x, 3x, 4x, 5x, 6x, 7x, and up to 10x repeats of the nucleic acid sequence comprising SEQ ID NO: 625, with or without a spacer sequence between each iteration.

[00381] A summary of these enhancers that can be utilized in ceDNA FVIII constructs is listed in Table 7.

Table 7. Enhancers

GE-##	Name (Abbreviation)	Description	SEQ ID NO.
GE-1115	Human Serpin Enhancer (hSerpEnh)	Enhancer region for Serpin1 gene as reporte Chuah, M., et al. (2014). Liver-Specific Transcriptional Modules Identified by Genor Wide In Silico Analysis Enable Efficient Ge Therapy in Mice and Non-Human Primates Molecular Therapy 22(9), 1605-1613. dx.doi.org/10.1038/mt.2014.114	198
GE-1116	TTRe	Enhancer region for Transthyretin gene	199
GE-1117	HNF1	Hepatic Nuclear Factor 1 binding site	200
GE-1118	HNF4	Hepatic Nuclear Factor 4 binding site	201
GE-1119	ApoE_Enh	Human apolipoprotein E/C-I liver specific enhancer	202
GE-1120	ProEnh	Enhancer region from Pro-albumin gene	203
GE-1129	ApoE_Enh_C03	CpG minimized version of the ApoE_Enh (Human apolipoprotein E/C-I liver specific enhancer)	204
GE-1130	ApoE_Enh_C04	CpG minimized version of the ApoE_Enh (Human apolipoprotein E/C-I liver specific enhancer)	205
GE-1131	ApoE_Enh_C09	CpG minimized version of the ApoE_Enh (Human apolipoprotein E/C-I liver specific enhancer)	206
GE-1132	ApoE_Enh_C10	CpG minimized version of the ApoE_Enh (Human apolipoprotein E/C-I liver specific enhancer)	207

GE-1127	Embedded_HCR1_footprint 123	HCR1 footprint123 embedded in GE-856 (aka between GE-859/GE-860)	208
GE-1128	Embedded_enhancer_HNF_ array	Hepatic nuclear factor enhancer array embedded in GE-856 (aka between GE-859/GE-860)	209
GE-1237	ApoE_Enh_v2	Derivative of Human apolipoprotein E/C-I liver specific enhancer	485
	3x_HNF4_FOXA_v1	3x repeat of the Human SERPINA1 enhancer with FOXA & HNF4 consensus sites spacer in bold	558
	3x_HNF4_FOXA_v1_CpGmi n	3x repeat of HNF4_FOXA_v1 with CpG minimization	559
	3x_HNF4_FOXA_v1_Second aryStruct_min_v1	3x repeat of HNF4_FOXA_v1 with poly-C/poly-G minimization v1	560
	3x_HNF4_FOXA_v1_Second aryStruct_min_v1_CpG_min	3x repeat of HNF4_FOXA_v1 with poly-C/poly-G minimization and CpG minimization v1	561
	3x_HNF4_FOXA_v1_Second aryStruct_min_v2	(3x repeat of HNF4_FOXA_v1 with poly-C/poly-G minimization v2 (“C” spacer))	562
	3x_HNF4_FOXA_v1_Second aryStruct_min_v2_CpG_min	(3x repeat of HNF4_FOXA_v1 with poly-C/poly-G minimization and CpG minimization v2 (“A” spacer))	563
	3x_HNF4_FOXA_v1_Second aryStruct_min_v3	(3x repeat of HNF4_FOXA_v1 with poly-C/poly-G minimization v3 (“C” spacer))	564
	3x_HNF4_FOXA_v1_Second aryStruct_min_v3_CpG_min	(3x repeat of HNF4_FOXA_v1 with poly-C/poly-G minimization and CpG minimization v3 (“A” spacer))	565
	3x_HNF4_FOXA_v1_Second aryStruct_min_v4_Aspacers	(“A” spacer inbetween the repeats) (3x repeat of HNF4_FOXA_v1 with poly-C/poly-G minimization v4 (2585))	566
	3x_HNF4_FOXA_v1_Second aryStruct_min_v5_Aspacers	(“A” spacer inbetween the repeats) (3x repeat of HNF4_FOXA_v1 with poly-C/poly-G minimization v5)	567
	3x_HNF4_FOXA_v1_Second aryStruct_min_v6_Aspacers	(“A” spacer inbetween the repeats) (3x repeat of HNF4_FOXA_v1 with poly-C/poly-G minimization v6)	568
	3x_ChineseTreeShrew	(3x repeat of the Chinese Tree Shrew SERPINA1 enhancer (“C” spancer inbetween the repeats))	569
	3x_ChineseTreeShrew_CpG min	(3x repeat of the Chinese Tree Shrew SERPINA1 enhancer with CpG minimization)	570

	3x_hSerpEnh_Aspacers	(3x repeat of the human SERPINA1 enhancer with 1 adenine between the repeats ("A" spacer))	571
	3x_Bushbaby_Aspacers	(3x repeat of the Bushbaby SERPINA1 enhancer with adenine nucleotide spacer ("A" spacer))	572
	5x_HNF4_FOXA_v1	(5x repeat of HNF4_FOXA_v1 ("C" spacer))	573
	5x_HNF4_FOXA_v1_SecondaryStruct_min_v1 (	5x repeat of HNF4_FOXA_v1 with poly-C/poly-G minimization v1 ("C" spacer))	574
	5x_HNF4_FOXA_v1_SecondaryStruct_min_v1_CpG_min	5x repeat of HNF4_FOXA_v1 with poly-C/poly-G minimization and CpG minimization v1 ("AG" spacer))	575
	5x_HNF4_FOXA_v1_SecondaryStruct_min_v2	(5x repeat of HNF4_FOXA_v1 with poly-C/poly-G minimization v2 ("C" spacer))	576
	5x_HNF4_FOXA_v1_SecondaryStruct_min_v2_CpG_min	5x repeat of HNF4_FOXA_v1 with poly-C/poly-G minimization and CpG minimization v2 ("A" spacer))	577
	5x_HNF4_FOXA_v1_SecondaryStruct_min_v3	(5x repeat of HNF4_FOXA_v1 with poly-C/poly-G minimization v3 ("C" spacer))	578
	5x_HNF4_FOXA_v1_SecondaryStruct_min_v3_CpG_min	5x repeat of HNF4_FOXA_v1 with poly-C/poly-G minimization and CpG minimization v3)	579
	5x_HNF4_FOXA_v1_SecondaryStruct_min_v4_Aspacers	(5x repeat of HNF4_FOXA_v1 with poly-C/poly-G minimization v4)	580
	5x_HNF4_FOXA_v1_SecondaryStruct_min_v5_Aspacers)	(5x repeat of HNF4_FOXA_v1 with poly-C/poly-G minimization v5	581
	5x_HNF4_FOXA_v1_SecondaryStruct_min_v6_Aspacers	(5x repeat of HNF4_FOXA_v1 with poly-C/poly-G minimization v6)	582
	5x_ChineseTreeShrew	(5x repeat of the Chinese Tree Shrew SERPINA1 enhancer)	583

	5x_ChineseTreeShrew_CpG min	(5x repeat of the Chinese Tree Shrew SERPINA1 enhancer with CpG minimization)	584
	5x_Bushbaby_Aspacers	(5x repeat of the Bushbaby SERPINA1 enhancer with adenenine nucleotide spacer)	585
	10x_HNF4_FOXA_v1	(10x repeat of HNF4_FOXA_v1)	586
	10x_HNF4_FOXA_v1_SecondaryStruct_min_v1	(10x repeat of HNF4_FOXA_v1 with poly-C/poly-G minimization v1)	587
	10x_HNF4_FOXA_v1_SecondaryStruct_min_v1_CpG_min	(10x repeat of HNF4_FOXA_v1 with poly-C/poly-G minimization and CpG minimization v1)	588
	10x_HNF4_FOXA_v1_SecondaryStruct_min_v2	(10x repeat of HNF4_FOXA_v1 with poly-C/poly-G minimization v2)	589
	10x_HNF4_FOXA_v1_SecondaryStruct_min_v2_CpG_min	(10x repeat of HNF4_FOXA_v1 with poly-C/poly-G minimization and CpG minimization v2)	590
	10x_HNF4_FOXA_v1_SecondaryStruct_min_v3	(10x repeat of HNF4_FOXA_v1 with poly-C/poly-G minimization v3)	591
	10x_HNF4_FOXA_v1_SecondaryStruct_min_v3_CpG_min	(10x repeat of HNF4_FOXA_v1 with poly-C/poly-G minimization and CpG minimization v3)	592
	10x_hSerpEnh	(10x repeat of the human SERPINA1 enhancer ("C" spacer))	593
	10x_Bushbaby_Aspacers	(10x repeat of the Bushbaby SERPINA1 enhancer with *adenenine nucleotide spacer)*	594
	Bushbaby_HN4F/FOXv1_HNF4mod	(Bushbaby SERPINA1 enhancer, FOXA_HNF4_v1 enhancer, HNF4 consensus binding site enhancer)	595
	HNF4mod_BushbabyMod_HN4F/FOXv1	(HNF4 consensus binding site enhancer, Bushbaby SERPINA1 enhancer, FOXA_HNF4_v1 enhancer)	596

	3x_hSerpEnh_2mer_spacers_v1	(3x repeat of hSerpEnh with 2mer spacers v1 (bold underlined))	597
	3x_hSerpEnh_2mer_spacers_v4	(3x repeat of hSerpEnh with 2mer spacers v4 (bold underlined))	598
	3x_hSerpEnh_2mer_spacers_v8	(3x repeat of hSerpEnh with 2mer spacers v8 (bold underlined))	599
	3x_hSerpEnh_2mer_spacers_v9	(3x repeat of hSerpEnh with 2mer spacers v9 (bold underlined))	600
	3x_hSerpEnh_2mer_spacers_v10	(3x repeat of hSerpEnh with 2mer spacers v10 (bold underlined))	601
	3x_hSerpEnh_2mer_spacers_v12	(3x repeat of hSerpEnh with 2mer spacers v12 (bold underlined))	602
	3x_hSerpEnh_2mer_spacers_v17	(3x repeat of hSerpEnh with 2mer spacers v17 (bold underlined))	603
	3x_hSerpEnh_3mer_spacers_v1	(3x repeat of hSerpEnh with 3mer spacers v1 (bold underlined))	604
	3x_hSerpEnh_3mer_spacers_v2	(3x repeat of hSerpEnh with 3mer spacers v2 (bold underlined))	605
	3x_hSerpEnh_5mer_spacers_v1	(3x repeat of hSerpEnh with 5mer spacers v1 (bold underlined))	606
	3x_hSerpEnh_5mer_spacers_v2	(3x repeat of hSerpEnh with 5mer spacers v2 (bold underlined))	607
	3x_hSerpEnh_5mer_spacers_v3	(3x repeat of hSerpEnh with 5mer spacers v3 (bold underlined))	608
	3x_hSerpEnh_11mer_spacers_v1	(3x repeat of hSerpEnh with 11mer spacers v1 (bold underlined))	609
	3x_hSerpEnh_11mer_spacers_v2	(3x repeat of hSerpEnh with 11mer spacers v2 (bold underlined))	610

	3x_hSerpEnh_11mer_spacers _v3	(3x repeat of hSerpEnh with 11mer spacers v3 (bold underlined))	611
	3x_hSerpEnh_11mer_spacers _HNF4former_spacers_FOX Afor	(3x repeat of hSerpEnh with 11mer spacers (bold underlined) with HNF4 binding site in orientation 1 & FOXA binding site in orientation 1)	612
	3x_hSerpEnh_11mer_spacers _HNF4former_spacers_FOX Arev (3x repeat of hSerpEnh with 11mer spacers (bold underlined) with HNF4 binding site in orientation 1 & FOXA binding site in orientation 2)	11mer spacers (bold underlined) with HNF4 binding site in orientation 2 & FOXA binding site in orientation 1)	613
	3x_hSerpEnh_11mer_spacers _HNF4revmer_spacers_FOX Afor (3x repeat of hSerpEnh with	11mer spacers (bold underlined) with HNF4 binding site in orientation 2 & FOXA binding site in orientation 1)	614
	3x_hSerpEnh_11mer_spacers _HNF4revmer_spacers_FOX Arev	(3x repeat of hSerpEnh with 11mer spacers (bold underlined) with HNF4 binding site in orientation 2 & FOXA binding site in orientation 2)	615
	3x_hSerpEnh_30mer_spacers _v1	(3x repeat of hSerpEnh with 30mer spacers v1 (bold underlined))	616

[00382] In some other embodiments, the enhancers can be used in tandem.

Promoter sets

[00383] According to some embodiments, the promoter comprises a synthetic liver specific promoter set including enhancers and core promoter, without 5pUTR, referred to as a promoter set.

[00384] According to some embodiments, the 3xHNF1-4_ProEnh (Pro-albumin enhancer) enhancer fused to TTR promoter comprises the sequence set forth in SEQ ID NO:184.

According to some embodiments, the 3xHNF1-4_ProEnh (Pro-albumin enhancer) enhancer fused to 3x VanD-TTRe and TTR promoter comprises the sequence set forth in SEQ ID NO: 185.

[00385] According to some embodiments, the 5xHNF1_ProEnh_enhancer fused to TTR promoter comprises the sequence set forth in SEQ ID NO: 186. According to some embodiments, the 5xHNF1_ProEnh_enhancer fused to 3x SerpEnh VD-TTRe and TTR promoter comprises the sequence set forth in SEQ ID NO:187.

[00386] According to some embodiments, the promoter set (promoter set 1471) comprises the sequence set forth as SEQ ID NO: 184.

[00387] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 184. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 184. According to some

embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 184. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 184. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 184. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 184. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 184. According to some embodiments, the promoter consists of the nucleic acid sequence of SEQ ID NO: 184.

[00388] According to some embodiments, the promoter set (promoter set 1472) comprises the sequence set forth as SEQ ID NO: 185.

[00389] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 185. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 185. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 185. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 185. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 185. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 185. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 185. According to some embodiments, the promoter consists of the nucleic acid sequence of SEQ ID NO: 185.

[00390] According to some embodiments, the promoter set (promoter set 1473) comprises the sequence set forth as SEQ ID NO: 186.

[00391] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 186. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 186. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 186. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 186. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 186. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 186. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 186. According to some embodiments, the promoter consists of the nucleic acid sequence of SEQ ID NO: 186.

[00392] According to some embodiments, the promoter set (promoter set 1474) comprises the sequence set forth as SEQ ID NO: 187.

[00393] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 187. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 187. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 187. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 187. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 187. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 187. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 187. According to some embodiments, the promoter consists of the nucleic acid sequence of SEQ ID NO: 187.

[00394] According to some embodiments, the promoter set (promoter set 1475) comprises the sequence set forth as SEQ ID NO: 484.

[00395] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 484. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 484. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 484. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 484. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 484. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 484. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 484. According to some embodiments, the promoter consists of the nucleic acid sequence of SEQ ID NO: 484.

[00396] According to some embodiments, the promoter set (promoter set 1476) comprises the sequence set forth as SEQ ID NO: 189.

[00397] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 189. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 189. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 189. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 189. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 189. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 189. According to some embodiments, the promoter comprises a nucleic acid sequence at least

about 99% identical to SEQ ID NO: 189. According to some embodiments, the promoter consists of the nucleic acid sequence of SEQ ID NO: 189.

[00398] According to some embodiments, the promoter set (promoter set 1477) comprises the sequence set forth as SEQ ID NO: 190.

[00399] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 190. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 190. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 190. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 190. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 190. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 190. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 190. According to some embodiments, the promoter consists of the nucleic acid sequence of SEQ ID NO: 190.

[00400] According to some embodiments, the promoter set (promoter set 1478) comprises the sequence set forth as SEQ ID NO: 191.

[00401] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 191. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 191. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 191. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 191. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 191. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 191. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 191. According to some embodiments, the promoter consists of the nucleic acid sequence of SEQ ID NO: 191.

[00402] According to some embodiments, the promoter set (promoter set 1479) comprises the sequence set forth as SEQ ID NO: 192.

[00403] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 192. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 192. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 192. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 192. According to some embodiments, the promoter comprises a

nucleic acid sequence at least about 97% identical to SEQ ID NO: 192. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 192. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 192. According to some embodiments, the promoter consists of the nucleic acid sequence of SEQ ID NO: 192.

[00404] According to some embodiments, the promoter set (promoter set 1480) comprises the sequence set forth as SEQ ID NO: 193.

[00405] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 193. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 193. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 193. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 193. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 193. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 193. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 193. According to some embodiments, the promoter consists of the nucleic acid sequence of SEQ ID NO: 193.

[00406] According to some embodiments, the promoter set (promoter set 1368) comprises the sequence set forth as SEQ ID NO: 194.

[00407] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 194. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 194. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 194. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 194. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 194. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 194. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 194. According to some embodiments, the promoter consists of the nucleic acid sequence of SEQ ID NO: 194.

[00408] According to some embodiments, the promoter set (promoter set 1648) comprises the sequence set forth as SEQ ID NO: 195).

[00409] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 195. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 195. According to some

embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 195. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 195. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 195. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 195. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 195. According to some embodiments, the promoter consists of the nucleic acid sequence of SEQ ID NO: 195.

[00410] According to some embodiments, the promoter set (promoter set 1657) comprises the sequence set forth as SEQ ID NO: 196.

[00411] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 196. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 196. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 196. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 196. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 196. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 196. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 196. According to some embodiments, the promoter consists of the nucleic acid sequence of SEQ ID NO: 196.

[00412] According to some embodiments, the promoter set (promoter set 1622) comprises the sequence set forth as SEQ ID NO: 197.

[00413] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 197. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 197. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 197. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 197. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 197. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 197. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 197. According to some embodiments, the promoter consists of the nucleic acid sequence of SEQ ID NO: 197.

[00414] According to some embodiments, the promoter set (promoter set 1664) comprises the sequence set forth as SEQ ID NO: 400.

[00415] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 400. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 400. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 400. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 400. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 400. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 400. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 400. According to some embodiments, the promoter consists of the nucleic acid sequence of SEQ ID NO: 400.

[00416] According to some embodiments, the promoter set (promoter set 979) comprises the sequence set forth as SEQ ID NO: 401.

[00417] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 401. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 401. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 401. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 401. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 401. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 401. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 401. According to some embodiments, the promoter comprises, or consists of, the nucleic acid sequence of SEQ ID NO: 401.

[00418] According to some embodiments, the promoter set (promoter set 2558) comprises the sequence set forth as SEQ ID NO: 617.

[00419] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 617. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 617. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 617. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 617. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 617. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 617. According to some embodiments, the promoter comprises a nucleic acid sequence at least

about 99% identical to SEQ ID NO: 617. According to some embodiments, the promoter comprises, or consists of, the nucleic acid sequence of SEQ ID NO: 617.

[00420] According to some embodiments, the promoter set (promoter set 2559) comprises the sequence set forth as SEQ ID NO: 618.

[00421] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 618. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 618. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 618. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 618. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 618. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 618. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 618. According to some embodiments, the promoter comprises, or consists of, the nucleic acid sequence of SEQ ID NO: 618.

[00422] According to some embodiments, the promoter set (promoter set 2560) comprises the sequence set forth as SEQ ID NO: 619.

[00423] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 619. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 619. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 619. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 619. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 619. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 619. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 619. According to some embodiments, the promoter comprises, or consists of, the nucleic acid sequence of SEQ ID NO: 619.

[00424] According to some embodiments, the promoter set (promoter set 2580) comprises the sequence set forth as SEQ ID NO: 620.

[00425] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 620. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 620. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 620. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 620. According to some embodiments, the promoter comprises a

nucleic acid sequence at least about 97% identical to SEQ ID NO: 620. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 620. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 620. According to some embodiments, the promoter comprises, or consists of, the nucleic acid sequence of SEQ ID NO: 620.

[00426] According to some embodiments, the promoter set (promoter set 2583) comprises the sequence set forth as SEQ ID NO: 621.

[00427] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 621. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 621. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 621. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 621. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 621. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 621. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 621. According to some embodiments, the promoter comprises, or consists of, the nucleic acid sequence of SEQ ID NO: 621.

[00428] According to some embodiments, the promoter set (promoter set 2584) comprises the sequence set forth as SEQ ID NO: 622.

[00429] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 622. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 622. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 622. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 622. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 622. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 622. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 622. According to some embodiments, the promoter comprises, or consists of, the nucleic acid sequence of SEQ ID NO: 622.

[00430] According to some embodiments, the promoter set (promoter set 2588) comprises the sequence set forth as SEQ ID NO: 623.

[00431] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 623. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 623. According to some

embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 623. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 623. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 623. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 623. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 623. According to some embodiments, the promoter comprises, or consists of, the nucleic acid sequence of SEQ ID NO: 623.

[00432] According to some embodiments, the promoter set (promoter set 2589) comprises the sequence set forth as SEQ ID NO: 624.

[00433] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 624. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 624. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 624. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 624. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 624. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 624. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 624. According to some embodiments, the promoter comprises, or consists of, the nucleic acid sequence of SEQ ID NO: 624.

[00434] A summary of promoter sets that can be utilized in ceDNA FVIII constructs are shown in **Table 8** and in **Table 9**.

Table 8: Promoter Sets

GE	Name	Description	SEQ ID NO.
GE-1223	PromoterSet - 1471	Synthetic Liver specific PromoterSet including enhancers and core promoter (without 5pUTR)	184
GE-1224	PromoterSet - 1472	Synthetic Liver specific PromoterSet including enhancers and core promoter (without 5pUTR)	185
GE-1225	PromoterSet - 1473	Synthetic Liver specific PromoterSet including enhancers and core promoter (without 5pUTR)	186
GE-1226	PromoterSet - 1474	Synthetic Liver specific PromoterSet including enhancers and core promoter (without 5pUTR)	187
GE-1227	PromoterSet - 1475	Synthetic Liver specific PromoterSet including enhancers and core promoter (without 5pUTR)	188
GE-1228	PromoterSet - 1476	Synthetic Liver specific PromoterSet including enhancers and core promoter (without 5pUTR)	189

GE-1229	PromoterSet - 1477	Synthetic Liver specific PromoterSet including enhancers and core promoter (without 5pUTR)	190
GE-1230	PromoterSet - 1478	Synthetic Liver specific PromoterSet including enhancers and core promoter (without 5pUTR)	191
GE-1231	PromoterSet - 1479	Synthetic Liver specific PromoterSet including enhancers and core promoter (without 5pUTR)	192
GE-1232	PromoterSet - 1480	Synthetic Liver specific PromoterSet including enhancers and core promoter (without 5pUTR)	193
GE-1233	PromoterSet - 1368	Synthetic Liver specific PromoterSet including enhancers and core promoter (without 5pUTR)	194
GE-1234	PromoterSet-1648	Synthetic Liver specific PromoterSet including enhancers and core promoter (without 5pUTR)	195
GE-1235	PromoterSet for ceDNA1657	Synthetic Liver specific PromoterSet including enhancers and core promoter (without 5pUTR)	196
GE-1236	PromoterSet-1622	Synthetic Liver specific PromoterSet including enhancers and core promoter (without 5pUTR)	197
GE-1270	PromoterSet - 1664	Synthetic Liver specific PromoterSet including enhancers and core promoter (without 5pUTR)	400
GE-1271	PromoterSet - 979	Synthetic Liver specific PromoterSet including enhancers and core promoter (without 5pUTR)	401
GE-1690	PromoterSet-2558	Promoter formed by contentation of 1) 3x repeat of HNF4_FOXA_v1 with reduction of poly-C/poly-G sequences and reduction of CpGs introduced by multimerization and concatenation with backbone sequences (v1). Repeats are separated by an adenine. 2) KpnI site 3) enhancer region for the murine transthyretin gene 4) XbaI site and BamHI site 5) murine transthyretin promoter	641
GE-1691	PromoterSet-2559	Promoter formed by contentation of 1) 3x repeat of HNF4_FOXA_v1 with reduction of poly-C/poly-G sequences (v2). Repeats are separated by a cytosine. 2) KpnI site 3) enhancer region for the murine transthyretin gene 4) XbaI site and BamHI site 5) murine transthyretin promoter	618
GE-1692	PromoterSet-2560	Promoter formed by contentation of 1) 3x repeat of HNF4_FOXA_v1 with reduction of poly-C/poly-G sequences and reduction of CpGs introduced by multimerization and concatenation with backbone sequences (v2.) Repeats are separated by an adenine. 2) KpnI site 3) enhancer region for the murine transthyretin gene 4) XbaI site and BamHI site 5) murine transthyretin promoter	619
GE-1693	PromoterSet-2580	Promoter formed by contentation of 1) 3x repeat of the SerpEnh_Bushbaby enhancer with adenine nucleotide spacer. Repeats are separated by an adenine. 2) KpnI site 3) enhancer region for the murine transthyretin gene 4) XbaI site and BamHI site 5) murine transthyretin promoter	620

GE-1694	PromoterSet-2583	Promoter formed by contentation of 1) 3 SERPINA1 enhancer variants: a) SerpEnh_Bushbaby, b) HNF4_FOXA_v1, c) human SERPINA1 enhancer with an HNF4 consensus site, internal CpG removed, and poly-C/poly-G regions reduced 2) KpnI site 3) enhancer region for the murine transthyretin gene 4) XbaI site and BamHI site 5) murine transthyretin promoter	621
GE-1695	PromoterSet-2584	Promoter formed by contentation of 1) 3 SERPINA1 enhancer variants: a) human SERPINA1 enhancer with an HNF4 consensus site, internal CpG removed, and poly-C/poly-G regions reduced, b) SerpEnh_Bushbaby with the second G changed to A, c) FOXA_HNF4_v1, 2) KpnI site 3) enhancer region for the murine transthyretin gene 4) XbaI site and BamHI site 5) murine transthyretin promoter	622
GE-1696	PromoterSet-2588	Promoter formed by contentation of 1) 3x repeat of the Chinese Tree Shrew SERPINA1 enhancer. Repeats separated by a cytosine. 2) KpnI site 3) enhancer region for the murine transthyretin gene 4) XbaI site and BamHI site 5) murine transthyretin promoter	623
GE-1697	PromoterSet-2589	Promoter formed by contentation of 1) 3x repeat of the Chinese Tree Shrew SERPINA1 enhancer with CpG reduction. Repeats separated by an adenine. 2) KpnI site 3) enhancer region for the murine transthyretin gene 4) XbaI site and BamHI site 5) murine transthyretin promoter	624

Table 9. Promoter sets: Combinations of the hAAT CpG minimized enhancer and core promoters CpG minimized hAAT core_C10 (hAAT_979) or hAAT_core_C06); combinations of the HNF4/FOXA - TTRe and TTR promoter; combinations of the bushbaby variant enhancer repeats and TTRe and TTR promoter; combinations of the Chinese tree shrew enhancer repeats and TTRe and TTR promoter.

Name	SEQ ID NO.
PromoterSet-970	402
PromoterSet-971	403
PromoterSet-972	404
PromoterSet-973	405
PromoterSet-974	406
PromoterSet-975	407
PromoterSet-976	408
PromoterSet-977	409
PromoterSet-978	410
PromoterSet-2558	641
PromoterSet-2559	618
PromoterSet-2560	619
PromoterSet-2580	620
PromoterSet-2583	621
PromoterSet-2584	622

PromoterSet-2588	623
PromoterSet-2589	624

[00435] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 402. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 402. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 402. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 402. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 402. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 402. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 402. According to some embodiments, the promoter comprises, or consists of the nucleic acid sequence of SEQ ID NO: 402.

[00436] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 403. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 403. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 403. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 403. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 403. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 403. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 403. According to some embodiments, the promoter comprises, or consists of the nucleic acid sequence of SEQ ID NO: 403.

[00437] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 404. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 404. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 404. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 404. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 404. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 404. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 404. According to some embodiments, the promoter comprises, or consists of the nucleic acid sequence of SEQ ID NO: 404.

[00438] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 405. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 405. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 405. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 405. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 405. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 405. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 405. According to some embodiments, the promoter comprises, or consists of the nucleic acid sequence of SEQ ID NO: 405.

[00439] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 406. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 406. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 406. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 406. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 406. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 406. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 406. According to some embodiments, the promoter comprises, or consists of the nucleic acid sequence of SEQ ID NO: 406.

[00440] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 407. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 407. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 407. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 407. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 407. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 407. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 407. According to some embodiments, the promoter comprises, or consists of the nucleic acid sequence of SEQ ID NO: 407.

[00441] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 408. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 408. According to some

embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 408. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 408. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 408. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 408. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 408. According to some embodiments, the promoter comprises, or consists of the nucleic acid sequence of SEQ ID NO: 408.

[00442] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 409. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 409. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 409. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 409. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 409. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 409. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 409. According to some embodiments, the promoter comprises, or consists of the nucleic acid sequence of SEQ ID NO: 409.

[00443] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 410. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 410. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 410. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 410. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 410. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 410. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 410. According to some embodiments, the promoter comprises, or consists of the nucleic acid sequence of SEQ ID NO: 410.

[00444] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 617. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 617. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 617. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 617. According to some embodiments, the promoter comprises a

nucleic acid sequence at least about 97% identical to SEQ ID NO: 617. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 617. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 617. According to some embodiments, the promoter comprises, or consists of the nucleic acid sequence of SEQ ID NO: 617.

[00445] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 618. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 618. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 618. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 618. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 618. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 618. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 618. According to some embodiments, the promoter comprises, or consists of the nucleic acid sequence of SEQ ID NO: 618.

[00446] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 619. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 619. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 619. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 619. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 619. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 619. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 619. According to some embodiments, the promoter comprises, or consists of the nucleic acid sequence of SEQ ID NO: 619.

[00447] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 620. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 620. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 620. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 620. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 620. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 620. According to some embodiments, the promoter comprises a nucleic acid sequence at least

about 99% identical to SEQ ID NO: 620. According to some embodiments, the promoter comprises, or consists of the nucleic acid sequence of SEQ ID NO: 620.

[00448] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 621. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 621. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 621. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 621. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 621. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 621. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 621. According to some embodiments, the promoter comprises, or consists of the nucleic acid sequence of SEQ ID NO: 621.

[00449] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 622. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 622. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 622. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 622. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 622. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 622. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 622. According to some embodiments, the promoter comprises, or consists of the nucleic acid sequence of SEQ ID NO: 622.

[00450] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 623. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 623. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 623. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 623. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 623. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 623. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 623. According to some embodiments, the promoter comprises, or consists of the nucleic acid sequence of SEQ ID NO: 623.

[00451] According to some embodiments, the promoter comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 624. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 624. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 624. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 624. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 624. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 624. According to some embodiments, the promoter comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 624. According to some embodiments, the promoter comprises, or consists of the nucleic acid sequence of SEQ ID NO: 624.

(iii) 5' UTR sequences and intron sequences

[00452] In some embodiments, a ceDNA vector comprises a 5' UTR sequence and/or an intron sequence that located 3' of the 5' ITR sequence. In some embodiments, the 5' UTR is located 5' of the transgene, *e.g.,* sequence encoding the FVIII protein. According to some embodiments, the 5' UTR sequence is selected from those listed in **Table 10** below and in International Application No. PCT/US2020/021328, for example in Table 9A, incorporated by reference in its entirety herein.

Table 10. 5' UTR

GE ##	Name	Description	SEQ ID NO.
GE-1124	TTR-MVM-PmeI-Consensus-5pUTR	5pUTR formed form concatenation of 1) the Transthyretin promoter 5pUTR, 2) Minute Virus of Mouse Intron, 3) PmeI restriction site, and 4) consensus kozak sequence	411
GE-1125	TTR-MVM_v2-PmeI-Consensus-5pUTR	5pUTR formed form concatenation of 1) the Transthyretin promoter 5pUTR, 2) Minute Virus of Mouse Intron_v2, 3) PmeI restriction site, and 4) consensus kozak sequence	412
GE-1126	TTR-MVM-PmeI*-Consensus-5pUTR	5pUTR formed form concatenation of 1) the Transthyretin promoter 5pUTR, 2) Minute Virus of Mouse Intron, 3) Mutated PmeI restriction site, and 4) consensus kozak sequence	413
GE-1138	hAAT-5pUTR_v2	5pUTR region derived from SERPINA1 (A1AT) gene	414
GE-1167	TTR-MVMspliced-PmeI-Consensus-5pUTR	5pUTR formed form concatenation of 1) the Transthyretin promoter 5pUTR, 2) Spliced form of Minute Virus of Mouse Intron, 3) PmeI restriction site, and 4) consensus kozak sequence	415
GE-772	5pUTR-325243	5pUTR variable region #325243	416

GE-774	5pUTR-constant	5pUTR constant region	417
GE-1208	hAAT-SV40-PmeI-Mod-5pUTR	5pUTR formed form concatenation of 1) the hAAT promoter 5pUTR, 2) SV40 intron, 3) PmeI restriction site, and 4) modified kozak sequence	418
GE-1209	hAAT-SV40-PmeI-Mod2-5pUTR	5pUTR formed form concatenation of 1) the hAAT promoter 5pUTR, 2) SV40 intron, 3) PmeI restriction site, and 4) modified kozak sequence v2	419
GE-1210	hAAT-SV40-PmeI-Con-5pUTR	5pUTR formed form concatenation of 1) the hAAT promoter 5pUTR, 2) SV40 intron, 3) PmeI restriction site, and 4) consensus kozak sequence	420
GE-1211	hAAT-SV40-PmeI-325243-5pUTR	5pUTR formed form concatenation of 1) the hAAT promoter 5pUTR, 2) SV40 intron, 3) PmeI restriction site, and 4) 325243-5pUTR	421
GE-1212	hAAT-SV40-PmeI-536-5pUTR	5pUTR formed form concatenation of 1) the hAAT promoter 5pUTR, 2) SV40 intron, 3) PmeI restriction site, and 4) 536-kozak	422
GE-1219	TTR-Xbal-MVM-PmeI-Consensus-5pUTR	5pUTR formed form concatenation of 1) the Transthyretin promoter 5pUTR, 2) XbaI restriction site, 3)Minute Virus of Mouse Intron, 4) PmeI restriction site, and 5) consensus kozak sequence	423
GE-1220	TTR-XbaI-MVM_v2-PmeI-Consensus-5pUTR	5pUTR formed form concatenation of 1) the Transthyretin promoter 5pUTR, 2) XbaI restriction site, 3) Minute Virus of Mouse Intron_v2, 4) PmeI restriction site, and 5) consensus kozak seqeunce	424
GE-1221	TTR-XbaI-MVM-PmeI*-Consensus-5pUTR	5pUTR formed form concatenation of 1) the Transthyretin promoter 5pUTR, 2) XbaI restriction site, 3) Minute Virus of Mouse Intron, 4) Mutated PmeI restriction site, and 5) consensus kozak sequence	425
GE-1122	TTR-5pUTR	5pUTR from mouse Transthyretin gene	426
GE-1260	hAAT-PmeI-Mod2-5pUTR	5pUTR formed by concatenation of 1) the hAAT promoter 5pUTR, 3) PmeI restriction site, and 4) modified kozak sequence v2	427
GE-1261	TTR-MVM_v2-PmeI-Mod2-5pUTR	5pUTR formed by concatenation of 1) the Transthyretin promoter 5pUTR, 2) Minute Virus of Mouse Intron_v2, 3) PmeI restriction site, and 4) Mod_Minimum_Consensus_Kozak_v2	428
GE-1262	TTR-MVM-PmeI-325243-5pUTR Copy	5pUTR formed by concatenation of 1) the Transthyretin promoter 5pUTR, 2) Minute Virus of Mouse Intron, 3) PmeI restriction site, and 4) 325243-5pUTR	429
GE-1263	TTR-MVM-PmeI*-Mod2-5pUTR	5pUTR formed by concatenation of 1) the Transthyretin promoter 5pUTR, 2) Minute Virus of Mouse Intron, 3) Mutated PmeI	430

		restriction site, and 4) Mod_Minimum_Consensus_Kozak_v2	
GE-1264	TTR-MVM-PmeI-Mod2-5pUTR	5pUTR formed by concatenation of 1) the Transthyretin promoter 5pUTR, 2) Minute Virus of Mouse Intron, 3) PmeI restriction site, and 4) Mod_Minimum_Consensus_Kozak_v2	431
GE-1265	TTR-MVMspliced-PmeI-Mod2-5pUTR	5pUTR formed by concatenation of 1) the Transthyretin promoter 5pUTR, 2) Spliced form of Minute Virus of Mouse Intron, 3) PmeI restriction site, and 4) Mod_Minimum_Consensus_Kozak_v2	432
GE-1266	TTR-XbaI-MVM_v2-PmeI-Mod2-5pUTR	5pUTR formed by concatenation of 1) the Transthyretin promoter 5pUTR, 2) XbaI restriction site, 3) Minute Virus of Mouse Intron_v2, 4) PmeI restriction site, and 5) Mod_Minimum_Consensus_Kozak_v2	433
GE-1267	TTR-XbaI-MVM-PmeI*-Mod2-5pUTR	5pUTR formed by concatenation of 1) the Transthyretin promoter 5pUTR, 2) XbaI restriction site, 3) Minute Virus of Mouse Intron, 4) Mutated PmeI restriction site, and 5) Mod_Minimum_Consensus_Kozak_v2	434
GE-1268	TTR-XbaI-MVM-PmeI-Mod2-5pUTR	5pUTR formed by concatenation of 1) the Transthyretin promoter 5pUTR, 2) XbaI restriction site, 3) Minute Virus of Mouse Intron, 4) PmeI restriction site, and 5) Mod_Minimum_Consensus_Kozak_v2	435
GE-1269	hAAT-PmeI-Con-5pUTR	5pUTR formed by concatenation of 1) the hAAT promoter 5pUTR, 3) PmeI restriction site, and 4) Consensus Kozak Sequence	436

[00453] According to some embodiments, the 5'-UTR sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of any one of the sequences set forth as SEQ ID NOS: 411-436.

[00454] According to some embodiment, a ceDNA vector comprises an intron sequence that is located 3' of the 5' ITR sequence. According to some embodiment, a ceDNA vector comprises an intron sequence that is located within the ORF of FVIII, inbetween two exons. According to some embodiments, the intron sequence is selected from those listed in **Table 11** below, which provides the sequence identifier and a description of the intron.

Table 11. Introns

SEQ ID NO	Description
235	Intron from Minute Virus of Mouse (MVM)
236	Intron from Minute Virus of Mouse with additional 'G' residue included in Splice Acceptor flanking sequence
237	Modified intron from SV40 virus
238	mini Factor VIII intron 1 chimera, 50 nucleotides from 5'-end of intron, 100 nucleotides from 3'-end of intron

239	mini Factor VIII intron 1 chimera, 50 nucleotides from 5'-end of intron, 200 nucleotides from 3'-end of intron
240	mini Factor VIII intron 1 chimera, 200 nucleotides from 5'-end of intron, 200 nucleotides from 3'-end of intron
241	mini Factor VIII intron 1 chimera, 500 nucleotides from 5'-end of intron, 500 nucleotides from 3'-end of intron
242	human beta globin intron 1
243	Human Factor VIII intron 8
244	Human Factor VIII intron 16
245	first (5') 200 bps of Factor VIII intron 1, used for annotation of embedded enhancers
246	last (3') 200 bps of Factor VIII intron 1, used for annotation of embedded enhancers
247	Predicted Sequence of MVM intron (GE-023) post splicing
248	Intron from Minute Virus of Mouse with flanking exon regions removed

[00455] According to some embodiments, the intron sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 235. According to some embodiments, the intron sequence comprises, or consists of SEQ ID NO: 235. According to some embodiments, the intron sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 236. According to some embodiments, the intron sequence comprises, or consists of SEQ ID NO: 236. According to some embodiments, the intron sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 237. According to some embodiments, the intron sequence comprises, or consists of SEQ ID NO: 237. According to some embodiments, the intron sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 238. According to some embodiments, the intron sequence comprises, or consists of SEQ ID NO: 238. According to some embodiments, the intron sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 239. According to some embodiments, the intron sequence comprises, or consists of SEQ ID NO: 239. According to some embodiments, the intron sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 240. According to some embodiments, the intron sequence comprises, or consists of SEQ ID NO: 240. According to some embodiments, the intron sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 241. According to some embodiments, the intron sequence comprises, or consists of SEQ ID NO: 241. According to some embodiments, the intron sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 242. According to some embodiments, the intron sequence comprises, or consists of SEQ ID NO: 242. According to some embodiments, the intron sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 243. According to some embodiments, the intron sequence comprises, or consists of SEQ ID NO: 243. According to some

embodiments, the intron sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 244. According to some embodiments, the intron sequence consists of SEQ ID NO: 244. According to some embodiments, the intron sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 245. According to some embodiments, the intron sequence comprises, or consists of SEQ ID NO: 245. According to some embodiments, the intron sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 246. According to some embodiments, the intron sequence comprises, or consists of SEQ ID NO: 246. According to some embodiments, the intron sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 247. According to some embodiments, the intron sequence comprises, or consists of SEQ ID NO: 247. According to some embodiments, the intron sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 248. According to some embodiments, the intron sequence comprises, or consists of SEQ ID NO: 248.

(iv) Exon sequences

[00456] According to some embodiment, a ceDNA vector comprises an exon sequence. According to some embodiments, the exon sequence is selected from those listed in **Table 12** below, which provides the sequence identifier and a description of the exon.

Table 12.

SEQ ID NO	Description
293	Exon1 from FVIII ORF hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD with 33bp of WT ORF sequence upstream of the splice donor site of intron1
294	Exon2-26 from FVIII ORF hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD with 33bp of WT ORF sequence downstream of the splice acceptor site of intron1
295	Exon1 from FVIII ORF _hFVIII-F309S-BD226seq124-Afstyla-BDD with 33bp of WT ORF sequence upstream of the splice donor site of intron1
296	Exon2-26 from FVIII ORF _hFVIII-F309S-BD226seq124-Afstyla-BDD with 33bp of WT ORF sequence downstream of the splice acceptor site of intron1
297	exon1 from FVIII ORF: hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD
298	exon2-26 from FVIII ORF: hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD
299	exon1 from FVIII ORF: hFVIII-F309S-BD226seq124-Afstyla-BDD
300	exon2-26 from FVIII ORF: _hFVIII-F309S-BD226seq124-Afstyla-BDD
301	33bp of WT hFVIII ORF sequence downstream of the splice acceptor site of intron1
302	33bp of WT hFVIII ORF sequence upstream of the splice donor site of intron1

[00457] According to some embodiments, the exon sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 293. According to some embodiments, the exon sequence comprises, or consists of SEQ ID NO: 293. According to some embodiments, the exon sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%,

96%, 97%, 98%, 99% identical to SEQ ID NO: 294. According to some embodiments, the exon sequence comprises, or consists of SEQ ID NO: 294. According to some embodiments, the exon sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 295. According to some embodiments, the exon sequence comprises, or consists of SEQ ID NO: 295. According to some embodiments, the exon sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 296. According to some embodiments, the exon sequence comprises, or consists of SEQ ID NO: 296. According to some embodiments, the exon sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 297. According to some embodiments, the exon sequence comprises, or consists of SEQ ID NO: 297. According to some embodiments, the exon sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 298. According to some embodiments, the exon sequence comprises, or consists of SEQ ID NO: 298. According to some embodiments, the exon sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 299. According to some embodiments, the exon sequence comprises, or consists of SEQ ID NO: 299. According to some embodiments, the exon sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 300. According to some embodiments, the exon sequence comprises, or consists of SEQ ID NO: 300. According to some embodiments, the exon sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 301. According to some embodiments, the exon sequence comprises, or consists of SEQ ID NO: 301. According to some embodiments, the exon sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 302. According to some embodiments, the exon sequence comprises, or consists of SEQ ID NO: 302.

(v) 3' UTR sequences

[00458] In some embodiments, a ceDNA vector comprises a 3' UTR sequence that located 5' of the 3' ITR sequence. In some embodiments, the 3' UTR is located 3' of the transgene, *e.g.,* sequence encoding the FVIII protein. According to some embodiments, the 3' UTR sequence is selected from those listed in **Table 13** below, which provides the sequence identifier and a description of the 3' UTR.

Table 13.

SEQ ID NO	Description (name)
283	Poly A signal derived from gene encoding bovine growth hormone (bGH)
284	Postranscriptional regularoty element derived from Woodchuck Hepatitis Virus (WPRE_3pUTR)
285	PolyA region from SV40 virus (SV40_polyA)

286	Derived from Human hemoglobin beta (HBB) gene 3pUTR (HBB_3pUTR)
287	Derived from Human hemoglobin beta (HBB) gene 3pUTR (HBBv3_3pUTR)
288	Derived from Human hemoglobin beta (HBB) gene 3pUTR (HBBv2_3pUTR)
289	Derived from Human hemoglobin beta (HBB) gene 3pUTR (HBBv3_CpGmin)
290	Derived from Human hemoglobin beta (HBB) gene 3pUTR (HBBv2_CpGmin)
291	Derived from Human hemoglobin beta (HBB) gene 3pUTR (HBB-3pUTR-CpGmin_v1)
634	Shortened WPRE3 sequence with minimal gamma and alpha element (ref: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3975461/), modified to remove ATG's generating cryptic ORFs > 25 aa (WPRE_3pUTR_v3-ATG)

WPRE_3pUTR_v3-ATG

GTTAATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGATATTCTTAACTATGTTG CTCCTTTTACGCTGTGTGGATACGCTGCTTTATAGCCTCTGTATCTAGCTATTGCTTCCCGT ACGGCTTTCGTTTTCTCCTCCTTGTATAAATCCTGGTTAGTTCTTGCCACGGCGGAACTCAT CGCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGCTGTTGGGCACTGACAATTCCGT GG (SEQ ID NO: 634)

[00459] According to some embodiments, the 3' UTR sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 283. According to some embodiments, the 3' UTR sequence comprises, or consists of SEQ ID NO: 283. According to some embodiments, the 3' UTR sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 284. According to some embodiments, the 3' UTR sequence comprises, or consists of SEQ ID NO: 284. According to some embodiments, the 3' UTR sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 285. According to some embodiments, the 3' UTR sequence comprises, or consists of SEQ ID NO: 285. According to some embodiments, the 3' UTR sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 286. According to some embodiments, the 3' UTR sequence comprises, or consists of SEQ ID NO: 286. According to some embodiments, the 3' UTR sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 287. According to some embodiments, the 3' UTR sequence comprises, or consists of SEQ ID NO: 287. According to some embodiments, the 3' UTR sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 288. According to some embodiments, the 3' UTR sequence comprises, or consists of SEQ ID NO: 288. According to some embodiments, the 3' UTR sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 289. According to some embodiments, the 3' UTR sequence comprises, or consists of SEQ ID NO: 289. According to some embodiments, the 3' UTR sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 290. According to some embodiments, the 3' UTR sequence comprises, or consists of SEQ ID NO:

290. According to some embodiments, the 3' UTR sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 291. According to some embodiments, the 3' UTR sequence comprises, or consists of SEQ ID NO: 291. According to some embodiments, the 3' UTR sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 634. According to some embodiments, the 3' UTR sequence comprises, or consists of SEQ ID NO: 634.

(v) Polyadenylation Sequences:

[00460] A sequence encoding a polyadenylation sequence can be included in the ceDNA vector for expression of FVIII protein to stabilize an mRNA expressed from the ceDNA vector, and to aid in nuclear export and translation. In one embodiment, the ceDNA vector does not include a polyadenylation sequence. In other embodiments, the ceDNA vector for expression of FVIII protein includes at least 1, at least 2, at least 3, at least 4, at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, at least 40, least 45, at least 50 or more adenine dinucleotides. In some embodiments, the polyadenylation sequence comprises about 43 nucleotides, about 40-50 nucleotides, about 40-55 nucleotides, about 45-50 nucleotides, about 35-50 nucleotides, or any range there between.
The expression cassettes can include any poly-adenylation sequence known in the art or a variation thereof. In some embodiments, a poly-adenylation (polyA) sequence is selected from any of those listed in International Application No. PCT/US2020/021328, for example in Table 10, incorporated by reference in its entirety herein. Other polyA sequences commonly known in the art can also be used, *e.g.,* including but not limited to, naturally occurring sequence isolated from bovine BGHpA (*e.g.,* SEQ ID NO: 68) or a virus SV40pA (*e.g.,* SEQ ID NO: 86), or a synthetic sequence (*e.g.,* SEQ ID NO: 87). Some expression cassettes can also include SV40 late polyA signal upstream enhancer (USE) sequence. In some embodiments, a USE sequence can be used in combination with SV40pA or heterologous poly-A signal. PolyA sequences are located 3' of the transgene encoding the FVIII protein. The expression cassettes can also include a post-transcriptional element to increase the expression of a transgene. In some embodiments, Woodchuck Hepatitis Virus (WHP) posttranscriptional regulatory element (WPRE) (*e.g.,* SEQ ID NO: 67) is used to increase the expression of a transgene. Other posttranscriptional processing elements such as the post-transcriptional element from the thymidine kinase gene of herpes simplex virus, or hepatitis B virus (HBV) can be used. Secretory sequences can be linked to the transgenes, *e.g.,* VH-02 and VK-A26 sequences, *e.g.,* SEQ ID NO: 88 and SEQ ID NO: 89.

(vi) DNA Nuclear Targeting Sequences (DTS)

In some embodiments, the ceDNA vector for expression of FVIII protein comprises one or more DNA nuclear targeting sequences (DTS), for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more DTSs. In some embodiments, the one or more DTSs are located at or near the amino-terminus, at or near the carboxy-terminus, or a combination of these (*e.g.,* one or more NLS at the amino-terminus and/or one or more

NLS at the carboxy terminus). When more than one DTS is present, each can be selected independently of the others, such that a single DTS is present in more than one copy and/or in combination with one or more other DTSs present in one or more copies. According to some embodiments, the DTS is selected from those listed in **Table 14** below, which provides the sequence identifier, a description of the DTS, and name.

Table 14.

SEQ ID NO	Description	Name
303	"nuclear factor kappa B (NFκB) transcription factor binding site triplet, comprising three 10-bp κB sites (GGGACTTTCC (SEQ ID NO: 546)) separated by a 5-bp optimized spacer (AGCTG)"	3NF_DTS
304	CpG-minimized spacer optimized for priming in PCR	3'DTS_primer_pad
305	CpG-minimized spacer optimized for priming in PCR	5'DTS_primer_pad
306	5X repeat of Igk kB motif 5'-GGGGACTTTCC-3' (SEQ ID NO: 548), 3 bp spacer, as described by Mesika *et al.*, 2001 Mol Ther	5x_kB_mesika_DTS
307	2X repeat of glucocorticoid response element (GRE; origin not described), SalI restriction site as spacer, as described by Dames *et al.*, 2007 J Gene Med	2x_GRE_dames_DTS
308	Single CREB binding site as described by Badding *et al.*, 2012 Gene Ther	CREB_badding_DTS
309	5x 72bp tandem repeat from SV40 genome separated by random CpG free 20 mer sequences.	SV40DNA_DTS_10merRepeat
310	High activity, high affinity GRE binding site	2x_Cgt_GRE_meijsing_DTS
311	72 base pair single repeat region from SV40 genome.	SV40DNA_DTS_72bpSingleRepeat
312	72 base pair tandem repeat region from SV40 genome.	SV40DNA_DTS_72bpTandemRepeat
313	5x Dual SV40 Enhancer elements separated by CpGfree spacer elements	10xSV40-DTS-arrray

B. Additional Components of ceDNA vectors

[00461] The ceDNA vectors for expression of FVIII protein of the present disclosure may contain nucleotides that encode other components for gene expression.

(i) Ubiquitous Chromatin-opening Elements (UCOEs)

[00462] According to some embodiments, the ceDNA vectors may further comprise Ubiquitous Chromatin-opening Elements (UCOEs), which structurally consist of methylation-free CpG islands encompassing single or dual divergently-transcribed housekeeping gene promoters, and are defined by their ability to consistently confer stable, site of integration-independent transgene expression that is proportional to copy number (Neville *et al.*, Volume 35, Issue 5, September 2017, Pages 557-56).

[00463] According to some embodiments, the ceDNA vector for expression of FVIII protein comprises a minimal UCOE derived from CBX3 intergentic region, which comprises mutations to

eliminate splice sites in the CBX3 intron region (CBX3(674mut1). According to some emboidments, the minimal UCOE comprises, or consists of, SEQ ID NO: 292.

[00464] According to some embodiments, the UCOE comprises a nucleic acid sequence at least about 85% identical to SEQ ID NO: 292. According to some embodiments, the UCOE comprises a nucleic acid sequence at least about 90% identical to SEQ ID NO: 292. According to some embodiments, the UCOE comprises a nucleic acid sequence at least about 95% identical to SEQ ID NO: 292. According to some embodiments, the UCOE comprises a nucleic acid sequence at least about 96% identical to SEQ ID NO: 292. According to some embodiments, the UCOE comprises a nucleic acid sequence at least about 97% identical to SEQ ID NO: 292. According to some embodiments, the UCOE comprises a nucleic acid sequence at least about 98% identical to SEQ ID NO: 292. According to some embodiments, the UCOE comprises a nucleic acid sequence at least about 99% identical to SEQ ID NO: 292. According to some embodiments, the UCOE comprises, or consists of the nucleic acid sequence of SEQ ID NO: 292.

(ii) Kozak Sequences

[00465] According to some embodiments, the ceDNA vectors may further comprise one or more Kozak sequences. According to some embodiments, the Kozak sequence is a consensus Kozak sequence. According to some embodiments, the Kozak sequence is a modified Kozak sequence. According to some embodiments, the Kozak sequence is a minimal Kozak sequence.

[00466] According to some embodiments, the consensus Kozak sequence (Consensus_Kozak) comprises GCCGCCACC (SEQ ID NO: 314). According to some embodiments, the modified consensus Kozak sequence (Mod_Minimum_Consensus_Kozak_v1) comprises AGCCACC (SEQ ID NO: 315). According to some embodiments, the modified consensus Kozak sequence (Mod_Minimum_Consensus_Kozak_v2) comprises CGCAGCCACC (SEQ ID NO: 316). According to some embodiments, the minimal consensus Kozak sequence (536_Kozak) comprises ACC (SEQ ID NO: 317).

(iii) Spacer Sequences

[00467] According to some embodiments, the ceDNA vectors may further comprise one or more spacer sequences. According to some embodiments, the spacer sequence is selected from one or more of those listed in **Table 15** below, which provides the sequence identifier, a description of the spacer sequence and the name.

Table 15. Spacers

SEQ ID NO	Description	Name
318	Synthetic Spacer Sequence	spacer_left-ITR_v1
319	Synthetic Spacer Sequence	spacer_left-ITR_v2.1
320	Synthetic Spacer Sequence	spacer_right-ITR_v1
321	CpG-free 20 bp spacer sequence	CpGfree20mer_1

322	CpG-free 20 bp spacer sequence	CpGfree20mer_2
323	CpG-free 20 bp spacer sequence	CpGfree20mer_3
324	CpG-free 20 bp spacer sequence	CpGfree20mer_4
325	CpG-free 20 bp spacer sequence	CpGfree20mer_5
326	CpG-free 20 bp spacer sequence	CpGfree20mer_6
327	CpG-free 20 bp spacer sequence	CpGfree20mer_6B
328	CpG-free synthetic spacer	Sp100-1
329	CpG-free synthetic spacer	Sp800-1
330	CpG-free synthetic spacer	Sp400-1
331	CpG-free synthetic spacer	Sp200-3
332	CpG-free synthetic spacer	Sp200-2
634	CpG-free Left ITR spacer with SbfI site	Spacer_Left-ITR_v7
635	Left ITR spacer with NotI site	Spacer_Left-ITR_v8
636	CpG-free Left ITR spacer with MfeI site v1	Spacer_Left-ITR_v9
637	CpG-free Left ITR spacer with MfeI site v2	Spacer_Left-ITR_v10
638	CpG-free Left ITR spacer with MfeI site v3	Spacer_Left-ITR_v11
639	CpG-free Right ITR spacer v1	Spacer_Right-ITR_v7
640	CpG-free Right ITR spacer v2	Spacer_Right-ITR_v8

[00468] According to some embodiments, the spacer sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 318. According to some embodiments, the spacer sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 319. According to some embodiments, the spacer sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 320. According to some embodiments, the spacer sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 321. According to some embodiments, the spacer sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 322. According to some embodiments, the spacer sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 323. According to some embodiments, the spacer sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 324. According to some embodiments, the spacer sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 325. According to some embodiments, the spacer sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 326. According to some embodiments, the spacer sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 327.

According to some embodiments, the spacer sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 328. According to some embodiments, the spacer sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 329. According to some embodiments, the spacer sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 330. According to some embodiments, the spacer sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 331. According to some embodiments, the spacer sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 332. According to some embodiments, the spacer sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 634. According to some embodiments, the spacer sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 635. According to some embodiments, the spacer sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 636. According to some embodiments, the spacer sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 637. According to some embodiments, the spacer sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 638. According to some embodiments, the spacer sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 639. According to some embodiments, the spacer sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 640.

(iv) Leader Sequences

[00469] According to some embodiments, the ceDNA vectors may further comprise one or more leader sequences. According to some embodiments, the leader sequence is selected from one or more of those listed in **Table 16** below, which provides the sequence identifier, a description of the leader sequence and the name.

Table 16. Leader Sequences

SEQ ID NO	Description	Name
249	Albumin leader sequence codon optimization #1	ALB-NS-CAI-v2
250	Albumin leader sequence codon optimization #2	ALB-NS-struct
251	Albumin leader sequence codon optimization #3	ALB-SSv1
252	CD33 leader sequence codon optimization #1	CD33-NS-CAI-v2
253	CD33 leader sequence codon optimization #2	CD33-NS-struct
254	CD33 leader sequence codon optimization #3	CD33-SSv1

255	Chymotrypsinogen leader sequence codon optimization #1	CHY-NS-CAI-v2
256	Chymotrypsinogen leader sequence codon optimization #2	CHY-NS-struct
257	Chymotrypsinogen leader sequence codon optimization #3	CHY-SSv1
258	Gaussia leader sequence codon optimization #1	Gaus-CAI-v2
259	Gaussia leader sequence codon optimization #2	Gaus-NS-struct-v2
260	Gaussia leader sequence codon optimization #3	Gaus-SSv1
261	IL-2 leader sequence codon optimization #1	IL2-NS-CAI
262	IL-2 leader sequence codon optimization #2	IL2-NS-struct
263	IL-2 leader sequence codon optimization #3	IL2-SSv1
264	Fibroin-L leader sequence codon optimization #1	Lonz-NS-CAI-v1
265	Fibroin-L leader sequence codon optimization #2	Lonz-NS-struct-v2
266	Fibroin-L leader sequence codon optimization #3	Lonz-SSv1
267	Secrecon leader sequence v1 codon optimization #1	Secrecon-v1-NS-CAI-v2
268	Secrecon leader sequence v1 codon optimization #2	Secrecon-v1-NS-struct
269	Secrecon leader sequence v1 codon optimization #3	Secrecon-SSv1
270	Secrecon leader sequence v2 codon optimization #3	Secrecon-SSv2
271	trans plasminogen activator leader sequence codon optimization #1	tPA-NS-CAI-v2
272	trans plasminogen activator leader sequence codon optimization #2	tPA-NS-struct
273	trans plasminogen activator leader sequence codon optimization #3	tPA-SSv1
274	Trypsinogen leader sequence codon optimization #1	TRYP-NS-CAI-v2
275	Trypsinogen leader sequence codon optimization #2	TRYP-NS-struct-v2
276	Trypsinogen leader sequence codon optimization #3	TRYP-SSv2
277	Fibroin-L leader sequence codon optimization #1 truncated to remove terminal 'QV' residues	LonzB-NS-CAI-v1
278	Fibroin-L leader sequence codon optimization #2 truncated to remove terminal 'QV' residues	LonzB-NS-struct-v2
279	Fibroin-L leader sequence codon optimization #3 truncated to remove terminal 'QV' residues	LonzB-SSv1
280	A1AT leader sequence codon optimization #1	A1AT-NS-CAI-v2
281	A1AT leader sequence codon optimization #2	A1AT-NS-struct
282	A1AT leader sequence codon optimization #3	A1AT-SSv3

[00470] According to some embodiments, the leader sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 249. According to some embodiments, the leader sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 250. According to some embodiments, the leader sequence comprises a nucleic acid sequence at least about

85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 251. According to some embodiments, the leader sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 252. According to some embodiments, the leader sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 253. According to some embodiments, the leader sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 254. According to some embodiments, the leader sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 255. According to some embodiments, the leader sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 256. According to some embodiments, the leader sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 257. According to some embodiments, the leader sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 258. According to some embodiments, the leader sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 259. According to some embodiments, the leader sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 260. According to some embodiments, the leader sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 261. According to some embodiments, the leader sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 262. According to some embodiments, the leader sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 263. According to some embodiments, the leader sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 264. According to some embodiments, the leader sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 265. According to some embodiments, the leader sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 266. According to some embodiments, the leader sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 267. According to some embodiments, the leader sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 268. According to some embodiments, the leader sequence comprises a nucleic acid sequence at least about

85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 269. According to some embodiments, the leader sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 270. According to some embodiments, the leader sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 271. According to some embodiments, the leader sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 272. According to some embodiments, the leader sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 273. According to some embodiments, the leader sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 274. According to some embodiments, the leader sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 275. According to some embodiments, the leader sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 276. According to some embodiments, the leader sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 277. According to some embodiments, the leader sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 278. According to some embodiments, the leader sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 279. According to some embodiments, the leader sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 280. According to some embodiments, the leader sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 281. According to some embodiments, the leader sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 282. According to some embodiments, the leader sequence comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 283.

[00471] In some embodiments, the ceDNA vector for expression of FVIII protein may comprise one or more micro RNA (MIR) sequences involved in immune responses or hepato-homestasis as shown in **Table 17** below.

[00472] **Table 17**. MIR Sequences

GE#	Name	SEQ ID NO	Description
GE-699	mir122_4x	542	micro-RNA involved in regulation of immune reponses

GE-020	mir-142_3pUTR	543	micro-RNA involved in liver homeostasis; Triplet repeat of mir-142 binding site

[00473] According to some embodiments, to select for specific gene targeting events, a protective shRNA may be embedded in a microRNA and inserted into a recombinant ceDNA vector designed to integrate site-specifically into the highly active locus, such as an albumin locus. Such embodiments may provide a system for *in vivo* selection and expansion of gene-modified hepatocytes in any genetic background such as described in Nygaard *et al.*, *A universal system to select gene-modified hepatocytes in vivo, Gene Therapy*, June 8, 2016. The ceDNA vectors of the present disclosure may contain one or more selectable markers that permit selection of transformed, transfected, transduced, or the like cells. A selectable marker is a gene the product of which provides for biocide or viral resistance, resistance to heavy metals, prototrophy to auxotrophs, NeoR, and the like. In certain embodiments, positive selection markers are incorporated into the donor sequences such as NeoR. Negative selections markers may be incorporated downstream the donor sequences, for example a nucleic acid sequence HSV-tk encoding a negative selection marker may be incorporated into a nucleic acid construct downstream the donor sequence.

C. Regulatory Switches

[00474] A molecular regulatory switch is one which generates a measurable change in state in response to a signal. Such regulatory switches can be usefully combined with the ceDNA vectors for expression of FVIII protein as described herein to control the output of expression of FVIII protein from the ceDNA vector. In some embodiments, the ceDNA vector for expression of FVIII protein comprises a regulatory switch that serves to fine tune expression of the FVIII protein. For example, it can serve as a biocontainment function of the ceDNA vector. In some embodiments, the switch is an "ON/OFF" switch that is designed to start or stop (*i.e.,* shut down) expression of FVIII protein in the ceDNA vector in a controllable and regulatable fashion. In some embodiments, the switch can include a "kill switch" that can instruct the cell comprising the ceDNA vector to undergo cell programmed death once the switch is activated. Exemplary regulatory switches encompassed for use in a ceDNA vector for expression of FVIII protein can be used to regulate the expression of a transgene, and are more fully discussed in International application PCT/US18/49996, which is incorporated herein in its entirety by reference

(i) Binary Regulatory Switches

[00475] In some embodiments, the ceDNA vector for expression of FVIII protein comprises a regulatory switch that can serve to controllably modulate expression of FVIII protein. For example, the expression cassette located between the ITRs of the ceDNA vector may additionally comprise a regulatory region, *e.g.,* a promoter, cis-element, repressor, enhancer etc., that is operatively linked to the nucleic acid sequence encoding FVIII protein, where the regulatory region is regulated by one or more cofactors or exogenous agents. By way of example only, regulatory regions can be modulated by

small molecule switches or inducible or repressible promoters. Non-limiting examples of inducible promoters are hormone-inducible or metal-inducible promoters. Other exemplary inducible promoters/enhancer elements include, but are not limited to, an RU486-inducible promoter, an ecdysone-inducible promoter, a rapamycin-inducible promoter, and a metallothionein promoter.

(ii) Small molecule Regulatory Switches

[00476] A variety of art-known small-molecule based regulatory switches are known in the art and can be combined with the ceDNA vectors for expression of FVIII protein as disclosed herein to form a regulatory-switch controlled ceDNA vector. In some embodiments, the regulatory switch can be selected from any one or a combination of: an orthogonal ligand/nuclear receptor pair, for example retinoid receptor variant/LG335 and GRQCIMFI, along with an artificial promoter controlling expression of the operatively linked transgene, such as that as disclosed in Taylor, *et al.* BMC Biotechnology 10 (2010): 15; engineered steroid receptors, *e.g.,* modified progesterone receptor with a C-terminal truncation that cannot bind progesterone but binds RU486 (mifepristone) (US Patent No. 5,364,791); an ecdysone receptor from *Drosophila* and their ecdysteroid ligands (Saez, *et al.*, PNAS, 97(26)(2000), 14512–14517; or a switch controlled by the antibiotic trimethoprim (TMP), as disclosed in Sando R 3rd; Nat Methods. 2013, 10(11):1085-8. In some embodiments, the regulatory switch to control the transgene or expressed by the ceDNA vector is a pro-drug activation switch, such as that disclosed in US patents 8,771,679, and 6,339,070, the contents of all of which are incorporated by reference in their entireties herein.

(iii) "Passcode" Regulatory Switches

[00477] In some embodiments the regulatory switch can be a "passcode switch" or "passcode circuit". Passcode switches allow fine tuning of the control of the expression of the transgene from the ceDNA vector when specific conditions occur – that is, a combination of conditions need to be present for transgene expression and/or repression to occur. For example, for expression of a transgene to occur at least conditions A and B must occur. A passcode regulatory switch can be any number of conditions, *e.g.,* at least 2, or at least 3, or at least 4, or at least 5, or at least 6 or at least 7 or more conditions to be present for transgene expression to occur. In some embodiments, at least 2 conditions (*e.g.,* A, B conditions) need to occur, and in some embodiments, at least 3 conditions need to occur (*e.g.,* A, B and C, or A, B and D). By way of an example only, for gene expression from a ceDNA to occur that has a passcode "ABC" regulatory switch, conditions A, B and C must be present. Conditions A, B and C could be as follows; condition A is the presence of a condition or disease, condition B is a hormonal response, and condition C is a response to the transgene expression. For example, if the transgene edits a defective EPO gene, Condition A is the presence of Chronic Kidney Disease (CKD), Condition B occurs if the subject has hypoxic conditions in the kidney, Condition C is that Erythropoietin-producing cells (EPC) recruitment in the kidney is impaired; or alternatively, HIF-

2 activation is impaired. Once the oxygen levels increase or the desired level of EPO is reached, the transgene turns off again until 3 conditions occur, turning it back on.

[00478] In some embodiments, a passcode regulatory switch or "Passcode circuit" encompassed for use in the ceDNA vector comprises hybrid transcription factors (TFs) to expand the range and complexity of environmental signals used to define biocontainment conditions. As opposed to a deadman switch which triggers cell death in the presence of a predetermined condition, the "passcode circuit" allows cell survival or transgene expression in the presence of a particular "passcode", and can be easily reprogrammed to allow transgene expression and/or cell survival only when the predetermined environmental condition or passcode is present.

[00479] Any and all combinations of regulatory switches disclosed herein, *e.g.,* small molecule switches, nucleic acid-based switches, small molecule-nucleic acid hybrid switches, post-transcriptional transgene regulation switches, post-translational regulation, radiation-controlled switches, hypoxia-mediated switches and other regulatory switches known by persons of ordinary skill in the art as disclosed herein can be used in a passcode regulatory switch as disclosed herein. Regulatory switches encompassed for use are also discussed in the review article Kis *et al.*, J R Soc Interface. 12: 20141000 (2015), and summarized in Table 1 of Kis, the contents of which are incorporated by reference in its entirety herein. In some embodiments, a regulatory switch for use in a passcode system can be selected from any or a combination of the switches disclosed in Table 11 of International Patent Application PCT/US18/49996, which is incorporated herein in its entirety by reference.

(iv) Nucleic acid-based regulatory switches to control transgene expression

[00480] In some embodiments, the regulatory switch to control the expression of FVIII protein by the ceDNA is based on a nucleic acid based control mechanism. Exemplary nucleic acid control mechanisms are known in the art and are envisioned for use. For example, such mechanisms include riboswitches, such as those disclosed in, *e.g.,* US2009/0305253, US2008/0269258, US2017/0204477, WO2018026762A1, US patent 9,222,093 and EP application EP288071, and disclosed in the review by Villa JK *et al.*, Microbiol Spectr. 2018 May;6(3). Also included are metabolite-responsive transcription biosensors, such as those disclosed in WO2018/075486 and WO2017/147585. Other art-known mechanisms envisioned for use include silencing of the transgene with an siRNA or RNAi molecule (*e.g.,* miR, shRNA). For example, the ceDNA vector can comprise a regulatory switch that encodes a RNAi molecule that is complementary to the two part of the transgene expressed by the ceDNA vector. When such RNAi is expressed even if the transgene (*e.g.,* FVIII protein) is expressed by the ceDNA vector, it will be silenced by the complementary RNAi molecule, and when the RNAi is not expressed when the transgene is expressed by the ceDNA vector the transgene (*e.g.,* FVIII protein) is not silenced by the RNAi.

[00481] In some embodiments, the regulatory switch is a tissue-specific self-inactivating regulatory switch, for example as disclosed in US2002/0022018, whereby the regulatory switch deliberately switches transgene (*e.g.,* FVIII protein) off at a site where transgene expression might otherwise be disadvantageous. In some embodiments, the regulatory switch is a recombinase reversible gene expression system, for example as disclosed in US2014/0127162 and US Patent 8,324,436.

(v) Post-transcriptional and post-translational regulatory switches.

[00482] In some embodiments, the regulatory switch to control the expression of FVIII protein by the ceDNA vector is a post-transcriptional modification system. For example, such a regulatory switch can be an aptazyme riboswitch that is sensitive to tetracycline or theophylline, as disclosed in US2018/0119156, GB201107768, WO2001/064956A3, EP Patent 2707487 and Beilstein *et al.*, ACS Synth. Biol., 2015, 4 (5), pp 526–534; Zhong *et al.*, Elife. 2016 Nov 2;5. pii: e18858. In some embodiments, it is envisioned that a person of ordinary skill in the art could encode both the transgene and an inhibitory siRNA which contains a ligand sensitive (OFF-switch) aptamer, the net result being a ligand sensitive ON-switch.

(vi) Other exemplary regulatory switches

[00483] Any known regulatory switch can be used in the ceDNA vector to control the expression of FVIII protein by the ceDNA vector, including those triggered by environmental changes. Additional examples include, but are not limited to; the BOC method of Suzuki *et al.*, Scientific Reports 8; 10051 (2018); genetic code expansion and a non-physiologic amino acid; radiation-controlled or ultra-sound controlled on/off switches (see, *e.g.,* Scott S *et al.*, Gene Ther. 2000 Jul;7(13):1121-5; US patents 5,612,318; 5,571,797; 5,770,581; 5,817,636; and WO1999/025385A1, the contents of each of which is incorporated by reference in its entirety herein). In some embodiments, the regulatory switch is controlled by an implantable system, *e.g.,* as disclosed in US patent 7,840,263; US2007/0190028A1 where gene expression is controlled by one or more forms of energy, including electromagnetic energy, that activates promoters operatively linked to the transgene in the ceDNA vector.

[00484] In some embodiments, a regulatory switch envisioned for use in the ceDNA vector is a hypoxia-mediated or stress-activated switch, *e.g.,* such as those disclosed in WO1999060142A2, US patent 5,834,306; 6,218,179; 6,709,858; US2015/0322410; Greco *et al.*, (2004) Targeted Cancer Therapies 9, S368, as well as FROG, TOAD and NRSE elements and conditionally inducible silence elements, including hypoxia response elements (HREs), inflammatory response elements (IREs) and shear-stress activated elements (SSAEs), *e.g.,* as disclosed in U.S. Patent 9,394,526. Such an embodiment is useful for turning on expression of the transgene from the ceDNA vector after ischemia or in ischemic tissues, and/or tumors.

(vii) Kill Switches

[00485] Other embodiments described herein relate to a ceDNA vector for expression of FVIII protein as described herein comprising a kill switch. A kill switch as disclosed herein enables a cell

comprising the ceDNA vector to be killed or undergo programmed cell death as a means to permanently remove an introduced ceDNA vector from the subject's system. It will be appreciated by one of ordinary skill in the art that use of kill switches in the ceDNA vectors for expression of FVIII protein would be typically coupled with targeting of the ceDNA vector to a limited number of cells that the subject can acceptably lose or to a cell type where apoptosis is desirable (*e.g.,* cancer cells). In all aspects, a "kill switch" as disclosed herein is designed to provide rapid and robust cell killing of the cell comprising the ceDNA vector in the absence of an input survival signal or other specified condition. Stated another way, a kill switch encoded by a ceDNA vector for expression of FVIII protein as described herein can restrict cell survival of a cell comprising a ceDNA vector to an environment defined by specific input signals. Such kill switches serve as a biological biocontainment function should it be desirable to remove the ceDNA vector e expression of FVIII protein in a subject or to ensure that it will not express the encoded FVIII protein.

[00486] Other kill switches known to a person of ordinary skill in the art are encompassed for use in the ceDNA vector for expression of FVIII protein as disclosed herein, *e.g.,* as disclosed in US2010/0175141; US2013/0009799; US2011/0172826; US2013/0109568, as well as kill switches disclosed in Jusiak *et al.*, Reviews in Cell Biology and molecular Medicine; 2014; 1-56; Kobayashi *et al.*, PNAS, 2004; 101; 8419-9; Marchisio *et al.*, Int. Journal of Biochem and Cell Biol., 2011; 43; 310-319; and in Reinshagen *et al.*, Science Translational Medicine, 2018, 11.

[00487] Accordingly, in some embodiments, the ceDNA vector for expression of FVIII protein can comprise a kill switch nucleic acid construct, which comprises the nucleic acid encoding an effector toxin or reporter protein, where the expression of the effector toxin (*e.g.,* a death protein) or reporter protein is controlled by a predetermined condition. For example, a predetermined condition can be the presence of an environmental agent, such as, *e.g.,* an exogenous agent, without which the cell will default to expression of the effector toxin (*e.g.,* a death protein) and be killed. In alternative embodiments, a predetermined condition is the presence of two or more environmental agents, *e.g.,* the cell will only survive when two or more necessary exogenous agents are supplied, and without either of which, the cell comprising the ceDNA vector is killed.

[00488] In some embodiments, the ceDNA vector for expression of FVIII protein is modified to incorporate a kill-switch to destroy the cells comprising the ceDNA vector to effectively terminate the *in vivo* expression of the transgene being expressed by the ceDNA vector (*e.g.,* expression of FVIII protein). Specifically, the ceDNA vector is further genetically engineered to express a switch-protein that is not functional in mammalian cells under normal physiological conditions. Only upon administration of a drug or environmental condition that specifically targets this switch-protein, the cells expressing the switch-protein will be destroyed thereby terminating the expression of the therapeutic protein or peptide. For instance, it was reported that cells expressing HSV-thymidine kinase can be killed upon administration of drugs, such as ganciclovir and cytosine deaminase. See, for

example, Dey and Evans, Suicide Gene Therapy by Herpes Simplex Virus-1 Thymidine Kinase (HSV-TK), in Targets in Gene Therapy, edited by You (2011); and Beltinger *et al.*, Proc. Natl. Acad. Sci. USA 96(15):8699-8704 (1999). In some embodiments the ceDNA vector can comprise a siRNA kill switch referred to as DISE (Death Induced by Survival gene Elimination) (Murmann *et al.*, Oncotarget. 2017; 8:84643-84658. Induction of DISE in ovarian cancer cells *in vivo*).

D. Constructs

[00489] Provided herein are FVIII ceDNA contructs comprising a nucleic acid sequence as set forth in Table 1, in combination with one of more of a promoter sequence, an enhancer sequence, a 5' UTR sequence, an intron sequence, a leader sequence, a 3'UTR sequence, a UCOE sequence, an exon sequence, a DNA nuclear targeting sequences (DTS) sequence, a Kozak sequence and/or a spacer sequence. According to some embodiments, the FVIII ceDNA construct comprises a sequence as set forth in **Table 18** below.

Table 18. ceDNA FVIII constructs

SEQ ID NO	ceDNA Construct Identifier
1	692
2	693
3	694
4	933
5	1270
6	1362
7	1367
8	1368
9	1373
10	1374
11	1375
12	1377
13	1378
14	1381
15	1387
16	1391
17	1572
18	1574
19	1579
20	1582
21	1585
22	1593
23	1598
24	1602
25	1611
26	1612
27	1615

28	1616
29	1620
30	1622
31	1627
32	1628
33	1632
34	1636
35	1637
36	1638
37	1645
38	1646
39	1647
40	1648
41	1649
42	1651
43	1652
44	1655
45	1657
46	1664
47	1668
48	1695
49	1700
50	1701
51	1708
52	1712
53	1725
54	1738
55	1740
56	1741
57	1742
58	1743
59	1744
60	1838
61	1840
62	1886
63	1918
64	1919
65	1920
66	1921
67	1922
68	1923
69	1930
70	1931
442	1658

443	1666
444	1880
445	1885
446	1948
447	1949
448	1950
449	ceDNA fusion 1476:: 1923ORF
450	ceDNA fusion 1477:: 1923ORF
451	ceDNA fusion 1478:: 1923ORF
452	ceDNA fusion 1479:: 1923ORF
453	ceDNA fusion 1480::1923ORF
454	ceDNA fusion 1649:: 3xG-1923ORF
455	ceDNA fusion 1649:: 3xG-Min-Con-1923ORF
456	ceDNA fusion 1649:: 3xG-mod-Con-1923ORF
457	ceDNA fusion 1649::325243-1923ORF
458	ceDNA fusion 1649::Min-Con-1923ORF
459	ceDNA fusion 1668::3xG-Mod-Con-1923ORF
460	ceDNA fusion 1668::1923ORF
461	ceDNA fusion 1668::Mod-Con-1923ORF
462	ceDNA fusion 1471::1923ORF
463	ceDNA fusion 1471::con-1923ORF
464	ceDNA fusion 1472::1923ORF
465	ceDNA fusion 1472::Con-1923ORF
466	ceDNA fusion 1473::1923ORF
467	ceDNA fusion 1473::Con-1923ORF
468	ceDNA fusion 1474::1923ORF
469	ceDNA fusion 1474::Con-1923ORF
470	ceDNA fusion 1475::1923ORF
471	ceDNA fusion 1622::1923ORF
472	ceDNA fusion 1627::1923ORF
473	ceDNA fusion 1628::1923ORF
474	ceDNA fusion 1632::1923 ORF
475	ceDNA fusion 1636::1923ORF
476	ceDNA fusion 1637::1923ORF
477	ceDNA fusion 1637::Con-1923ORF
478	ceDNA fusion 1638::1923ORF
479	ceDNA fusion 1645::1923ORF
480	ceDNA Fusion 1646::1923ORF
481	ceDNA Fusion 1649::1923ORF
482	ceDNA Fusion 1649::mod-Con-1923ORF
483	1719
642	ceDNA construct 10 (3x hSerpEnh VD, 1651)

643	ceDNA construct 60 (w/ 3x hSerpEnh_2mer_spacers_v17)
644	ceDNA construct 61 (w/ 3x hSerpEnh_11mer_spacers_v3)
645	ceDNA construct 62(w/ 3x Bushbaby SerpEnh_Aspacers)
646	ceDNA construct 39 (wild-type left ITR and right ITR truncation)

[00490] According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 1. According to some embodiments, the ceDNA construct comprises, or consists of SEQ ID NO: 1. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 2. According to some embodiments, the ceDNA construct comprises, or consists of SEQ ID NO: 2. According to some embodiments, a ceDNA construct comprises comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 3. According to some embodiments, the ceDNA construct comprises, or consists of SEQ ID NO: 3. According to some embodiments, a ceDNA construct comprises comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 4. According to some embodiments, the ceDNA construct comprises, or consists of SEQ ID NO: 4. According to some embodiments, a ceDNA construct comprises comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 5. According to some embodiments, the ceDNA construct comprises, or consists of SEQ ID NO: 5. According to some embodiments, a ceDNA construct comprises comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 6. According to some embodiments, the ceDNA construct comprises, or consists of SEQ ID NO: 6. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 7. According to some embodiments, the ceDNA construct comprises, or consists of SEQ ID NO: 7. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO:8. According to some embodiments, the ceDNA construct comprises, or consists of SEQ ID NO: 8. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 9. According to some embodiments, the ceDNA construct comprises, or consists of SEQ ID NO: 9. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 10. According to some embodiments, the ceDNA construct comprises, or consists of SEQ ID NO: 10. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 11. According to some embodiments, the ceDNA

construct comprises, or consists of SEQ ID NO: 11. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 12. According to some embodiments, the ceDNA construct comprises, or consists of SEQ ID NO: 12. According to some embodiments, a ceDNA construct comprises comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 13. According to some embodiments, the ceDNA construct comprises, or consists of SEQ ID NO: 13. According to some embodiments, a ceDNA construct comprises comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 14. According to some embodiments, the ceDNA construct comprises, or consists of SEQ ID NO: 14. According to some embodiments, a ceDNA construct comprises comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 15. According to some embodiments, the ceDNA construct comprises, or consists of SEQ ID NO: 15. According to some embodiments, a ceDNA construct comprises comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 16. According to some embodiments, the ceDNA construct comprises, or consists of SEQ ID NO: 16. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 17. According to some embodiments, the ceDNA construct comprises, or consists of SEQ ID NO: 17. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 18. According to some embodiments, the ceDNA construct comprises, or consists of SEQ ID NO: 18. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 19. According to some embodiments, the ceDNA construct comprises, or consists of SEQ ID NO: 19. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 20. According to some embodiments, the ceDNA construct comprises, or consists of SEQ ID NO: 20. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 21. According to some embodiments, the ceDNA construct comprises, or consists of SEQ ID NO: 21. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 22. According to some embodiments, the ceDNA construct comprises, or consists of SEQ ID NO: 22. According to some embodiments, a ceDNA construct comprises comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 23. According to some embodiments, the ceDNA construct comprises, or consists of SEQ ID NO: 23. According to some embodiments, a ceDNA construct comprises comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 24. According to some embodiments, the

ceDNA construct comprises, or consists of SEQ ID NO: 24. According to some embodiments, a ceDNA construct comprises comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 25. According to some embodiments, the ceDNA construct comprises, or consists of SEQ ID NO: 25. According to some embodiments, a ceDNA construct comprises comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 26. According to some embodiments, the ceDNA construct comprises, or consists of SEQ ID NO: 26. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 27. According to some embodiments, the ceDNA construct comprises, or consists of SEQ ID NO: 7. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO:28. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 28. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 29. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 29. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 30. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 30. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 31. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 31. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 32. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 32. According to some embodiments, a ceDNA construct comprises comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 33. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 33. According to some embodiments, a ceDNA construct comprises comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 34. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 34. According to some embodiments, a ceDNA construct comprises comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 35. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 35. According to some embodiments, a ceDNA construct comprises comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 36. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 36. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%,

95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 37. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 37. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 38. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 38. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 39. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 39. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 40. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 40. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 41. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 41. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 42. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 42. According to some embodiments, a ceDNA construct comprises comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 43. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 43. According to some embodiments, a ceDNA construct comprises comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 44. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 44. According to some embodiments, a ceDNA construct comprises comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 45. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 45. According to some embodiments, a ceDNA construct comprises comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 46. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 46. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 47. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 47. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 48. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 48. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 49. According to some embodiments, the ceDNA construct consists of SEQ ID NO:

49. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 50. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 50. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 51. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 51. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 52. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 52. According to some embodiments, a ceDNA construct comprises comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 53. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 53. According to some embodiments, a ceDNA construct comprises comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 54. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 54. According to some embodiments, a ceDNA construct comprises comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 55. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 55. According to some embodiments, a ceDNA construct comprises comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 56. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 56. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 57. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 57. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 58. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 58. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, comprises, or consists of SEQ ID NO: 59. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 59. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 60. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 60. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 61. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 61. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 62.

According to some embodiments, the ceDNA construct consists of SEQ ID NO: 62. According to some embodiments, a ceDNA construct comprises comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 63. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 63. According to some embodiments, a ceDNA construct comprises comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 64. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 64. According to some embodiments, a ceDNA construct comprises comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 65. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 65. According to some embodiments, a ceDNA construct comprises comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 66. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 66. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 67. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 67. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 68. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 68. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 69. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 69. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 70. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 70. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 442. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 442. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 443. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 443. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 444. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 444. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 445. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 445. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO:

446. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 446. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 447. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 447. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 448. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 448. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 449. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 449. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 450. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 450. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 451. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 451. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 452. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 452. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 453. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 453. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 454. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 454. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 455. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 455. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 456. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 456. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 457. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 457. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 458. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 458. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 459. According to some

embodiments, the ceDNA construct consists of SEQ ID NO: 459. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 460. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 460. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 461. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 461. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 462. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 462. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 463. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 463. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 464. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 464. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 465. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 465. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 466. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 466. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 467. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 467. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 468. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 468. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 469. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 469. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 470. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 470. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 471. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 471. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 472. According to some embodiments, the ceDNA

construct consists of SEQ ID NO: 472. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 473. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 473. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 474. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 474. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 475. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 475. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 476. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 476. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 477. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 477. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 478. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 478. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 479. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 479. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 480. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 480. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 481. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 481. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises SEQ ID NO: 482. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 482. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises, SEQ ID NO: 483. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 483. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises, SEQ ID NO: 642. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 642. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises, SEQ ID NO: 643. According to some embodiments, the ceDNA construct consists of

SEQ ID NO: 643. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises, SEQ ID NO: 644. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 644. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises, SEQ ID NO: 645. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 645. According to some embodiments, a ceDNA construct comprises a nucleic acid sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, or comprises, SEQ ID NO: 646. According to some embodiments, the ceDNA construct consists of SEQ ID NO: 646.

VI. Detailed method of Production of a ceDNA Vector

A. Production in General

[00491] Certain methods for the production of a ceDNA vector for expression of FVIII protein comprising an asymmetrical ITR pair or symmetrical ITR pair as defined herein is described in section IV of International application PCT/US18/49996 filed September 7, 2018, which is incorporated herein in its entirety by reference. In some embodiments, a ceDNA vector for expression of FVIII protein as disclosed herein can be produced using insect cells, as described herein. In alternative embodiments, a ceDNA vector for expression of FVIII protein as disclosed herein can be produced synthetically and in some embodiments, in a cell-free method, as disclosed on International Application PCT/US19/14122, filed January 18, 2019, which is incorporated herein in its entirety by reference.

[00492] As described herein, in one embodiment, a ceDNA vector for expression of FVIII protein can be obtained, for example, by the process comprising the steps of: a) incubating a population of host cells (*e.g.,* insect cells) harboring the polynucleotide expression construct template (*e.g.,* a ceDNA-plasmid, a ceDNA-Bacmid, and/or a ceDNA-baculovirus), which is devoid of viral capsid coding sequences, in the presence of a Rep protein under conditions effective and for a time sufficient to induce production of the ceDNA vector within the host cells, and wherein the host cells do not comprise viral capsid coding sequences; and b) harvesting and isolating the ceDNA vector from the host cells. The presence of Rep protein induces replication of the vector polynucleotide with a modified ITR to produce the ceDNA vector in a host cell. However, no viral particles (*e.g.,* AAV virions) are expressed. Thus, there is no size limitation such as that naturally imposed in AAV or other viral-based vectors.

[00493] The presence of the ceDNA vector isolated from the host cells can be confirmed by digesting DNA isolated from the host cell with a restriction enzyme having a single recognition site on the ceDNA vector and analyzing the digested DNA material on a non-denaturing gel to confirm the

presence of characteristic bands of linear and continuous DNA as compared to linear and non-continuous DNA.

[00494] In yet another aspect, the disclosure provides for use of host cell lines that have stably integrated the DNA vector polynucleotide expression template (ceDNA template) into their own genome in production of the non-viral DNA vector, *e.g.,* as described in Lee, L. *et al.* (2013) Plos One 8(8): e69879. Preferably, Rep is added to host cells at an MOI of about 3. When the host cell line is a mammalian cell line, *e.g.,* HEK293 cells, the cell lines can have polynucleotide vector template stably integrated, and a second vector such as herpes virus can be used to introduce Rep protein into cells, allowing for the excision and amplification of ceDNA in the presence of Rep and helper virus.

[00495] In one embodiment, the host cells used to make the ceDNA vectors for expression of FVIII protein as described herein are insect cells, and baculovirus is used to deliver both the polynucleotide that encodes Rep protein and the non-viral DNA vector polynucleotide expression construct template for ceDNA, *e.g.,* as described in **FIGS. 4A-4C** and Example 1. In some embodiments, the host cell is engineered to express Rep protein.

[00496] The ceDNA vector is then harvested and isolated from the host cells. The time for harvesting and collecting ceDNA vectors described herein from the cells can be selected and optimized to achieve a high-yield production of the ceDNA vectors. For example, the harvest time can be selected in view of cell viability, cell morphology, cell growth, etc. In one embodiment, cells are grown under sufficient conditions and harvested a sufficient time after baculoviral infection to produce ceDNA vectors but before a majority of cells start to die because of the baculoviral toxicity. The DNA vectors can be isolated using plasmid purification kits such as Qiagen Endo-Free Plasmid kits. Other methods developed for plasmid isolation can be also adapted for DNA vectors. Generally, any nucleic acid purification methods can be adopted.

[00497] The DNA vectors can be purified by any means known to those of skill in the art for purification of DNA. In one embodiment, ceDNA vectors are purified as DNA molecules. In another embodiment, the ceDNA vectors are purified as exosomes or microparticles.

[00498] The presence of the ceDNA vector for expression of FVIII protein can be confirmed by digesting the vector DNA isolated from the cells with a restriction enzyme having a single recognition site on the DNA vector and analyzing both digested and undigested DNA material using gel electrophoresis to confirm the presence of characteristic bands of linear and continuous DNA as compared to linear and non-continuous DNA. **FIG. 4C** and **FIG. 4D** illustrate one embodiment for identifying the presence of the closed ended ceDNA vectors produced by the processes herein.

B. ceDNA Plasmid

[00499] A ceDNA-plasmid is a plasmid used for later production of a ceDNA vector for expression of FVIII protein. In some embodiments, a ceDNA-plasmid can be constructed using known techniques to provide at least the following as operatively linked components in the direction of transcription: (1)

a modified 5' ITR sequence; (2) an expression cassette containing a cis-regulatory element, for example, a promoter, inducible promoter, regulatory switch, enhancers and the like; and (3) a modified 3' ITR sequence, where the 3' ITR sequence is symmetric relative to the 5' ITR sequence. In some embodiments, the expression cassette flanked by the ITRs comprises a cloning site for introducing an exogenous sequence. The expression cassette replaces the rep and cap coding regions of the AAV genomes.

[00500] In one aspect, a ceDNA vector for expression of FVIII protein is obtained from a plasmid, referred to herein as a "ceDNA-plasmid" encoding in this order: a first adeno-associated virus (AAV) inverted terminal repeat (ITR), an expression cassette comprising a transgene, and a mutated or modified AAV ITR, wherein said ceDNA-plasmid is devoid of AAV capsid protein coding sequences. In alternative embodiments, the ceDNA-plasmid encodes in this order: a first (or 5') modified or mutated AAV ITR, an expression cassette comprising a transgene, and a second (or 3') modified AAV ITR, wherein said ceDNA-plasmid is devoid of AAV capsid protein coding sequences, and wherein the 5' and 3' ITRs are symmetric relative to each other. In alternative embodiments, the ceDNA-plasmid encodes in this order: a first (or 5') modified or mutated AAV ITR, an expression cassette comprising a transgene, and a second (or 3') mutated or modified AAV ITR, wherein said ceDNA-plasmid is devoid of AAV capsid protein coding sequences, and wherein the 5' and 3' modified ITRs are have the same modifications (*i.e.,* they are inverse complement or symmetric relative to each other).

[00501] In a further embodiment, the ceDNA-plasmid system is devoid of viral capsid protein coding sequences (*i.e.* it is devoid of AAV capsid genes but also of capsid genes of other viruses). In addition, in a particular embodiment, the ceDNA-plasmid is also devoid of AAV Rep protein coding sequences. Accordingly, in a preferred embodiment, ceDNA-plasmid is devoid of functional AAV cap and AAV rep genes GG-3′ for AAV2) plus a variable palindromic sequence allowing for hairpin formation.

[00502] A ceDNA-plasmid of the present disclosure can be generated using natural nucleotide sequences of the genomes of any AAV serotypes well known in the art. In one embodiment, the ceDNA-plasmid backbone is derived from the AAV1, AAV2, AAV3, AAV4, AAV5, AAV 5, AAV7, AAV8, AAV9, AAV10, AAV 11, AAV12, AAVrh8, AAVrh10, AAV-DJ, and AAV-DJ8 genome. *E.g.,* NCBI: NC 002077; NC 001401; NC001729; NC001829; NC006152; NC 006260; NC 006261; Kotin and Smith, The Springer Index of Viruses, available at the URL maintained by Springer (at www web address: oesys.springer.de/viruses/database/mkchapter.asp?virID=42.04.)(note -references to a URL or database refer to the contents of the URL or database as of the effective filing date of this application) In a particular embodiment, the ceDNA-plasmid backbone is derived from the AAV2 genome. In another particular embodiment, the ceDNA-plasmid backbone is a synthetic backbone genetically engineered to include at its 5' and 3' ITRs derived from one of these AAV genomes.

[00503] A ceDNA-plasmid can optionally include a selectable or selection marker for use in the establishment of a ceDNA vector-producing cell line. In one embodiment, the selection marker can be inserted downstream (*i.e.,* 3') of the 3' ITR sequence. In another embodiment, the selection marker can be inserted upstream (*i.e.,* 5') of the 5' ITR sequence. Appropriate selection markers include, for example, those that confer drug resistance. Selection markers can be, for example, a blasticidin S-resistance gene, kanamycin, geneticin, and the like. In a preferred embodiment, the drug selection marker is a blasticidin S-resistance gene.

[00504] An exemplary ceDNA (*e.g.,* rAAV0) vector for expression of FVIII protein is produced from an rAAV plasmid. A method for the production of a rAAV vector, can comprise: (a) providing a host cell with a rAAV plasmid as described above, wherein both the host cell and the plasmid are devoid of capsid protein encoding genes, (b) culturing the host cell under conditions allowing production of an ceDNA genome, and (c) harvesting the cells and isolating the AAV genome produced from said cells.

C. Exemplary method of making the ceDNA vectors from ceDNA plasmids

[00505] Methods for making capsid-less ceDNA vectors for expression of FVIII protein are also provided herein, notably a method with a sufficiently high yield to provide sufficient vector for *in vivo* experiments.

[00506] In some embodiments, a method for the production of a ceDNA vector for expression of FVIII protein comprises the steps of: (1) introducing the nucleic acid construct comprising an expression cassette and two symmetric ITR sequences into a host cell (*e.g.,* Sf9 cells), (2) optionally, establishing a clonal cell line, for example, by using a selection marker present on the plasmid, (3) introducing a Rep coding gene (either by transfection or infection with a baculovirus carrying said gene) into said insect cell, and (4) harvesting the cell and purifying the ceDNA vector. The nucleic acid construct comprising an expression cassette and two ITR sequences described above for the production of ceDNA vector can be in the form of a ceDNA plasmid, or Bacmid or Baculovirus generated with the ceDNA plasmid as described below. The nucleic acid construct can be introduced into a host cell by transfection, viral transduction, stable integration, or other methods known in the art.

D. Cell lines

[00507] Host cell lines used in the production of a ceDNA vector for expression of FVIII protein can include insect cell lines derived from Spodoptera frugiperda, such as Sf9 Sf21, or Trichoplusia ni cell, or other invertebrate, vertebrate, or other eukaryotic cell lines including mammalian cells. Other cell lines known to an ordinarily skilled artisan can also be used, such as HEK293, Huh-7, HeLa, HepG2, HeplA, 911, CHO, COS, MeWo, NIH3T3, A549, HT1 180, monocytes, and mature and immature dendritic cells. Host cell lines can be transfected for stable expression of the ceDNA-plasmid for high yield ceDNA vector production.

[00508] CeDNA-plasmids can be introduced into Sf9 cells by transient transfection using reagents (*e.g.,* liposomal, calcium phosphate) or physical means (*e.g.,* electroporation) known in the art. Alternatively, stable Sf9 cell lines which have stably integrated the ceDNA-plasmid into their genomes can be established. Such stable cell lines can be established by incorporating a selection marker into the ceDNA -plasmid as described above. If the ceDNA -plasmid used to transfect the cell line includes a selection marker, such as an antibiotic, cells that have been transfected with the ceDNA-plasmid and integrated the ceDNA-plasmid DNA into their genome can be selected for by addition of the antibiotic to the cell growth media. Resistant clones of the cells can then be isolated by single-cell dilution or colony transfer techniques and propagated.

***E. Isolating and Purifying ceDNA vectors*:**

[00509] Examples of the process for obtaining and isolating ceDNA vectors are described in **FIGS. 4A-4E** and the specific examples below. ceDNA-vectors for expression of FVIII protein disclosed herein can be obtained from a producer cell expressing AAV Rep protein(s), further transformed with a ceDNA-plasmid, ceDNA-bacmid, or ceDNA-baculovirus. Plasmids useful for the production of ceDNA vectors include plasmids that encode FVIII protein, or plasmids encoding one or more REP proteins.

[00510] In one aspect, a polynucleotide encodes the AAV Rep protein (Rep 78 or 68) delivered to a producer cell in a plasmid (Rep-plasmid), a bacmid (Rep-bacmid), or a baculovirus (Rep-baculovirus). The Rep-plasmid, Rep-bacmid, and Rep-baculovirus can be generated by methods described above.

[00511] Methods to produce a ceDNA vector for expression of FVIII protein are described herein. Expression constructs used for generating a ceDNA vector for expression of FVIII protein as described herein can be a plasmid (*e.g.,* ceDNA-plasmids), a Bacmid (*e.g.,* ceDNA-bacmid), and/or a baculovirus (*e.g.,* ceDNA-baculovirus). By way of an example only, a ceDNA-vector can be generated from the cells co-infected with ceDNA-baculovirus and Rep-baculovirus. Rep proteins produced from the Rep-baculovirus can replicate the ceDNA-baculovirus to generate ceDNA-vectors. Alternatively, ceDNA vectors for expression of FVIII protein can be generated from the cells stably transfected with a construct comprising a sequence encoding the AAV Rep protein (Rep78/52) delivered in Rep-plasmids, Rep-bacmids, or Rep-baculovirus. CeDNA-Baculovirus can be transiently transfected to the cells, be replicated by Rep protein and produce ceDNA vectors.

[00512] The bacmid (*e.g.,* ceDNA-bacmid) can be transfected into permissive insect cells such as Sf9, Sf21, Tni (Trichoplusia ni) cell, High Five cell, and generate ceDNA-baculovirus, which is a recombinant baculovirus including the sequences comprising the symmetric ITRs and the expression cassette. ceDNA-baculovirus can be again infected into the insect cells to obtain a next generation of the recombinant baculovirus. Optionally, the step can be repeated once or multiple times to produce the recombinant baculovirus in a larger quantity.

[00513] The time for harvesting and collecting ceDNA vectors for expression of FVIII protein as described herein from the cells can be selected and optimized to achieve a high-yield production of the ceDNA vectors. For example, the harvest time can be selected in view of cell viability, cell morphology, cell growth, etc. Usually, cells can be harvested after sufficient time after baculoviral infection to produce ceDNA vectors (*e.g.,* ceDNA vectors) but before majority of cells start to die because of the viral toxicity. The ceDNA-vectors can be isolated from the Sf9 cells using plasmid purification kits such as Qiagen ENDO-FREE PLASMID® kits. Other methods developed for plasmid isolation can be also adapted for ceDNA vectors. Generally, any art-known nucleic acid purification methods can be adopted, as well as commercially available DNA extraction kits.

[00514] Alternatively, purification can be implemented by subjecting a cell pellet to an alkaline lysis process, centrifuging the resulting lysate and performing chromatographic separation. As one non-limiting example, the process can be performed by loading the supernatant on an ion exchange column (*e.g.,* SARTOBIND Q®) which retains nucleic acids, and then eluting (*e.g.,* with a 1.2 M NaCl solution) and performing a further chromatographic purification on a gel filtration column (*e.g.,* 6 fast flow GE). The capsid-free AAV vector is then recovered by, *e.g.,* precipitation.

[00515] In some embodiments, ceDNA vectors for expression of FVIII protein can also be purified in the form of exosomes, or microparticles. It is known in the art that many cell types release not only soluble proteins, but also complex protein/nucleic acid cargoes via membrane microvesicle shedding (Cocucci et al, 2009; EP 10306226.1) Such vesicles include microvesicles (also referred to as microparticles) and exosomes (also referred to as nanovesicles), both of which comprise proteins and RNA as cargo. Microvesicles are generated from the direct budding of the plasma membrane, and exosomes are released into the extracellular environment upon fusion of multivesicular endosomes with the plasma membrane. Thus, ceDNA vector-containing microvesicles and/or exosomes can be isolated from cells that have been transduced with the ceDNA-plasmid or a bacmid or baculovirus generated with the ceDNA-plasmid.

[00516] Microvesicles can be isolated by subjecting culture medium to filtration or ultracentrifugation at 20,000 x g, and exosomes at 100,000 x g. The optimal duration of ultracentrifugation can be experimentally-determined and will depend on the particular cell type from which the vesicles are isolated. Preferably, the culture medium is first cleared by low-speed centrifugation (*e.g.,* at 2000 x g for 5-20 minutes) and subjected to spin concentration using, *e.g.,* an AMICON® spin column (Millipore, Watford, UK). Microvesicles and exosomes can be further purified via FACS or MACS by using specific antibodies that recognize specific surface antigens present on the microvesicles and exosomes. Other microvesicle and exosome purification methods include, but are not limited to, immunoprecipitation, affinity chromatography, filtration, and magnetic beads coated with specific antibodies or aptamers. Upon purification, vesicles are washed with, *e.g.,* phosphate-buffered saline. One advantage of using microvesicles or exosome to deliver ceDNA-

containing vesicles is that these vesicles can be targeted to various cell types by including on their membrane proteins recognized by specific receptors on the respective cell types. (See also EP 10306226)

[00517] Another aspect of the disclosure herein relates to methods of purifying ceDNA vectors from host cell lines that have stably integrated a ceDNA construct into their own genome. In one embodiment, ceDNA vectors are purified as DNA molecules. In another embodiment, the ceDNA vectors are purified as exosomes or microparticles.

[00518] **FIG. 5** of International application PCT/US18/49996 shows a gel confirming the production of ceDNA from multiple ceDNA-plasmid constructs using the method described in the Examples. The ceDNA is confirmed by a characteristic band pattern in the gel, as discussed with respect to **FIG. 4D** in the Examples.

VII. Pharmaceutical Compositions

[00519] In another aspect, pharmaceutical compositions are provided. The pharmaceutical composition comprises a ceDNA vector for expression of FVIII protein as described herein and a pharmaceutically acceptable carrier or diluent.

[00520] The ceDNA vectors for expression of FVIII protein as disclosed herein can be incorporated into pharmaceutical compositions suitable for administration to a subject for *in vivo* delivery to cells, tissues, or organs of the subject. Typically, the pharmaceutical composition comprises a ceDNA-vector as disclosed herein and a pharmaceutically acceptable carrier. For example, the ceDNA vectors for expression of FVIII protein as described herein can be incorporated into a pharmaceutical composition suitable for a desired route of therapeutic administration (*e.g.,* parenteral administration). Passive tissue transduction via high pressure intravenous or intra-arterial infusion, as well as intracellular injection, such as intranuclear microinjection or intracytoplasmic injection, are also contemplated. Pharmaceutical compositions for therapeutic purposes can be formulated as a solution, microemulsion, dispersion, liposomes, or other ordered structure suitable to high ceDNA vector concentration. Sterile injectable solutions can be prepared by incorporating the ceDNA vector compound in the required amount in an appropriate buffer with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization including a ceDNA vector can be formulated to deliver a transgene in the nucleic acid to the cells of a recipient, resulting in the therapeutic expression of the transgene or donor sequence therein. The composition can also include a pharmaceutically acceptable carrier.

[00521] Pharmaceutically active compositions comprising a ceDNA vector for expression of FVIII protein can be formulated to deliver a transgene for various purposes to the cell, *e.g.,* cells of a subject.

[00522] Pharmaceutical compositions for therapeutic purposes typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposomes, or other ordered structure suitable to high ceDNA vector

concentration. Sterile injectable solutions can be prepared by incorporating the ceDNA vector compound in the required amount in an appropriate buffer with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization.

[00523] A ceDNA vector for expression of FVIII protein as disclosed herein can be incorporated into a pharmaceutical composition suitable for topical, systemic, intra-amniotic, intrathecal, intracranial, intra-arterial, intravenous, intralymphatic, intraperitoneal, subcutaneous, tracheal, intra-tissue (*e.g.,* intramuscular, intracardiac, intrahepatic, intrarenal, intracerebral), intrathecal, intravesical, conjunctival (*e.g.,* extra-orbital, intraorbital, retroorbital, intraretinal, subretinal, choroidal, sub-choroidal, intrastromal, intracameral and intravitreal), intracochlear, and mucosal (*e.g.,* oral, rectal, nasal) administration. Passive tissue transduction via high pressure intravenous or intraarterial infusion, as well as intracellular injection, such as intranuclear microinjection or intracytoplasmic injection, are also contemplated.

[00524] In some aspects, the methods provided herein comprise delivering one or more ceDNA vectors for expression of FVIII protein as disclosed herein to a host cell. Also provided herein are cells produced by such methods, and organisms (such as animals, plants, or fungi) comprising or produced from such cells. Methods of delivery of nucleic acids can include lipofection, nucleofection, microinjection, biolistics, liposomes, immunoliposomes, polycation or lipid:nucleic acid conjugates, naked DNA, and agent-enhanced uptake of DNA. Lipofection is described in *e.g.,* U.S. Pat. Nos. 5,049,386, 4,946,787; and 4,897,355, the contents of each of which are incorporated by reference in their entireties herein) and lipofection reagents are sold commercially (*e.g.,* TRANSFECTAM™ and LIPFECTIN™). Delivery can be to cells (*e.g., in vitro* or *ex vivo* administration) or target tissues (*e.g., in vivo* administration).

[00525] Various techniques and methods are known in the art for delivering nucleic acids to cells. For example, nucleic acids, such as ceDNA for expression of FVIII protein can be formulated into lipid nanoparticles (LNPs), lipidoids, liposomes, lipid nanoparticles, lipoplexes, or core-shell nanoparticles. Typically, LNPs are composed of nucleic acid (*e.g.,* ceDNA) molecules, one or more ionizable or cationic lipids (or salts thereof), one or more non-ionic or neutral lipids (*e.g.,* a phospholipid), a molecule that prevents aggregation (*e.g.,* PEG or a PEG-lipid conjugate), and optionally a sterol (*e.g.,* cholesterol).

[00526] Another method for delivering nucleic acids, such as ceDNA for expression of FVIII protein to a cell is by conjugating the nucleic acid with a ligand that is internalized by the cell. For example, the ligand can bind a receptor on the cell surface and internalized via endocytosis. The ligand can be covalently linked to a nucleotide in the nucleic acid. Exemplary conjugates for delivering nucleic acids into a cell are described, example, in WO2015/006740, WO2014/025805, WO2012/037254, WO2009/082606, WO2009/073809, WO2009/018332, WO2006/112872, WO2004/090108,

WO2004/091515 and WO2017/177326, the contents of each of which are incorporated by reference in their entireties herein.

[00527] Nucleic acids, such as ceDNA vectors for expression of FVIII protein can also be delivered to a cell by transfection. Useful transfection methods include, but are not limited to, lipid-mediated transfection, cationic polymer-mediated transfection, or calcium phosphate precipitation. Transfection reagents are well known in the art and include, but are not limited to, TurboFect Transfection Reagent (Thermo Fisher Scientific), Pro-Ject Reagent (Thermo Fisher Scientific), TRANSPASS™ P Protein Transfection Reagent (New England Biolabs), CHARIOT™ Protein Delivery Reagent (Active Motif), PROTEOJUICE™ Protein Transfection Reagent (EMD Millipore), 293fectin, LIPOFECTAMINE™ 2000, LIPOFECTAMINE™ 3000 (Thermo Fisher Scientific), LIPOFECTAMINE™ (Thermo Fisher Scientific), LIPOFECTIN™ (Thermo Fisher Scientific), DMRIE-C, CELLFECTIN™ (Thermo Fisher Scientific), OLIGOFECTAMINE™ (Thermo Fisher Scientific), LIPOFECTACE™, FUGENE™ (Roche, Basel, Switzerland), FUGENE™ HD (Roche), TRANSFECTAM™(Transfectam, Promega, Madison, Wis.), TFX-10™ (Promega), TFX-20™ (Promega), TFX-50™ (Promega), TRANSFECTIN™ (BioRad, Hercules, Calif.), SILENTFECT™ (Bio-Rad), Effectene™ (Qiagen, Valencia, Calif.), DC-chol (Avanti Polar Lipids), GENEPORTER™ (Gene Therapy Systems, San Diego, Calif.), DHARMAFECT 1™ (Dharmacon, Lafayette, Colo.), DHARMAFECT 2™ (Dharmacon), DHARMAFECT 3™ (Dharmacon), DHARMAFECT 4™ (Dharmacon), ESCORT™ III (Sigma, St. Louis, Mo.), and ESCORT™ IV (Sigma Chemical Co.). Nucleic acids, such as ceDNA, can also be delivered to a cell via microfluidics methods known to those of skill in the art.

[00528] ceDNA vectors for expression of FVIII protein as described herein can also be administered directly to an organism for transduction of cells *in vivo*. Administration is by any of the routes normally used for introducing a molecule into ultimate contact with blood or tissue cells including, but not limited to, injection, infusion, topical application and electroporation. Suitable methods of administering such nucleic acids are available and well known to those of skill in the art, and, although more than one route can be used to administer a particular composition, a particular route can often provide a more immediate and more effective reaction than another route.

[00529] Methods for introduction of a nucleic acid vector ceDNA vector for expression of FVIII protein as disclosed herein can be delivered into hematopoietic stem cells, for example, by the methods as described, for example, in U.S. Pat. No. 5,928,638, the contents of which is incorporated by reference in its entirety herein.

[00530] The ceDNA vectors for expression of FVIII protein in accordance with the present disclosure can be added to liposomes for delivery to a cell or target organ in a subject. Liposomes are vesicles that possess at least one lipid bilayer. Liposomes are typical used as carriers for drug/ therapeutic delivery in the context of pharmaceutical development. They work by fusing with a cellular membrane and repositioning its lipid structure to deliver a drug or active pharmaceutical

ingredient (API). Liposome compositions for such delivery are composed of phospholipids, especially compounds having a phosphatidylcholine group, however these compositions may also include other lipids. Exemplary liposomes and liposome formulations, including but not limited to polyethylene glycol (PEG)-functional group containing compounds are disclosed in International Application PCT/US2018/050042, filed on September 7, 2018 and in International application PCT/US2018/064242, filed on December 6, 2018, *e.g.,* see the section entitled "Pharmaceutical Formulations".

[00531] Various delivery methods known in the art or modification thereof can be used to deliver ceDNA vectors *in vitro* or *in vivo.* For example, in some embodiments, ceDNA vectors for expression of FVIII protein are delivered by making transient penetration in cell membrane by mechanical, electrical, ultrasonic, hydrodynamic, or laser-based energy so that DNA entrance into the targeted cells is facilitated. For example, a ceDNA vector can be delivered by transiently disrupting cell membrane by squeezing the cell through a size-restricted channel or by other means known in the art. In some cases, a ceDNA vector alone is directly injected as naked DNA into any one of: any one or more tissues selected from: liver, kidneys, gallbladder, prostate, adrenal gland, heart, intestine, lung, and stomach, skin, thymus, cardiac muscle or skeletal muscle. In some cases, a ceDNA vector is delivered by gene gun. Gold or tungsten spherical particles (1–3 μm diameter) coated with capsid-free AAV vectors can be accelerated to high speed by pressurized gas to penetrate into target tissue cells.

[00532] Compositions comprising a ceDNA vector for expression of FVIII protein and a pharmaceutically acceptable carrier are specifically contemplated herein. In some embodiments, the ceDNA vector is formulated with a lipid delivery system, for example, liposomes as described herein. In some embodiments, such compositions are administered by any route desired by a skilled practitioner. The compositions may be administered to a subject by different routes including orally, parenterally, sublingually, transdermally, rectally, transmucosally, topically, via inhalation, via buccal administration, intrapleurally, intravenous, intra-arterial, intraperitoneal, subcutaneous, intramuscular, intranasal intrathecal, and intraarticular or combinations thereof. For veterinary use, the composition may be administered as a suitably acceptable formulation in accordance with normal veterinary practice. The veterinarian may readily determine the dosing regimen and route of administration that is most appropriate for a particular animal. The compositions may be administered by traditional syringes, needleless injection devices, "microprojectile bombardment gene guns", or other physical methods such as electroporation ("EP"), hydrodynamic methods, or ultrasound.

[00533] In some cases, a ceDNA vector for expression of FVIII protein is delivered by hydrodynamic injection, which is a simple and highly efficient method for direct intracellular delivery of any water-soluble compounds and particles into internal organs and skeletal muscle in an entire limb.

[00534] In some cases, ceDNA vectors for expression of FVIII protein are delivered by ultrasound by making nanoscopic pores in membrane to facilitate intracellular delivery of DNA particles into cells of internal organs or tumors, so the size and concentration of plasmid DNA have great role in efficiency of the system. In some cases, ceDNA vectors are delivered by magnetofection by using magnetic fields to concentrate particles containing nucleic acid into the target cells.

[00535] In some cases, chemical delivery systems can be used, for example, by using nanomeric complexes, which include compaction of negatively charged nucleic acid by polycationic nanomeric particles, belonging to cationic liposome/micelle or cationic polymers. Cationic lipids used for the delivery method includes, but not limited to monovalent cationic lipids, polyvalent cationic lipids, guanidine containing compounds, cholesterol derivative compounds, cationic polymers, (*e.g.*, poly(ethylenimine), poly-L-lysine, protamine, other cationic polymers), and lipid-polymer hybrid.

A. Exosomes

[00536] In some embodiments, a ceDNA vector for expression of FVIII protein as disclosed herein is delivered by being packaged in an exosome. Exosomes are small membrane vesicles of endocytic origin that are released into the extracellular environment following fusion of multivesicular bodies with the plasma membrane. Their surface consists of a lipid bilayer from the donor cell's cell membrane, they contain cytosol from the cell that produced the exosome, and exhibit membrane proteins from the parental cell on the surface. Exosomes are produced by various cell types including epithelial cells, B and T lymphocytes, mast cells (MC) as well as dendritic cells (DC). Some embodiments, exosomes with a diameter between 10nm and 1μm, between 20nm and 500nm, between 30nm and 250nm, between 50nm and 100nm are envisioned for use. Exosomes can be isolated for a delivery to target cells using either their donor cells or by introducing specific nucleic acids into them. Various approaches known in the art can be used to produce exosomes containing capsid-free AAV vectors of the present disclosure.

B. Microparticle/Nanoparticles

[00537] In some embodiments, a ceDNA vector for expression of FVIII protein as disclosed herein is delivered by a lipid nanoparticle. Generally, lipid nanoparticles comprise an ionizable amino lipid (*e.g.,* heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate, DLin-MC3-DMA, a phosphatidylcholine (1,2-distearoyl-sn-glycero-3-phosphocholine, DSPC), cholesterol and a coat lipid (polyethylene glycol-dimyristolglycerol, PEG-DMG), for example as disclosed by Tam *et al.* (2013). *Advances in Lipid Nanoparticles for siRNA delivery*. Pharmaceuticals 5(3): 498-507.

[00538] In some embodiments, a lipid nanoparticle has a mean diameter between about 10 and about 1000 nm. In some embodiments, a lipid nanoparticle has a diameter that is less than 300 nm. In some embodiments, a lipid nanoparticle has a diameter between about 10 and about 300 nm. In some embodiments, a lipid nanoparticle has a diameter that is less than 200 nm. In some embodiments, a lipid nanoparticle has a diameter between about 25 and about 200 nm. In some embodiments, a lipid

nanoparticle preparation (*e.g.,* composition comprising a plurality of lipid nanoparticles) has a size distribution in which the mean size (*e.g.,* diameter) is about 70 nm to about 200 nm, and more typically the mean size is about 100 nm or less.

[00539] Various lipid nanoparticles known in the art can be used to deliver ceDNA vector for expression of FVIII protein as disclosed herein. For example, various delivery methods using lipid nanoparticles are described in U.S. Patent Nos. 9,404,127, 9,006,417 and 9,518,272.

[00540] In some embodiments, a ceDNA vector for expression of FVIII protein as disclosed herein is delivered by a gold nanoparticle. Generally, a nucleic acid can be covalently bound to a gold nanoparticle or non-covalently bound to a gold nanoparticle (*e.g.,* bound by a charge-charge interaction), for example as described by Ding *et al.* (2014). *Gold Nanoparticles for Nucleic Acid Delivery*. Mol. Ther. 22(6); 1075-1083. In some embodiments, gold nanoparticle-nucleic acid conjugates are produced using methods described, for example, in U.S. Patent No. 6,812,334.

C. Conjugates

[00541] In some embodiments, a ceDNA vector for expression of FVIII protein as disclosed herein is conjugated (*e.g.,* covalently bound to an agent that increases cellular uptake. An "agent that increases cellular uptake" is a molecule that facilitates transport of a nucleic acid across a lipid membrane. For example, a nucleic acid can be conjugated to a lipophilic compound (*e.g.,* cholesterol, tocopherol, *etc.*), a cell penetrating peptide (CPP) (*e.g.,* penetratin, TAT, Syn1B, *etc.*), and polyamines (*e.g.,* spermine). Further examples of agents that increase cellular uptake are disclosed, for example, in Winkler (2013). *Oligonucleotide conjugates for therapeutic applications*. Ther. Deliv. 4(7); 791-809.

[00542] In some embodiments, a ceDNA vector for expression of FVIII protein as disclosed herein is conjugated to a polymer (*e.g.,* a polymeric molecule) or a folate molecule (*e.g.,* folic acid molecule). Generally, delivery of nucleic acids conjugated to polymers is known in the art, for example as described in WO2000/34343 and WO2008/022309. In some embodiments, a ceDNA vector for expression of FVIII protein as disclosed herein is conjugated to a poly(amide) polymer, for example as described by U.S. Patent No. 8,987,377. In some embodiments, a nucleic acid described by the disclosure is conjugated to a folic acid molecule as described in U.S. Patent No. 8,507,455.

[00543] In some embodiments, a ceDNA vector for expression of FVIII protein as disclosed herein is conjugated to a carbohydrate, for example as described in U.S. Patent No. 8,450,467.

D. Nanocapsule

[00544] Alternatively, nanocapsule formulations of a ceDNA vector for expression of FVIII protein as disclosed herein can be used. Nanocapsules can generally entrap substances in a stable and reproducible way. To avoid side effects due to intracellular polymeric overloading, such ultrafine particles (sized around 0.1 μm) should be designed using polymers able to be degraded *in vivo*. Biodegradable polyalkyl-cyanoacrylate nanoparticles that meet these requirements are contemplated for use.

E. Liposomes

[00545] The ceDNA vectors for expression of FVIII protein in accordance with the present disclosure can be added to liposomes for delivery to a cell or target organ in a subject. Liposomes are vesicles that possess at least one lipid bilayer. Liposomes are typical used as carriers for drug/ therapeutic delivery in the context of pharmaceutical development. They work by fusing with a cellular membrane and repositioning its lipid structure to deliver a drug or active pharmaceutical ingredient (API). Liposome compositions for such delivery are composed of phospholipids, especially compounds having a phosphatidylcholine group, however these compositions may also include other lipids.

[00546] The formation and use of liposomes are generally known to those of skill in the art. Liposomes have been developed with improved serum stability and circulation half-times (U.S. Pat. No. 5,741,516). Further, various methods of liposome and liposome like preparations as potential drug carriers have been described (U.S. Pat. Nos. 5,567,434; 5,552,157; 5,565,213; 5,738,868 and 5,795,587).

F. Exemplary liposome and Lipid Nanoparticle (LNP) Compositions

[00547] The ceDNA vectors for expression of FVIII protein in accordance with the present disclosure can be added to liposomes for delivery to a cell, *e.g.,* a cell in need of expression of the transgene. Liposomes are vesicles that possess at least one lipid bilayer. Liposomes are typical used as carriers for drug/ therapeutic delivery in the context of pharmaceutical development. They work by fusing with a cellular membrane and repositioning its lipid structure to deliver a drug or active pharmaceutical ingredient (API). Liposome compositions for such delivery are composed of phospholipids, especially compounds having a phosphatidylcholine group, however these compositions may also include other lipids.

[00548] Lipid nanoparticles (LNPs) comprising ceDNA vectors are disclosed in International Application PCT/US2018/050042, filed on September 7, 2018, and International Application PCT/US2018/064242, filed on December 6, 2018 which are incorporated herein in their entirety and envisioned for use in the methods and compositions for ceDNA vectors for expression of FVIII protein as disclosed herein.

[00549] In some aspects, the disclosure provides for a liposome formulation that includes one or more compounds with a polyethylene glycol (PEG) functional group (so-called "PEG-ylated compounds") which can reduce the immunogenicity/ antigenicity of, provide hydrophilicity and hydrophobicity to the compound(s) and reduce dosage frequency. Or the liposome formulation simply includes polyethylene glycol (PEG) polymer as an additional component. In such aspects, the molecular weight of the PEG or PEG functional group can be from 62 Da to about 5,000 Da.

[00550] In some aspects, the disclosure provides for a liposome formulation that will deliver an API with extended release or controlled release profile over a period of hours to weeks. In some related

aspects, the liposome formulation may comprise aqueous chambers that are bound by lipid bilayers. In other related aspects, the liposome formulation encapsulates an API with components that undergo a physical transition at elevated temperature which releases the API over a period of hours to weeks.

[00551] In some aspects, the liposome formulation comprises sphingomyelin and one or more lipids disclosed herein. In some aspects, the liposome formulation comprises optisomes.

[00552] In some aspects, the disclosure provides for a liposome formulation that includes one or more lipids selected from: N-(carbonyl-methoxypolyethylene glycol 2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt, (distearoyl-sn-glycero-phosphoethanolamine), MPEG (methoxy polyethylene glycol)-conjugated lipid, HSPC (hydrogenated soy phosphatidylcholine); PEG (polyethylene glycol); DSPE (distearoyl-sn-glycero-phosphoethanolamine); DSPC (distearoylphosphatidylcholine); DOPC (dioleoylphosphatidylcholine); DPPG (dipalmitoylphosphatidylglycerol); EPC (egg phosphatidylcholine); DOPS (dioleoylphosphatidylserine); POPC (palmitoyloleoylphosphatidylcholine); SM (sphingomyelin); MPEG (methoxy polyethylene glycol); DMPC (dimyristoyl phosphatidylcholine); DMPG (dimyristoyl phosphatidylglycerol); DSPG (distearoylphosphatidylglycerol); DEPC (dierucoylphosphatidylcholine); DOPE (dioleoly-sn-glycero-phophoethanolamine); cholesteryl sulphate (CS); dipalmitoylphosphatidylglycerol (DPPG); DOPC (dioleoly-sn-glycero-phosphatidylcholine) or any combination thereof.

[00553] In some aspects, the disclosure provides for a liposome formulation comprising phospholipid, cholesterol and a PEG-ylated lipid in a molar ratio of 56:38:5. In some aspects, the liposome formulation's overall lipid content is from 2-16 mg/mL. In some aspects, the disclosure provides for a liposome formulation comprising a lipid containing a phosphatidylcholine functional group, a lipid containing an ethanolamine functional group and a PEG-ylated lipid. In some aspects, the disclosure provides for a liposome formulation comprising a lipid containing a phosphatidylcholine functional group, a lipid containing an ethanolamine functional group and a PEG-ylated lipid in a molar ratio of 3:0.015:2 respectively. In some aspects, the disclosure provides for a liposome formulation comprising a lipid containing a phosphatidylcholine functional group, cholesterol and a PEG-ylated lipid. In some aspects, the disclosure provides for a liposome formulation comprising a lipid containing a phosphatidylcholine functional group and cholesterol. In some aspects, the PEG-ylated lipid is PEG-2000-DSPE. In some aspects, the disclosure provides for a liposome formulation comprising DPPG, soy PC, MPEG-DSPE lipid conjugate and cholesterol.

[00554] In some aspects, the disclosure provides for a liposome formulation comprising one or more lipids containing a phosphatidylcholine functional group and one or more lipids containing an ethanolamine functional group. In some aspects, the disclosure provides for a liposome formulation comprising one or more: lipids containing a phosphatidylcholine functional group, lipids containing an

ethanolamine functional group, and sterols, *e.g.,* cholesterol. In some aspects, the liposome formulation comprises DOPC/ DEPC; and DOPE.

[00555] In some aspects, the disclosure provides for a liposome formulation further comprising one or more pharmaceutical excipients, *e.g.,* sucrose and/or glycine.

[00556] In some aspects, the disclosure provides for a liposome formulation that is either unilamellar or multilamellar in structure. In some aspects, the disclosure provides for a liposome formulation that comprises multi-vesicular particles and/or foam-based particles. In some aspects, the disclosure provides for a liposome formulation that are larger in relative size to common nanoparticles and about 150 to 250 nm in size. In some aspects, the liposome formulation is a lyophilized powder.

[00557] In some aspects, the disclosure provides for a liposome formulation that is made and loaded with ceDNA vectors disclosed or described herein, by adding a weak base to a mixture having the isolated ceDNA outside the liposome. This addition increases the pH outside the liposomes to approximately 7.3 and drives the API into the liposome. In some aspects, the disclosure provides for a liposome formulation having a pH that is acidic on the inside of the liposome. In such cases the inside of the liposome can be at pH 4-6.9, and more preferably pH 6.5. In other aspects, the disclosure provides for a liposome formulation made by using intra-liposomal drug stabilization technology. In such cases, polymeric or non-polymeric highly charged anions and intra-liposomal trapping agents are utilized, *e.g.,* polyphosphate or sucrose octasulfate.

[00558] In some aspects, the disclosure provides for a lipid nanoparticle comprising ceDNA and an ionizable lipid. For example, a lipid nanoparticle formulation that is made and loaded with ceDNA obtained by the process as disclosed in International Application PCT/US2018/050042, filed on September 7, 2018, which is incorporated herein. This can be accomplished by high energy mixing of ethanolic lipids with aqueous ceDNA at low pH which protonates the ionizable lipid and provides favorable energetics for ceDNA/lipid association and nucleation of particles. The particles can be further stabilized through aqueous dilution and removal of the organic solvent. The particles can be concentrated to the desired level.

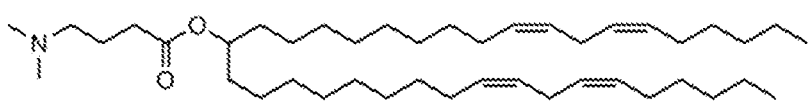
[00559] Generally, the lipid particles are prepared at a total lipid to ceDNA (mass or weight) ratio of from about 10:1 to 30:1. In some embodiments, the lipid to ceDNA ratio (mass/mass ratio; w/w ratio) can be in the range of from about 1:1 to about 25:1, from about 10:1 to about 14:1, from about 3:1 to about 15:1, from about 4:1 to about 10:1, from about 5:1 to about 9:1, or about 6:1 to about 9:1. The amounts of lipids and ceDNA can be adjusted to provide a desired N/P ratio, for example, N/P ratio of 3, 4, 5, 6, 7, 8, 9, 10 or higher. Generally, the lipid particle formulation's overall lipid content can range from about 5 mg/ml to about 30 mg/mL.

[00560] The ionizable lipid is typically employed to condense the nucleic acid cargo, *e.g.,* ceDNA at low pH and to drive membrane association and fusogenicity. Generally, ionizable lipids are lipids comprising at least one amino group that is positively charged or becomes protonated under acidic

conditions, for example at pH of 6.5 or lower. Ionizable lipids are also referred to as cationic lipids herein.

[00561] Exemplary ionizable lipids are described in International PCT patent publications WO2015/095340, WO2015/199952, WO2018/011633, WO2017/049245, WO2015/061467, WO2012/040184, WO2012/000104, WO2015/074085, WO2016/081029, WO2017/004143, WO2017/075531, WO2017/117528, WO2011/022460, WO2013/148541, WO2013/116126, WO2011/153120, WO2012/044638, WO2012/054365, WO2011/090965, WO2013/016058, WO2012/162210, WO2008/042973, WO2010/129709, WO2010/144740 , WO2012/099755, WO2013/049328, WO2013/086322, WO2013/086373, WO2011/071860, WO2009/132131, WO2010/048536, WO2010/088537, WO2010/054401, WO2010/054406 , WO2010/054405, WO2010/054384, WO2012/016184, WO2009/086558, WO2010/042877, WO2011/000106, WO2011/000107, WO2005/120152, WO2011/141705, WO2013/126803, WO2006/007712, WO2011/038160, WO2005/121348, WO2011/066651, WO2009/127060, WO2011/141704, WO2006/069782, WO2012/031043, WO2013/006825, WO2013/033563, WO2013/089151, WO2017/099823, WO2015/095346, and WO2013/086354, and US patent publications US2016/0311759, US2015/0376115, US2016/0151284, US2017/0210697, US2015/0140070, US2013/0178541, US2013/0303587, US2015/0141678, US2015/0239926, US2016/0376224, US2017/0119904, US2012/0149894, US2015/0057373, US2013/0090372, US2013/0274523, US2013/0274504, US2013/0274504, US2009/0023673, US2012/0128760, US2010/0324120, US2014/0200257, US2015/0203446, US2018/0005363, US2014/0308304, US2013/0338210, US2012/0101148, US2012/0027796, US2012/0058144, US2013/0323269, US2011/0117125, US2011/0256175, US2012/0202871, US2011/0076335, US2006/0083780, US2013/0123338, US2015/0064242, US2006/0051405, US2013/0065939, US2006/0008910, US2003/0022649, US2010/0130588, US2013/0116307, US2010/0062967, US2013/0202684, US2014/0141070, US2014/0255472, US2014/0039032, US2018/0028664, US2016/0317458, and US2013/0195920, the contents of all of which are incorporated herein by reference in their entireties.

[00562] In some embodiments, the ionizable lipid is MC3 (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino) butanoate (DLin-MC3-DMA or MC3) having the following structure:



DLin-M-C3-DMA ("MC3")

[00563] The lipid DLin-MC3-DMA is described in Jayaraman *et al.*, Angew. Chem. Int. Ed Engl. (2012), 51(34): 8529-8533, content of which is incorporated herein by reference in its entirety.

[00564] In some embodiments, the ionizable lipid is the lipid ATX-002 as described in WO2015/074085, content of which is incorporated herein by reference in its entirety.

[00565] In some embodiments, the ionizable lipid is (13*Z*,16*Z*)-*N*,*N*-dimethyl-3-nonyldocosa-13,16-dien-1-amine (Compound 32), as described in WO2012/040184, the contents of which is incorporated herein by reference in its entirety.

[00566] In some embodiments, the ionizable lipid is Compound 6 or Compound 22 as described in WO2015/199952, the contents of which is incorporated herein by reference in its entirety.

[00567] Without limitations, ionizable lipid can comprise 20-90% (mol) of the total lipid present in the lipid nanoparticle. For example, ionizable lipid molar content can be 20-70% (mol), 30-60% (mol) or 40-50% (mol) of the total lipid present in the lipid nanoparticle. In some embodiments, ionizable lipid comprises from about 50 mol % to about 90 mol % of the total lipid present in the lipid nanoparticle.

[00568] In some aspects, the lipid nanoparticle can further comprise a non-cationic lipid. Non-ionic lipids include amphipathic lipids, neutral lipids and anionic lipids. Accordingly, the non-cationic lipid can be a neutral uncharged, zwitterionic, or anionic lipid. Non-cationic lipids are typically employed to enhance fusogenicity.

[00569] Exemplary non-cationic lipids envisioned for use in the methods and compositions as disclosed herein are described in International Application PCT/US2018/050042, filed on September 7, 2018, and PCT/US2018/064242, filed on December 6, 2018 which is incorporated herein in its entirety. Exemplary non-cationic lipids are described in International Application Publication WO2017/099823 and US patent publication US2018/0028664, the contents of both of which are incorporated herein by reference in their entirety.

[00570] The non-cationic lipid can comprise 0-30% (mol) of the total lipid present in the lipid nanoparticle. For example, the non-cationic lipid content is 5-20% (mol) or 10-15% (mol) of the total lipid present in the lipid nanoparticle. In various embodiments, the molar ratio of ionizable lipid to the neutral lipid ranges from about 2:1 to about 8:1.

[00571] In some embodiments, the lipid nanoparticles do not comprise any phospholipids. In some aspects, the lipid nanoparticle can further comprise a component, such as a sterol, to provide membrane integrity.

[00572] One exemplary sterol that can be used in the lipid nanoparticle is cholesterol and derivatives thereof. Exemplary cholesterol derivatives are described in International application WO2009/127060 and US patent publication US2010/0130588, the contents of both of which are incorporated herein by reference in their entireties.

[00573] The component providing membrane integrity, such as a sterol, can comprise 0-50% (mol) of the total lipid present in the lipid nanoparticle. In some embodiments, such a component is 20-50% (mol) 30-40% (mol) of the total lipid content of the lipid nanoparticle.

[00574] In some aspects, the lipid nanoparticle can further comprise a polyethylene glycol (PEG) or a conjugated lipid molecule. Generally, these are used to inhibit aggregation of lipid nanoparticles and/or provide steric stabilization. Exemplary conjugated lipids include, but are not limited to, PEG-lipid conjugates, polyoxazoline (POZ)-lipid conjugates, polyamide-lipid conjugates (such as ATTA-lipid conjugates), cationic-polymer lipid (CPL) conjugates, and mixtures thereof. In some embodiments, the conjugated lipid molecule is a PEG-lipid conjugate, for example, a (methoxy polyethylene glycol)-conjugated lipid. Exemplary PEG-lipid conjugates include, but are not limited to, PEG-diacylglycerol (DAG) (such as l-(monomethoxy-polyethyleneglycol)-2,3-dimyristoylglycerol (PEG-DMG)), PEG-dialkyloxypropyl (DAA), PEG-phospholipid, PEG-ceramide (Cer), a pegylated phosphatidylethanoloamine (PEG-PE), PEG succinate diacylglycerol (PEGS-DAG) (such as 4-O-(2',3'-di(tetradecanoyloxy)propyl-1-O-(w-methoxy(polyethoxy)ethyl) butanedioate (PEG-S-DMG)), PEG dialkoxypropylcarbam, N-(carbonyl-methoxypolyethylene glycol 2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt, or a mixture thereof. Additional exemplary PEG-lipid conjugates are described, for example, in US5,885,613, US6,287,591, US2003/0077829, US2003/0077829, US2005/0175682, US2008/0020058, US2011/0117125, US2010/0130588, US2016/0376224, and US2017/0119904, the contents of all of which are incorporated herein by reference in their entireties.

[00575] In some embodiments, a PEG-lipid is a compound as defined in US2018/0028664, the contents of which is incorporated herein by reference in its entirety. In some embodiments, a PEG-lipid is disclosed in US20150376115 or in US2016/0376224, the content of both of which is incorporated herein by reference in its entirety.

[00576] The PEG-DAA conjugate can be, for example, PEG-dilauryloxypropyl, PEG-dimyristyloxypropyl, PEG-dipalmityloxypropyl, or PEG-distearyloxypropyl. The PEG-lipid can be one or more of PEG-DMG, PEG-dilaurylglycerol, PEG-dipalmitoylglycerol, PEG-disterylglycerol, PEG-dilaurylglycamide, PEG-dimyristylglycamide, PEG-dipalmitoylglycamide, PEG-disterylglycamide, PEG-cholesterol (1-[8'-(Cholest-5-en-3[beta]-oxy)carboxamido-3',6'-dioxaoctanyl] carbamoyl-[omega]-methyl-poly(ethylene glycol), PEG-DMB (3,4-Ditetradecoxylbenzyl- [omega]-methyl-poly(ethylene glycol) ether), and 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000]. In some examples, the PEG-lipid can be selected from the group consisting of PEG-DMG, 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000],

[00577] Lipids conjugated with a molecule other than a PEG can also be used in place of PEG-lipid. For example, polyoxazoline (POZ)-lipid conjugates, polyamide-lipid conjugates (such as ATTA-lipid conjugates), and cationic-polymer lipid (CPL) conjugates can be used in place of or in addition to the PEG-lipid. Exemplary conjugated lipids, *i.e.,* PEG-lipids, (POZ)-lipid conjugates, ATTA-lipid conjugates and cationic polymer-lipids are described in the International patent application

publications WO1996/010392, WO1998/051278, WO2002/087541, WO2005/026372, WO2008/147438, WO2009/086558, WO2012/000104, WO2017/117528, WO2017/099823, WO2015/199952, WO2017/004143, WO2015/095346, WO2012/000104, WO2012/000104, and WO2010/006282, US patent application publications US2003/0077829, US2005/0175682, US2008/0020058, US2011/0117125, US2013/0303587, US2018/0028664, US2015/0376115, US2016/0376224, US2016/0317458, US2013/0303587, US2013/0303587, and US20110123453, and US patents US5,885,613, US6,287,591, US6,320,017, and US6,586,559, the contents of all of which are incorporated herein by reference in their entireties.

[00578] In some embodiments, the one or more additional compound can be a therapeutic agent. The therapeutic agent can be selected from any class suitable for the therapeutic objective. In other words, the therapeutic agent can be selected from any class suitable for the therapeutic objective. In other words, the therapeutic agent can be selected according to the treatment objective and biological action desired. For example, if the ceDNA within the LNP is useful for treating hemophilia A, the additional compound can be an anti-hemophilia A agent (*e.g.,* a chemotherapeutic agent, or other hemophilia A therapy (including, but not limited to, a small molecule or an antibody). In another example, if the LNP containing the ceDNA is useful for treating an infection, the additional compound can be an antimicrobial agent (*e.g.,* an antibiotic or antiviral compound). In yet another example, if the LNP containing the ceDNA is useful for treating an immune disease or disorder, the additional compound can be a compound that modulates an immune response (*e.g.,* an immunosuppressant, immunostimulatory compound, or compound modulating one or more specific immune pathways). In some embodiments, different cocktails of different lipid nanoparticles containing different compounds, such as a ceDNA encoding a different protein or a different compound, such as a therapeutic may be used in the compositions and methods of the disclosure.

[00579] In some embodiments, the additional compound is an immune modulating agent. For example, the additional compound is an immunosuppressant. In some embodiments, the additional compound is immune stimulatory agent. Also provided herein is a pharmaceutical composition comprising the lipid nanoparticle-encapsulated insect-cell produced, or a synthetically produced ceDNA vector for expression of FVIII protein as described herein and a pharmaceutically acceptable carrier or excipient.

[00580] In some aspects, the disclosure provides for a lipid nanoparticle formulation further comprising one or more pharmaceutical excipients. In some embodiments, the lipid nanoparticle formulation further comprises sucrose, tris, trehalose and/or glycine.

[00581] The ceDNA vector can be complexed with the lipid portion of the particle or encapsulated in the lipid position of the lipid nanoparticle. In some embodiments, the ceDNA can be fully encapsulated in the lipid position of the lipid nanoparticle, thereby protecting it from degradation by a nuclease, *e.g.,* in an aqueous solution. In some embodiments, the ceDNA in the lipid nanoparticle is

not substantially degraded after exposure of the lipid nanoparticle to a nuclease at 37°C. for at least about 20, 30, 45, or 60 minutes. In some embodiments, the ceDNA in the lipid nanoparticle is not substantially degraded after incubation of the particle in serum at 37°C. for at least about 30, 45, or 60 minutes or at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, or 36 hours.

[00582] In certain embodiments, the lipid nanoparticles are substantially non-toxic to a subject, *e.g.*, to a mammal such as a human. In some aspects, the lipid nanoparticle formulation is a lyophilized powder.

[00583] In some embodiments, lipid nanoparticles are solid core particles that possess at least one lipid bilayer. In other embodiments, the lipid nanoparticles have a non-bilayer structure, *i.e.*, a non-lamellar (*i.e.*, non-bilayer) morphology. Without limitations, the non-bilayer morphology can include, for example, three dimensional tubes, rods, cubic symmetries, etc. For example, the morphology of the lipid nanoparticles (lamellar vs. non-lamellar) can readily be assessed and characterized using, *e.g.*, Cryo-TEM analysis as described in US2010/0130588, the content of which is incorporated herein by reference in its entirety.

[00584] In some further embodiments, the lipid nanoparticles having a non-lamellar morphology are electron dense. In some aspects, the disclosure provides for a lipid nanoparticle that is either unilamellar or multilamellar in structure. In some aspects, the disclosure provides for a lipid nanoparticle formulation that comprises multi-vesicular particles and/or foam-based particles.

[00585] By controlling the composition and concentration of the lipid components, one can control the rate at which the lipid conjugate exchanges out of the lipid particle and, in turn, the rate at which the lipid nanoparticle becomes fusogenic. In addition, other variables including, *e.g.*, pH, temperature, or ionic strength, can be used to vary and/or control the rate at which the lipid nanoparticle becomes fusogenic. Other methods which can be used to control the rate at which the lipid nanoparticle becomes fusogenic will be apparent to those of ordinary skill in the art based on this disclosure. It will also be apparent that by controlling the composition and concentration of the lipid conjugate, one can control the lipid particle size.

[00586] The pKa of formulated cationic lipids can be correlated with the effectiveness of the LNPs for delivery of nucleic acids (see Jayaraman *et al.*, Angewandte Chemie, International Edition (2012), 51(34), 8529-8533; Semple *et al.*, Nature Biotechnology 28, 172-176 (2010), both of which are incorporated by reference in their entirety). The preferred range of pKa is ~5 to ~ 7. The pKa of the cationic lipid can be determined in lipid nanoparticles using an assay based on fluorescence of 2-(p-toluidino)-6-napthalene sulfonic acid (TNS).

VIII. Methods of Use

[00587] A ceDNA vector for expression of FVIII protein as disclosed herein can also be used in a method for the delivery of a nucleic acid sequence of interest (*e.g.*, encoding FVIII protein) to a target

cell (*e.g.,* a host cell). In some embodiments, the method comprises a method for delivering FVIII protein to a cell of a subject in need thereof and treating hemophilia A. The disclosure allows for the *in vivo* expression of FVIII protein encoded in the ceDNA vector in a cell in a subject such that therapeutic effect of the expression of FVIII protein occurs. These results are seen with both *in vivo* and *in vitro* modes of ceDNA vector delivery.

[00588] In addition, the disclosure provides a method for the delivery of FVIII protein in a cell of a subject in need thereof, comprising multiple administrations of the ceDNA vector of the disclosure encoding said FVIII protein. Since the ceDNA vector of the disclosure does not induce an immune response like that typically observed against encapsidated viral vectors, such a multiple administration strategy will likely have greater success in a ceDNA-based system. The ceDNA vector are administered in sufficient amounts to transfect the cells of a desired tissue and to provide sufficient levels of gene transfer and expression of the FVIII protein without undue adverse effects. Conventional and pharmaceutically acceptable routes of administration include, but are not limited to, retinal administration (*e.g.,* subretinal injection, suprachoroidal injection or intravitreal injection), intravenous (*e.g.,* in a liposome formulation), direct delivery to the selected organ (*e.g.,* any one or more tissues selected from: liver, kidneys, gallbladder, prostate, adrenal gland, heart, intestine, lung, and stomach), intramuscular, and other parental routes of administration. Routes of administration may be combined, if desired.

[00589] Delivery of a ceDNA vector for expression of FVIII protein as described herein is not limited to delivery of the expressed FVIII protein. For example, conventionally produced (*e.g.,* using a cell-based production method (*e.g.,* insect-cell production methods) or synthetically produced ceDNA vectors as described herein may be used with other delivery systems provided to provide a portion of the gene therapy. One non-limiting example of a system that may be combined with the ceDNA vectors in accordance with the present disclosure includes systems which separately deliver one or more co-factors or immune suppressors for effective gene expression of the ceDNA vector expressing the FVIII protein.

[00590] The disclosure also provides for a method of treating hemophilia A in a subject comprising introducing into a target cell in need thereof (in particular a muscle cell or tissue) of the subject a therapeutically effective amount of a ceDNA vector, optionally with a pharmaceutically acceptable carrier. While the ceDNA vector can be introduced in the presence of a carrier, such a carrier is not required. The ceDNA vector selected comprises a nucleic acid sequence encoding an FVIII protein useful for treating hemophilia A. In particular, the ceDNA vector may comprise a desired FVIII protein sequence operably linked to control elements capable of directing transcription of the desired FVIII protein encoded by the exogenous DNA sequence when introduced into the subject. The ceDNA vector can be administered via any suitable route as provided above, and elsewhere herein.

[00591] The compositions and vectors provided herein can be used to deliver an FVIII protein for various purposes. In some embodiments, the transgene encodes an FVIII protein that is intended to be used for research purposes, *e.g.,* to create a somatic transgenic animal model harboring the transgene, *e.g.,* to study the function of the FVIII protein product. In another example, the transgene encodes an FVIII protein that is intended to be used to create an animal model of hemophilia A. In some embodiments, the encoded FVIII protein is useful for the treatment or prevention of hemophilia A states in a mammalian subject. The FVIII protein can be transferred (*e.g.,* expressed in) to a patient in a sufficient amount to treat hemophilia A associated with reduced expression, lack of expression or dysfunction of the gene.

[00592] In principle, the expression cassette can include a nucleic acid or any transgene that encodes an FVIII protein that is either reduced or absent due to a mutation or which conveys a therapeutic benefit when overexpressed is considered to be within the scope of the disclosure. Preferably, noninserted bacterial DNA is not present and preferably no bacterial DNA is present in the ceDNA compositions provided herein.

[00593] A ceDNA vector is not limited to one species of ceDNA vector. As such, in another aspect, multiple ceDNA vectors expressing different proteins or the same FVIII protein but operatively linked to different promoters or cis-regulatory elements can be delivered simultaneously or sequentially to the target cell, tissue, organ, or subject. Therefore, this strategy can allow for the gene therapy or gene delivery of multiple proteins simultaneously. It is also possible to separate different portions of a FVIII protein into separate ceDNA vectors (*e.g.,* different domains and/or co-factors required for functionality of a FVIII protein) which can be administered simultaneously or at different times, and can be separately regulatable, thereby adding an additional level of control of expression of a FVIII protein. Delivery can also be performed multiple times and, importantly for gene therapy in the clinical setting, in subsequent increasing or decreasing doses, given the lack of an anti-capsid host immune response due to the absence of a viral capsid. It is anticipated that no anti-capsid response will occur as there is no capsid.

[00594] The disclosure also provides for a method of treating hemophilia A in a subject comprising introducing into a target cell in need thereof (in particular a muscle cell or tissue) of the subject a therapeutically effective amount of a ceDNA vector as disclosed herein, optionally with a pharmaceutically acceptable carrier. While the ceDNA vector can be introduced in the presence of a carrier, such a carrier is not required. The ceDNA vector implemented comprises a nucleic acid sequence of interest useful for treating the hemophilia A. In particular, the ceDNA vector may comprise a desired exogenous DNA sequence operably linked to control elements capable of directing transcription of the desired polypeptide, protein, or oligonucleotide encoded by the exogenous DNA sequence when introduced into the subject. The ceDNA vector can be administered via any suitable route as provided above, and elsewhere herein.

IX. Methods of delivering ceDNA vectors for FVIII protein production

[00595] In some embodiments, a ceDNA vector for expression of FVIII protein can be delivered to a target cell *in vitro* or *in vivo* by various suitable methods. ceDNA vectors alone can be applied or injected. According to embodiments, ceDNA vectors can be delivered to a cell without the help of a transfection reagent or other physical means. Alternatively, according to other embodiments, ceDNA vectors for expression of FVIII protein can be delivered using any art-known transfection reagent or other art-known physical means that facilitates entry of DNA into a cell, *e.g.,* liposomes, alcohols, polylysine- rich compounds, arginine-rich compounds, calcium phosphate, microvesicles, microinjection, electroporation and the like.

[00596] The ceDNA vectors for expression of FVIII protein as disclosed herein can efficiently target cell and tissue-types that are normally difficult to transduce with conventional AAV virions using various delivery reagent.

[00597] One aspect of the technology described herein relates to a method of delivering an FVIII protein to a cell. Typically, for *in vivo* and *in vitro* methods, a ceDNA vector for expression of FVIII protein as disclosed herein may be introduced into the cell using the methods as disclosed herein, as well as other methods known in the art. A ceDNA vector for expression of FVIII protein as disclosed herein are preferably administered to the cell in a biologically-effective amount. If the ceDNA vector is administered to a cell *in vivo* (*e.g.,* to a subject), a biologically-effective amount of the ceDNA vector is an amount that is sufficient to result in transduction and expression of the FVIII protein in a target cell.

[00598] Exemplary modes of administration of a ceDNA vector for expression of FVIII protein as disclosed herein includes oral, rectal, transmucosal, intranasal, inhalation (*e.g.,* via an aerosol), buccal (*e.g.,* sublingual), vaginal, intrathecal, intraocular, transdermal, intraendothelial, in utero (or in ovo), parenteral (*e.g.,* intravenous, subcutaneous, intradermal, intracranial, intramuscular [including administration to skeletal, diaphragm and/or cardiac muscle], intrapleural, intracerebral, and intraarticular). Administration can be systemically or direct delivery to the liver or elsewhere (*e.g.,* any kidneys, gallbladder, prostate, adrenal gland, heart, intestine, lung, and stomach).

[00599] Administration can be topical (*e.g.,* to both skin and mucosal surfaces, including airway surfaces, and transdermal administration), intralymphatic, and the like, as well as direct tissue or organ injection (*e.g.,* but not limited to, liver, but also to eye, muscles, including skeletal muscle, cardiac muscle, diaphragm muscle, or brain).

[00600] Administration of the ceDNA vector can be to any site in a subject, including, without limitation, a site selected from the group consisting of the liver and/or also eyes, brain, a skeletal muscle, a smooth muscle, the heart, the diaphragm, the airway epithelium, the kidney, the spleen, the pancreas, the skin.

[00601] The most suitable route in any given case will depend on the nature and severity of the condition being treated, ameliorated, and/or prevented and on the nature of the particular ceDNA vector that is being used. Additionally, ceDNA permits one to administer more than one FVIII protein in a single vector, or multiple ceDNA vectors (*e.g.,* a ceDNA cocktail).

A. Intramuscular Administration of a ceDNA vector

[00602] In some embodiments, a method of treating a disease in a subject comprises introducing into a target cell in need thereof (in particular a muscle cell or tissue) of the subject a therapeutically effective amount of a ceDNA vector encoding an FVIII protein, optionally with a pharmaceutically acceptable carrier. In some embodiments, the ceDNA vector for expression of FVIII protein is administered to a muscle tissue of a subject.

[00603] In some embodiments, administration of the ceDNA vector can be to any site in a subject, including, without limitation, a site selected from the group consisting of a skeletal muscle, a smooth muscle, the heart, the diaphragm, or muscles of the eye.

[00604] Administration of a ceDNA vector for expression of FVIII protein as disclosed herein to a skeletal muscle according to the present disclosure includes but is not limited to administration to the skeletal muscle in the limbs (*e.g.,* upper arm, lower arm, upper leg, and/or lower leg), back, neck, head (*e.g.,* tongue), thorax, abdomen, pelvis/perineum, and/or digits. The ceDNA as disclosed herein vector can be delivered to skeletal muscle by intravenous administration, intra-arterial administration, intraperitoneal administration, limb perfusion, (optionally, isolated limb perfusion of a leg and/or arm; see, *e.g.,* Arruda *et al.*, (2005) Blood 105: 3458-3464), and/or direct intramuscular injection. In particular embodiments, the ceDNA vector as disclosed herein is administered to the liver, eye, a limb (arm and/or leg) of a subject (*e.g.,* a subject with muscular dystrophy such as DMD) by limb perfusion, optionally isolated limb perfusion (*e.g.,* by intravenous or intra-articular administration. In embodiments, the ceDNA vector as disclosed herein can be administered without employing "hydrodynamic" techniques.

[00605] For instance, tissue delivery (*e.g.,* to retina) of conventional viral vectors is often enhanced by hydrodynamic techniques (*e.g.,* intravenous/intravenous administration in a large volume), which increase pressure in the vasculature and facilitate the ability of the viral vector to cross the endothelial cell barrier. In particular embodiments, the ceDNA vectors described herein can be administered in the absence of hydrodynamic techniques such as high volume infusions and/or elevated intravascular pressure (*e.g.,* greater than normal systolic pressure, for example, less than or equal to a 5%, 10%, 15%, 20%, 25% increase in intravascular pressure over normal systolic pressure). Such methods may reduce or avoid the side effects associated with hydrodynamic techniques such as edema, nerve damage and/or compartment syndrome.

[00606] Furthermore, a composition comprising a ceDNA vector for expression of FVIII protein as disclosed herein that is administered to a skeletal muscle can be administered to a skeletal muscle in

the limbs (*e.g.,* upper arm, lower arm, upper leg, and/or lower leg), back, neck, head (*e.g.,* tongue), thorax, abdomen, pelvis/perineum, and/or digits. Suitable skeletal muscles include but are not limited to abductor digiti minimi (in the hand), abductor digiti minimi (in the foot), abductor hallucis, abductor ossis metatarsi quinti, abductor pollicis brevis, abductor pollicis longus, adductor brevis, adductor hallucis, adductor longus, adductor magnus, adductor pollicis, anconeus, anterior scalene, articularis genus, biceps brachii, biceps femoris, brachialis, brachioradialis, buccinator, coracobrachialis, corrugator supercilii, deltoid, depressor anguli oris, depressor labii inferioris, digastric, dorsal interossei (in the hand), dorsal interossei (in the foot), extensor carpi radialis brevis, extensor carpi radialis longus, extensor carpi ulnaris, extensor digiti minimi, extensor digitorum, extensor digitorum brevis, extensor digitorum longus, extensor hallucis brevis, extensor hallucis longus, extensor indicis, extensor pollicis brevis, extensor pollicis longus, flexor carpi radialis, flexor carpi ulnaris, flexor digiti minimi brevis (in the hand), flexor digiti minimi brevis (in the foot), flexor digitorum brevis, flexor digitorum longus, flexor digitorum profundus, flexor digitorum superficialis, flexor hallucis brevis, flexor hallucis longus, flexor pollicis brevis, flexor pollicis longus, frontalis, gastrocnemius, geniohyoid, gluteus maximus, gluteus medius, gluteus minimus, gracilis, iliocostalis cervicis, iliocostalis lumborum, iliocostalis thoracis, illiacus, inferior gemellus, inferior oblique, inferior rectus, infraspinatus, interspinalis, intertransversi, lateral pterygoid, lateral rectus, latissimus dorsi, levator anguli oris, levator labii superioris, levator labii superioris alaeque nasi, levator palpebrae superioris, levator scapulae, long rotators, longissimus capitis, longissimus cervicis, longissimus thoracis, longus capitis, longus colli, lumbricals (in the hand), lumbricals (in the foot), masseter, medial pterygoid, medial rectus, middle scalene, multifidus, mylohyoid, obliquus capitis inferior, obliquus capitis superior, obturator externus, obturator internus, occipitalis, omohyoid, opponens digiti minimi, opponens pollicis, orbicularis oculi, orbicularis oris, palmar interossei, palmaris brevis, palmaris longus, pectineus, pectoralis major, pectoralis minor, peroneus brevis, peroneus longus, peroneus tertius, piriformis, plantar interossei, plantaris, platysma, popliteus, posterior scalene, pronator quadratus, pronator teres, psoas major, quadratus femoris, quadratus plantae, rectus capitis anterior, rectus capitis lateralis, rectus capitis posterior major, rectus capitis posterior minor, rectus femoris, rhomboid major, rhomboid minor, risorius, sartorius, scalenus minimus, semimembranosus, semispinalis capitis, semispinalis cervicis, semispinalis thoracis, semitendinosus, serratus anterior, short rotators, soleus, spinalis capitis, spinalis cervicis, spinalis thoracis, splenius capitis, splenius cervicis, sternocleidomastoid, sternohyoid, sternothyroid, stylohyoid, subclavius, subscapularis, superior gemellus, superior oblique, superior rectus, supinator, supraspinatus, temporalis, tensor fascia lata, teres major, teres minor, thoracis, thyrohyoid, tibialis anterior, tibialis posterior, trapezius, triceps brachii, vastus intermedius, vastus lateralis, vastus medialis, zygomaticus major, and zygomaticus minor, and any other suitable skeletal muscle as known in the art.

[00607] Administration of a ceDNA vector for expression of FVIII protein as disclosed herein to diaphragm muscle can be by any suitable method including intravenous administration, intra-arterial administration, and/or intra-peritoneal administration. In some embodiments, delivery of an expressed transgene from the ceDNA vector to a target tissue can also be achieved by delivering a synthetic depot comprising the ceDNA vector, where a depot comprising the ceDNA vector is implanted into skeletal, smooth, cardiac and/or diaphragm muscle tissue or the muscle tissue can be contacted with a film or other matrix comprising the ceDNA vector as described herein. Such implantable matrices or substrates are described in U.S. Pat. No. 7,201,898.

[00608] Administration of a ceDNA vector for expression of FVIII protein as disclosed herein to cardiac muscle includes administration to the left atrium, right atrium, left ventricle, right ventricle and/or septum. The ceDNA vector as described herein can be delivered to cardiac muscle by intravenous administration, intra-arterial administration such as intra-aortic administration, direct cardiac injection (*e.g.,* into left atrium, right atrium, left ventricle, right ventricle), and/or coronary artery perfusion.

[00609] Administration of a ceDNA vector for expression of FVIII protein as disclosed herein to smooth muscle can be by any suitable method including intravenous administration, intra-arterial administration, and/or intra-peritoneal administration. In one embodiment, administration can be to endothelial cells present in, near, and/or on smooth muscle. Non-limiting examples of smooth muscles include the iris of the eye, bronchioles of the lung, laryngeal muscles (vocal cords), muscular layers of the stomach, esophagus, small and large intestine of the gastrointestinal tract, ureter, detrusor muscle of the urinary bladder, uterine myometrium, penis, or prostate gland.

[00610] In some embodiments, of a ceDNA vector for expression of FVIII protein as disclosed herein is administered to skeletal muscle, diaphragm muscle and/or cardiac muscle. In representative embodiments, a ceDNA vector according to the present disclosure is used to treat and/or prevent disorders of skeletal, cardiac and/or diaphragm muscle.

[00611] Specifically, it is contemplated that a composition comprising a ceDNA vector for expression of FVIII protein as disclosed herein can be delivered to one or more muscles of the eye (*e.g.,* Lateral rectus, Medial rectus, Superior rectus, Inferior rectus, Superior oblique, Inferior oblique), facial muscles (*e.g.,* Occipitofrontalis muscle, Temporoparietalis muscle, Procerus muscle, Nasalis muscle, Depressor septi nasi muscle, Orbicularis oculi muscle, Corrugator supercilii muscle, Depressor supercilii muscle, Auricular muscles, Orbicularis oris muscle, Depressor anguli oris muscle, Risorius, Zygomaticus major muscle, Zygomaticus minor muscle, Levator labii superioris, Levator labii superioris alaeque nasi muscle, Depressor labii inferioris muscle, Levator anguli oris, Buccinator muscle, Mentalis) or tongue muscles (*e.g.,* genioglossus, hyoglossus, chondroglossus, styloglossus, palatoglossus, superior longitudinal muscle, inferior longitudinal muscle, the vertical muscle, and the transverse muscle).

(i) Intramuscular injection: In some embodiments, a composition comprising a ceDNA vector for expression of FVIII protein as disclosed herein can be injected into one or more sites of a given muscle, for example, skeletal muscle (*e.g.,* deltoid, vastus lateralis, ventrogluteal muscle of dorsogluteal muscle, or anterolateral thigh for infants) in a subject using a needle. The composition comprising ceDNA can be introduced to other subtypes of muscle cells. Non-limiting examples of muscle cell subtypes include skeletal muscle cells, cardiac muscle cells, smooth muscle cells and/or diaphragm muscle cells.

[00612] Methods for intramuscular injection are known to those of skill in the art and as such are not described in detail herein. However, when performing an intramuscular injection, an appropriate needle size should be determined based on the age and size of the patient, the viscosity of the composition, as well as the site of injection. **Table 19** provides guidelines for exemplary sites of injection and corresponding needle size:

Table 19: Guidelines for intramuscular injection in human patients

Injection Site	***Needle Gauge***	***Needle Size***	***Max. vol. of composition***
Ventrogluteal site (gluteus medius and gluteus minimus)	*Aqueous solutions:* 20-25 gauge *Viscous or oil-based solution*: 18-21 gauge	Thin adult: 15 to 25 mm Average adult: 25 mm Larger adult (over 150 lbs): 25 to 38 mm. Children and infants: will require a smaller needle	3mL
Vastus lateralis	*Aqueous solutions:* 20-25 gauge *Viscous or oil-based solution*: 18-21 gauge Children/infants: 22 to 25 gauge	Adult: 25 mm to 38 mm	3mL
Deltoid	22 to 25 gauge	Males: 130-260lbs: 25 mm Females: <130 lbs: 16 mm 130-200 lbs: 25mm >200lbs: 38mm	1mL

[00613] In certain embodiments, a ceDNA vector for expression of FVIII protein as disclosed herein is formulated in a small volume, for example, an exemplary volume as outlined in **Table 8** for a given subject. In some embodiments, the subject can be administered a general or local anesthetic prior to the injection, if desired. This is particularly desirable if multiple injections are required or if a deeper muscle is injected, rather than the common injection sites noted above.

[00614] In some embodiments, intramuscular injection can be combined with electroporation, delivery pressure or the use of transfection reagents to enhance cellular uptake of the ceDNA vector.

(ii) Transfection Reagents: In some embodiments, a ceDNA vector for expression of FVIII protein as disclosed herein is formulated in compositions comprising one or more transfection reagents to facilitate uptake of the vectors into myotubes or muscle tissue. Thus, in one embodiment, the nucleic acids described herein are administered to a muscle cell, myotube or muscle tissue by transfection using methods described elsewhere herein.

(iii) Electroporation: In certain embodiments, a ceDNA vector for expression of FVIII protein as disclosed herein is administered in the absence of a carrier to facilitate entry of ceDNA into the cells, or in a physiologically inert pharmaceutically acceptable carrier (*i.e.,* any carrier that does not improve or enhance uptake of the capsid free, non-viral vectors into the myotubes). In such embodiments, the uptake of the capsid free, non-viral vector can be facilitated by electroporation of the cell or tissue.

[00615] Cell membranes naturally resist the passage of extracellular into the cell cytoplasm. One method for temporarily reducing this resistance is "electroporation", where electrical fields are used to create pores in cells without causing permanent damage to the cells. These pores are large enough to allow DNA vectors, pharmaceutical drugs, DNA, and other polar compounds to gain access to the interior of the cell. With time, the pores in the cell membrane close and the cell once again becomes impermeable.

[00616] Electroporation can be used in both *in vitro* and *in vivo* applications to introduce *e.g.,* exogenous DNA into living cells. *In vitro* applications typically mix a sample of live cells with the composition comprising *e.g.,* DNA. The cells are then placed between electrodes such as parallel plates and an electrical field is applied to the cell/composition mixture.

[00617] There are a number of methods for *in vivo* electroporation; electrodes can be provided in various configurations such as, for example, a caliper that grips the epidermis overlying a region of cells to be treated. Alternatively, needle-shaped electrodes may be inserted into the tissue, to access more deeply located cells. In either case, after the composition comprising *e.g.,* nucleic acids are injected into the treatment region, the electrodes apply an electrical field to the region. In some electroporation applications, this electric field comprises a single square wave pulse on the order of 100 to 500 V/cm. of about 10 to 60 ms duration. Such a pulse may be generated, for example, in known applications of the Electro Square Porator T820, made by the BTX Division of Genetronics, Inc.

[00618] Typically, successful uptake of *e.g.,* nucleic acids occurs only if the muscle is electrically stimulated immediately, or shortly after administration of the composition, for example, by injection into the muscle.

[00619] In certain embodiments, electroporation is achieved using pulses of electric fields or using low voltage/long pulse treatment regimens (*e.g.,* using a square wave pulse electroporation system). Exemplary pulse generators capable of generating a pulsed electric field include, for example, the ECM600, which can generate an exponential wave form, and the ElectroSquarePorator (T820), which can generate a square wave form, both of which are available from BTX, a division of Genetronics, Inc. (San Diego, Calif.). Square wave electroporation systems deliver controlled electric pulses that rise quickly to a set voltage, stay at that level for a set length of time (pulse length), and then quickly drop to zero.

[00620] In some embodiments, a local anesthetic is administered, for example, by injection at the site of treatment to reduce pain that may be associated with electroporation of the tissue in the presence of a composition comprising a capsid free, non-viral vector as described herein. In addition, one of skill in the art will appreciate that a dose of the composition should be chosen that minimizes and/or prevents excessive tissue damage resulting in fibrosis, necrosis or inflammation of the muscle.

(iv) Delivery Pressure: In some embodiments, delivery of a ceDNA vector for expression of FVIII protein as disclosed herein to muscle tissue is facilitated by delivery pressure, which uses a combination of large volumes and rapid injection into an artery supplying a limb (*e.g.,* iliac artery). This mode of administration can be achieved through a variety of methods that involve infusing limb vasculature with a composition comprising a ceDNA vector, typically while the muscle is isolated from the systemic circulation using a tourniquet of vessel clamps. In one method, the composition is circulated through the limb vasculature to permit extravasation into the cells. In another method, the intravascular hydrodynamic pressure is increased to expand vascular beds and increase uptake of the ceDNA vector into the muscle cells or tissue. In one embodiment, the ceDNA composition is administered into an artery.

(v) Lipid Nanoparticle Compositions: In some embodiments, a ceDNA vector for expression of FVIII protein as disclosed herein for intramuscular delivery are formulated in a composition comprising a liposome as described elsewhere herein.

(vi) Systemic Administration of a ceDNA Vector targeted to Muscle Tissue: In some embodiments, a ceDNA vector for expression of FVIII protein as disclosed herein is formulated to be targeted to the muscle via indirect delivery administration, where the ceDNA is transported to the muscle as opposed to the liver. Accordingly, the technology described herein encompasses indirect administration of compositions comprising a ceDNA vector for expression of FVIII protein as disclosed herein to muscle tissue, for example, by systemic administration. Such compositions can be administered topically, intravenously (by bolus or continuous infusion), intracellular injection, intratissue injection, orally, by inhalation, intraperitoneally, subcutaneously, intracavity, and can be delivered by peristaltic means, if desired, or by other means known by those skilled in the art. The agent can be administered systemically, for example, by intravenous infusion, if so desired.

[00621] In some embodiments, uptake of a ceDNA vector for expression of FVIII protein as disclosed herein into muscle cells/tissue is increased by using a targeting agent or moiety that preferentially directs the vector to muscle tissue. Thus, in some embodiments, a capsid free, ceDNA vector can be concentrated in muscle tissue as compared to the amount of capsid free ceDNA vectors present in other cells or tissues of the body.

[00622] In some embodiments, the composition comprising a ceDNA vector for expression of FVIII protein as disclosed herein further comprises a targeting moiety to muscle cells. In other embodiments, the expressed gene product comprises a targeting moiety specific to the tissue in which it is desired to act. The targeting moiety can include any molecule, or complex of molecules, which is/are capable of targeting, interacting with, coupling with, and/or binding to an intracellular, cell surface, or extracellular biomarker of a cell or tissue. The biomarker can include, for example, a cellular protease, a kinase, a protein, a cell surface receptor, a lipid, and/or fatty acid. Other examples of biomarkers that the targeting moieties can target, interact with, couple with, and/or bind to include molecules associated with a particular disease. For example, the biomarkers can include cell surface receptors implicated in cancer development, such as epidermal growth factor receptor and transferrin receptor. The targeting moieties can include, but are not limited to, synthetic compounds, natural compounds or products, macromolecular entities, bioengineered molecules (*e.g.,* polypeptides, lipids, polynucleotides, antibodies, antibody fragments), and small entities (*e.g.,* small molecules, neurotransmitters, substrates, ligands, hormones and elemental compounds) that bind to molecules expressed in the target muscle tissue.

[00623] In certain embodiments, the targeting moiety may further comprise a receptor molecule, including, for example, receptors, which naturally recognize a specific desired molecule of a target cell. Such receptor molecules include receptors that have been modified to increase their specificity of interaction with a target molecule, receptors that have been modified to interact with a desired target molecule not naturally recognized by the receptor, and fragments of such receptors (see, *e.g.,* Skerra, 2000, J. Molecular Recognition, 13:167-187). A preferred receptor is a chemokine receptor. Exemplary chemokine receptors have been described in, for example, Lapidot et al, 2002, Exp Hematol, 30:973-81 and Onuffer *et al.*, 2002, Trends Pharmacol Sci, 23:459-67.

[00624] In other embodiments, the additional targeting moiety may comprise a ligand molecule, including, for example, ligands which naturally recognize a specific desired receptor of a target cell, such as a Transferrin (Tf) ligand. Such ligand molecules include ligands that have been modified to increase their specificity of interaction with a target receptor, ligands that have been modified to interact with a desired receptor not naturally recognized by the ligand, and fragments of such ligands.

[00625] In still other embodiments, the targeting moiety may comprise an aptamer. Aptamers are oligonucleotides that are selected to bind specifically to a desired molecular structure of the target cell. Aptamers typically are the products of an affinity selection process similar to the affinity selection of

phage display (also known as *in vitro* molecular evolution). The process involves performing several tandem iterations of affinity separation, *e.g.,* using a solid support to which the diseased immunogen is bound, followed by polymerase chain reaction (PCR) to amplify nucleic acids that bound to the immunogens. Each round of affinity separation thus enriches the nucleic acid population for molecules that successfully bind the desired immunogen. In this manner, a random pool of nucleic acids may be "educated" to yield aptamers that specifically bind target molecules. Aptamers typically are RNA, but may be DNA or analogs or derivatives thereof, such as, without limitation, peptide nucleic acids (PNAs) and phosphorothioate nucleic acids.

[00626] In some embodiments, the targeting moiety can comprise a photo-degradable ligand (*i.e.,* a 'caged' ligand) that is released, for example, from a focused beam of light such that the capsid free, non-viral vectors or the gene product are targeted to a specific tissue.

[00627] It is also contemplated herein that the compositions be delivered to multiple sites in one or more muscles of the subject. That is, injections can be in at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 100 injections sites. Such sites can be spread over the area of a single muscle or can be distributed among multiple muscles.

B. Administration of the ceDNA vector for expression of FVIII protein to non-muscle locations

[00628] In another embodiment, a ceDNA vector for expression of FVIII protein is administered to the liver. The ceDNA vector may also be administered to different regions of the eye such as the cornea and/or optic nerve The ceDNA vector may also be introduced into the spinal cord, brainstem (medulla oblongata, pons), midbrain (hypothalamus, thalamus, epithalamus, pituitary gland, substantia nigra, pineal gland), cerebellum, telencephalon (corpus striatum, cerebrum including the occipital, temporal, parietal and frontal lobes, cortex, basal ganglia, hippocampus and portaamygdala), limbic system, neocortex, corpus striatum, cerebrum, and inferior colliculus.. The ceDNA vector may be delivered into the cerebrospinal fluid (*e.g.,* by lumbar puncture). The ceDNA vector for expression of FVIII protein may further be administered intravascularly to the CNS in situations in which the blood-brain barrier has been perturbed (*e.g.,* brain tumor or cerebral infarct).

[00629] In some embodiments, the ceDNA vector for expression of FVIII protein can be administered to the desired region(s) of the eye by any route known in the art, including but not limited to, intrathecal, intra-ocular, intracerebral, intraventricular, intravenous (*e.g.,* in the presence of a sugar such as mannitol), intranasal, intra-aural, intra-ocular (*e.g.,* intra-vitreous, sub-retinal, anterior chamber) and peri-ocular (*e.g.,* sub-Tenon's region) delivery as well as intramuscular delivery with retrograde delivery to motor neurons.

[00630] In some embodiments, the ceDNA vector for expression of FVIII protein is administered in a liquid formulation by direct injection (*e.g.,* stereotactic injection) to the desired region or compartment in the CNS. In other embodiments, the ceDNA vector can be provided by topical application to the desired region or by intra-nasal administration of an aerosol formulation. Administration to the eye may be by topical application of liquid droplets. As a further alternative, the ceDNA vector can be administered as a solid, slow-release formulation (see, *e.g.,* U.S. Pat. No. 7,201,898). In yet additional embodiments, the ceDNA vector can used for retrograde transport to treat, ameliorate, and/or prevent diseases and disorders involving motor neurons (*e.g.,* amyotrophic lateral sclerosis (ALS); spinal muscular atrophy (SMA), etc.). For example, the ceDNA vector can be delivered to muscle tissue from which it can migrate into neurons.

C. Ex vivo treatment

[00631] In some embodiments, cells are removed from a subject, a ceDNA vector for expression of FVIII protein as disclosed herein is introduced therein, and the cells are then replaced back into the subject. Methods of removing cells from subject for treatment *ex vivo*, followed by introduction back into the subject are known in the art (see, *e.g.,* U.S. Pat. No. 5,399,346; the disclosure of which is incorporated herein in its entirety). Alternatively, a ceDNA vector is introduced into cells from another subject, into cultured cells, or into cells from any other suitable source, and the cells are administered to a subject in need thereof.

[00632] Cells transduced with a ceDNA vector for expression of FVIII protein as disclosed herein are preferably administered to the subject in a "therapeutically-effective amount" in combination with a pharmaceutical carrier. Those skilled in the art will appreciate that the therapeutic effects need not be complete or curative, as long as some benefit is provided to the subject.

[00633] In some embodiments, a ceDNA vector for expression of FVIII protein as disclosed herein can encode an FVIII protein as described herein (sometimes called a transgene or heterologous nucleic acid sequence) that is to be produced in a cell *in vitro, ex vivo,* or *in vivo*. For example, in contrast to the use of the ceDNA vectors described herein in a method of treatment as discussed herein, in some embodiments a ceDNA vector for expression of FVIII protein may be introduced into cultured cells and the expressed FVIII protein isolated from the cells, *e.g.,* for the production of antibodies and fusion proteins. In some embodiments, the cultured cells comprising a ceDNA vector for expression of FVIII protein as disclosed herein can be used for commercial production of antibodies or fusion proteins, *e.g.,* serving as a cell source for small or large scale biomanufacturing of antibodies or fusion proteins. In alternative embodiments, a ceDNA vector for expression of FVIII protein as disclosed herein is introduced into cells in a host non-human subject, for *in vivo* production of antibodies or fusion proteins, including small scale production as well as for commercial large scale FVIII protein production.

[00634] The ceDNA vectors for expression of FVIII protein as disclosed herein can be used in both veterinary and medical applications. Suitable subjects for *ex vivo* gene delivery methods as described above include both avians (*e.g.,* chickens, ducks, geese, quail, turkeys and pheasants) and mammals (*e.g.,* humans, bovines, ovines, caprines, equines, felines, canines, and lagomorphs), with mammals being preferred. Human subjects are most preferred. Human subjects include neonates, infants, juveniles, and adults.

D. Dose ranges

[00635] Provided herein are methods of treatment comprising administering to the subject an effective amount of a composition comprising a ceDNA vector encoding an FVIII protein as described herein. As will be appreciated by a skilled practitioner, the term "effective amount" refers to the amount of the ceDNA composition administered that results in expression of the FVIII protein in a "therapeutically effective amount" for the treatment of hemophilia A.

[00636] *In vivo* and/or *in vitro* assays can optionally be employed to help identify optimal dosage ranges for use. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the condition, and should be decided according to the judgment of the person of ordinary skill in the art and each subject's circumstances. Effective doses can be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

[00637] A ceDNA vectors for expression of FVIII protein as disclosed herein is administered in sufficient amounts to transfect the cells of a desired tissue and to provide sufficient levels of gene transfer and expression without undue adverse effects. Conventional and pharmaceutically acceptable routes of administration include, but are not limited to, those described above in the "Administration" section, such as direct delivery to the selected organ (*e.g.,* intraportal delivery to the liver), oral, inhalation (including intranasal and intratracheal delivery), intraocular, intravenous, intramuscular, subcutaneous, intradermal, intratumoral, and other parental routes of administration. Routes of administration can be combined, if desired.

[00638] The dose of the amount of a ceDNA vectors for expression of FVIII protein as disclosed herein required to achieve a particular "therapeutic effect," will vary based on several factors including, but not limited to: the route of nucleic acid administration, the level of gene or RNA expression required to achieve a therapeutic effect, the specific disease or disorder being treated, and the stability of the gene(s), RNA product(s), or resulting expressed protein(s). One of skill in the art can readily determine a ceDNA vector dose range to treat a patient having a particular disease or disorder based on the aforementioned factors, as well as other factors that are well known in the art.

[00639] Dosage regime can be adjusted to provide the optimum therapeutic response. For example, the oligonucleotide can be repeatedly administered, *e.g.,* several doses can be administered daily, or the dose can be proportionally reduced as indicated by the exigencies of the therapeutic situation. One of ordinary skill in the art will readily be able to determine appropriate doses and schedules of

administration of the subject oligonucleotides, whether the oligonucleotides are to be administered to cells or to subjects.

[00640] A "therapeutically effective dose" will fall in a relatively broad range that can be determined through clinical trials and will depend on the particular application (neural cells will require very small amounts, while systemic injection would require large amounts). For example, for direct *in vivo* injection into skeletal or cardiac muscle of a human subject, a therapeutically effective dose will be on the order of from about 1 μg to 100 g of the ceDNA vector. If exosomes or microparticles are used to deliver the ceDNA vector, then a therapeutically effective dose can be determined experimentally, but is expected to deliver from 1 μg to about 100 g of vector. Moreover, a therapeutically effective dose is an amount ceDNA vector that expresses a sufficient amount of the transgene to have an effect on the subject that results in a reduction in one or more symptoms of the disease, but does not result in significant off-target or significant adverse side effects. In one embodiment, a "therapeutically effective amount" is an amount of an expressed FVIII protein that is sufficient to produce a statistically significant, measurable change in expression of hemophilia A biomarker or reduction of a given disease symptom. Such effective amounts can be gauged in clinical trials as well as animal studies for a given ceDNA vector composition.

[00641] Formulation of pharmaceutically acceptable excipients and carrier solutions is well-known to those of skill in the art, as is the development of suitable dosing and treatment regimens for using the particular compositions described herein in a variety of treatment regimens.

[00642] For *in vitro* transfection, an effective amount of a ceDNA vectors for expression of FVIII protein as disclosed herein to be delivered to cells ($1x10^6$ cells) will be on the order of 0.1 to 100 μg ceDNA vector, preferably 1 to 20 μg, and more preferably 1 to 15 μg or 8 to 10 μg. Larger ceDNA vectors will require higher doses. If exosomes or microparticles are used, an effective *in vitro* dose can be determined experimentally but would be intended to deliver generally the same amount of the ceDNA vector.

[00643] For the treatment of hemophilia A, the appropriate dosage of a ceDNA vector that expresses an FVIII protein as disclosed herein will depend on the specific type of disease to be treated, the type of a FVIII protein, the severity and course of the hemophilia A disease, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The ceDNA vector encoding a FVIII protein is suitably administered to the patient at one time or over a series of treatments. Various dosing schedules including, but not limited to, single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

[00644] Depending on the type and severity of the disease, a ceDNA vector is administered in an amount that the encoded FVIII protein is expressed at about 0.3 mg/kg to 100 mg/kg (*e.g.,* 15 mg/kg-100 mg/kg, or any dosage within that range), by one or more separate administrations, or by continuous infusion. One typical daily dosage of the ceDNA vector is sufficient to result in the

expression of the encoded FVIII protein at a range from about 15 mg/kg to 100 mg/kg or more, depending on the factors mentioned above. One exemplary dose of the ceDNA vector is an amount sufficient to result in the expression of the encoded FVIII protein as disclosed herein in a range from about 10 mg/kg to about 50 mg/kg. Thus, one or more doses of a ceDNA vector in an amount sufficient to result in the expression of the encoded FVIII protein at about 0.5 mg/kg, 1 mg/kg, 1.5 mg/kg, 2.0 mg/kg, 3 mg/kg, 4.0 mg/kg, 5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg, 35 mg/kg, 40 mg/kg, 50 mg/kg, 60 mg/kg, 70 mg/kg, 80 mg/kg, 90 mg/kg, or 100 mg/kg (or any combination thereof) may be administered to the patient. In some embodiments, the ceDNA vector is an amount sufficient to result in the expression of the encoded FVIII protein for a total dose in the range of 50 mg to 2500 mg. An exemplary dose of a ceDNA vector is an amount sufficient to result in the total expression of the encoded FVIII protein at about 50 mg, about 100 mg, 200 mg, 300 mg, 400 mg, about 500 mg, about 600 mg, about 700 mg, about 720 mg, about 1000 mg, about 1050 mg, about 1100 mg, about 1200 mg, about 1300 mg, about 1400 mg, about 1500 mg, about 1600 mg, about 1700 mg, about 1800 mg, about 1900 mg, about 2000 mg, about 2050 mg, about 2100 mg, about 2200 mg, about 2300 mg, about 2400 mg, or about 2500 mg (or any combination thereof). As the expression of the FVIII protein from ceDNA vector can be carefully controlled by regulatory switches herein, or alternatively multiple dose of the ceDNA vector administered to the subject, the expression of the FVIII protein from the ceDNA vector can be controlled in such a way that the doses of the expressed FVIII protein may be administered intermittently, *e.g.,* every week, every two weeks, every three weeks, every four weeks, every month, every two months, every three months, or every six months from the ceDNA vector. The progress of this therapy can be monitored by conventional techniques and assays.

[00645] In certain embodiments, a ceDNA vector is administered an amount sufficient to result in the expression of the encoded FVIII protein at a dose of 15 mg/kg, 30 mg/kg, 40 mg/kg, 45 mg/kg, 50 mg/kg, 60 mg/kg or a flat dose, *e.g.,* 300 mg, 500 mg, 700 mg, 800 mg, or higher. In some embodiments, the expression of the FVIII protein from the ceDNA vector is controlled such that the FVIII protein is expressed every day, every other day, every week, every 2 weeks or every 4 weeks for a period of time. In some embodiments, the expression of the FVIII protein from the ceDNA vector is controlled such that the FVIII protein is expressed every 2 weeks or every 4 weeks for a period of time. In certain embodiments, the period of time is 6 months, one year, eighteen months, two years, five years, ten years, 15 years, 20 years, or the lifetime of the patient.

[00646] Treatment can involve administration of a single dose or multiple doses. In some embodiments, more than one dose can be administered to a subject; in fact, multiple doses can be administered as needed, because the ceDNA vector elicits does not elicit an anti-capsid host immune response due to the absence of a viral capsid. As such, one of skill in the art can readily determine an

appropriate number of doses. The number of doses administered can, for example, be on the order of 1-100, preferably 2-20 doses.

[00647] Without wishing to be bound by any particular theory, the lack of typical anti-viral immune response elicited by administration of a ceDNA vector as described by the disclosure (*i.e.,* the absence of capsid components) allows the ceDNA vector for expression of FVIII protein to be administered to a host on multiple occasions. In some embodiments, the number of occasions in which a nucleic acid is delivered to a subject is in a range of 2 to 10 times (*e.g.,* 2, 3, 4, 5, 6, 7, 8, 9, or 10 times). In some embodiments, a ceDNA vector is delivered to a subject more than 10 times.

In some embodiments, a dose of a ceDNA vector for expression of FVIII protein as disclosed herein is administered to a subject no more than once per calendar day (*e.g.,* a 24-hour period). In some embodiments, a dose of a ceDNA vector is administered to a subject no more than once per 2, 3, 4, 5, 6, or 7 calendar days. In some embodiments, a dose of a ceDNA vector for expression of FVIII protein as disclosed herein is administered to a subject no more than once per calendar week (*e.g.,* 7 calendar days). In some embodiments, a dose of a ceDNA vector is administered to a subject no more than bi-weekly (*e.g.,* once in a two calendar week period). In some embodiments, a dose of a ceDNA vector is administered to a subject no more than once per calendar month (*e.g.,* once in 30 calendar days). In some embodiments, a dose of a ceDNA vector is administered to a subject no more than once per six calendar months. In some embodiments, a dose of a ceDNA vector is administered to a subject no more than once per calendar year (*e.g.,* 365 days or 366 days in a leap year). In particular embodiments, more than one administration (*e.g.,* two, three, four or more administrations) of a ceDNA vector for expression of FVIII protein as disclosed herein may be employed to achieve the desired level of gene expression over a period of various intervals, *e.g.,* daily, weekly, monthly, yearly, etc.

[00648] Administration of the ceDNA compositions described herein can be repeated for a limited period of time. In some embodiments, the doses are given periodically or by pulsed administration. In a preferred embodiment, the doses recited above are administered over several months. The duration of treatment depends upon the subject's clinical progress and responsiveness to therapy. Booster treatments over time are contemplated. Further, the level of expression can be titrated as the subject grows.

[00649] An FVIII therapeutic protein can be expressed in a subject for at least 1 week, at least 2 weeks, at least 1 month, at least 2 months, at least 6 months, at least 12 months/one year, at least 2 years, at least 5 years, at least 10 years, at least 15 years, at least 20 years, at least 30 years, at least 40 years, at least 50 years or more. Long-term expression can be achieved by repeated administration of the ceDNA vectors described herein at predetermined or desired intervals.

[00650] In some embodiments, a therapeutic a FVIII protein encoded by a ceDNA vector as disclosed herein can be regulated by a regulatory switch, inducible or repressible promotor so that it is

expressed in a subject for at least 1 hour, at least 2 hours, at least 5 hours, at least 10 hours, at least 12 hours, at least 18 hours, at least 24 hours, at least 36 hours, at least 48 hours, at least 72 hours, at least 1 week, at least 2 weeks, at least 1 month, at least 2 months, at least 6 months, at least 12 months/one year, at least 2 years, at least 5 years, at least 10 years, at least 15 years, at least 20 years, at least 30 years, at least 40 years, at least 50 years or more. In one embodiment, the expression can be achieved by repeated administration of the ceDNA vectors described herein at predetermined or desired intervals. Alternatively, a ceDNA vector for expression of FVIII protein as disclosed herein can further comprise components of a gene editing system (*e.g.,* CRISPR/Cas, TALENs, zinc finger endonucleases etc.) to permit insertion of the one or more nucleic acid sequences encoding the FVIII protein for substantially permanent treatment or "curing" the disease. Such ceDNA vectors comprising gene editing components are disclosed in International Application PCT/US18/64242, and can include the 5' and 3' homology arms (*e.g.,* SEQ ID NO: 151-154, or sequences with at least 40%, 50%, 60%, 70% or 80% homology thereto) for insertion of the nucleic acid encoding the a FVIII protein into safe harbor regions, such as, but not including albumin gene or CCR5 gene. By way of example, a ceDNA vector expressing a FVIII protein can comprise at least one genomic safe harbor (GSH)-specific homology arms for insertion of the FVIII transgene into a genomic safe harbor is disclosed in International Patent Application PCT/US2019/020225, filed on March 1, 2019, which is incorporated herein in its entirety by reference.

[00651] The duration of treatment depends upon the subject's clinical progress and responsiveness to therapy. Continuous, relatively low maintenance doses are contemplated after an initial higher therapeutic dose.

E. Unit dosage forms

[00652] In some embodiments, the pharmaceutical compositions comprising a ceDNA vector for expression of FVIII protein as disclosed herein can conveniently be presented in unit dosage form. A unit dosage form will typically be adapted to one or more specific routes of administration of the pharmaceutical composition. In some embodiments, the unit dosage form is adapted for droplets to be administered directly to the eye. In some embodiments, the unit dosage form is adapted for administration by inhalation. In some embodiments, the unit dosage form is adapted for administration by a vaporizer. In some embodiments, the unit dosage form is adapted for administration by a nebulizer. In some embodiments, the unit dosage form is adapted for administration by an aerosolizer. In some embodiments, the unit dosage form is adapted for oral administration, for buccal administration, or for sublingual administration. In some embodiments, the unit dosage form is adapted for intravenous, intramuscular, or subcutaneous administration. In some embodiments, the unit dosage form is adapted for subretinal injection, suprachoroidal injection or intravitreal injection.

[00653] In some embodiments, the unit dosage form is adapted for intrathecal or intracerebroventricular administration. In some embodiments, the pharmaceutical composition is formulated for topical administration. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect.

X. Methods of Treatment

[00654] The technology described herein also demonstrates methods for making, as well as methods of using the disclosed ceDNA vectors for expression of FVIII protein in a variety of ways, including, for example, *ex vivo*, *ex situ*, *in vitro and in vivo* applications, methodologies, diagnostic procedures, and/or gene therapy regimens.

[00655] In one embodiment, the expressed therapeutic FVIII protein expressed from a ceDNA vector as disclosed herein is functional for the treatment of disease. In a preferred embodiment, the therapeutic FVIII protein does not cause an immune system reaction, unless so desired.

[00656] Provided herein is a method of treating hemophilia A in a subject comprising introducing into a target cell in need thereof (for example, a muscle cell or tissue, or other affected cell type) of the subject a therapeutically effective amount of a ceDNA vector for expression of FVIII protein as disclosed herein, optionally with a pharmaceutically acceptable carrier. While the ceDNA vector can be introduced in the presence of a carrier, such a carrier is not required. The ceDNA vector implemented comprises a nucleic acid sequence encoding an FVIII protein as described herein useful for treating the disease. In particular, a ceDNA vector for expression of FVIII protein as disclosed herein may comprise a desired FVIII protein DNA sequence operably linked to control elements capable of directing transcription of the desired FVIII protein encoded by the exogenous DNA sequence when introduced into the subject. The ceDNA vector for expression of FVIII protein as disclosed herein can be administered via any suitable route as provided above, and elsewhere herein.

[00657] Disclosed herein are ceDNA vector compositions and formulations for expression of FVIII protein as disclosed herein that include one or more of the ceDNA vectors of the present disclosure together with one or more pharmaceutically-acceptable buffers, diluents, or excipients. Such compositions may be included in one or more diagnostic or therapeutic kits, for diagnosing, preventing, treating or ameliorating one or more symptoms of hemophilia A. In one aspect the disease, injury, disorder, trauma or dysfunction is a human disease, injury, disorder, trauma or dysfunction.

[00658] Another aspect of the technology described herein provides a method for providing a subject in need thereof with a diagnostically- or therapeutically-effective amount of a ceDNA vector for expression of FVIII protein as disclosed herein, the method comprising providing to a cell, tissue or organ of a subject in need thereof, an amount of the ceDNA vector as disclosed herein; and for a time effective to enable expression of the FVIII protein from the ceDNA vector thereby providing the

subject with a diagnostically- or a therapeutically-effective amount of the FVIII protein expressed by the ceDNA vector. In a further aspect, the subject is human.

[00659] Another aspect of the technology described herein provides a method for diagnosing, preventing, treating, or ameliorating at least one or more symptoms of hemophilia A, a disorder, a dysfunction, an injury, an abnormal condition, or trauma in a subject. In an overall and general sense, the method includes at least the step of administering to a subject in need thereof one or more of the disclosed ceDNA vector for FVIII protein production, in an amount and for a time sufficient to diagnose, prevent, treat or ameliorate the one or more symptoms of the disease, disorder, dysfunction, injury, abnormal condition, or trauma in the subject. In such an embodiment, the subject can be evaluated for efficacy of the FVIII protein, or alternatively, detection of the FVIII protein or tissue location (including cellular and subcellular location) of the FVIII protein in the subject. As such, the ceDNA vector for expression of FVIII protein as disclosed herein can be used as an *in vivo* diagnostic tool, *e.g.,* for the detection of cancer or other indications. In a further aspect, the subject is human.

[00660] Another aspect is use of a ceDNA vector for expression of FVIII protein as disclosed herein as a tool for treating or reducing one or more symptoms of hemophilia A or disease states. There are a number of inherited diseases in which defective genes are known, and typically fall into two classes: deficiency states, usually of enzymes, which are generally inherited in a recessive manner, and unbalanced states, which may involve regulatory or structural proteins, and which are typically but not always inherited in a dominant manner. For unbalanced disease states, a ceDNA vector for expression of FVIII protein as disclosed herein can be used to create hemophilia A state in a model system, which could then be used in efforts to counteract the disease state. Thus, the ceDNA vector for expression of FVIII protein as disclosed herein permit the treatment of genetic diseases. As used herein, hemophilia A state is treated by partially or wholly remedying the deficiency or imbalance that causes the disease or makes it more severe.

[00661] As used herein, the term “therapeutically effective amount” is an amount of an expressed FVIII therapeutic protein, or functional fragment thereof that is sufficient to produce a statistically significant, measurable change in expression of a disease biomarker or reduction in a given disease symptom (see “Efficacy Measurement" below). Such effective amounts can be gauged in clinical trials as well as animal studies for a given ceDNA composition.

[00662] The efficacy of a given treatment for hemophilia A, can be determined by the skilled clinician. However, a treatment is considered “effective treatment," as the term is used herein, if any one or all of the signs or symptoms of the disease or disorder is/are altered in a beneficial manner, or other clinically accepted symptoms or markers of disease are improved, or ameliorated, *e.g.,* by at least 10% following treatment with a ceDNA vector encoding FVIII, or a functional fragment thereof. Efficacy can also be measured by failure of an individual to worsen as assessed by stabilization of the disease, or the need for medical interventions (*i.e.*, progression of the disease is halted or at least

slowed). Methods of measuring these indicators are known to those of skill in the art and/or described herein. Treatment includes any treatment of a disease in an individual or an animal (some non-limiting examples include a human, or a mammal) and includes: (1) inhibiting the disease, *e.g.,* arresting, or slowing progression of the disease or disorder; or (2) relieving the disease, *e.g.,* causing regression of symptoms; and (3) preventing or reducing the likelihood of the development of the disease, or preventing secondary diseases/disorders associated with the disease, such as liver or kidney failure. An effective amount for the treatment of a disease means that amount which, when administered to a mammal in need thereof, is sufficient to result in effective treatment as that term is defined herein, for that disease.

[00663] Efficacy of an agent can be determined by assessing physical indicators that are particular to hemophilia A. Standard methods of analysis of hemophilia A indicators are known in the art.

A. Host cells

[00664] In some embodiments, a ceDNA vector for expression of FVIII protein as disclosed herein delivers the FVIII protein transgene into a subject host cell. In some embodiments, the cells are photoreceptor cells. In some embodiments, the cells are RPE cells. In some embodiments, the subject host cell is a human host cell, including, for example blood cells, stem cells, hematopoietic cells, $CD34^+$ cells, liver cells, cancer cells, vascular cells, muscle cells, pancreatic cells, neural cells, ocular or retinal cells, epithelial or endothelial cells, dendritic cells, fibroblasts, or any other cell of mammalian origin, including, without limitation, hepatic (*i.e.,* liver) cells, lung cells, cardiac cells, pancreatic cells, intestinal cells, diaphragmatic cells, renal (*i.e.,* kidney) cells, neural cells, blood cells, bone marrow cells, or any one or more selected tissues of a subject for which gene therapy is contemplated. In one aspect, the subject host cell is a human host cell.

[00665] The present disclosure also relates to recombinant host cells as mentioned above, including a ceDNA vector for expression of FVIII protein as disclosed herein. Thus, one can use multiple host cells depending on the purpose as is obvious to the skilled artisan. A construct or a ceDNA vector for expression of FVIII protein as disclosed herein including donor sequence is introduced into a host cell so that the donor sequence is maintained as a chromosomal integrant as described earlier. The term host cell encompasses any progeny of a parent cell that is not identical to the parent cell due to mutations that occur during replication. The choice of a host cell will to a large extent depend upon the donor sequence and its source.

[00666] The host cell may also be a eukaryote, such as a mammalian, insect, plant, or fungal cell. In one embodiment, the host cell is a human cell (*e.g.,* a primary cell, a stem cell, or an immortalized cell line). In some embodiments, the host cell can be administered a ceDNA vector for expression of FVIII protein as disclosed herein *ex vivo* and then delivered to the subject after the gene therapy event. A host cell can be any cell type, *e.g.,* a somatic cell or a stem cell, an induced pluripotent stem cell, or a blood cell, *e.g.,* T-cell or B-cell, or bone marrow cell. In certain embodiments, the host cell is an

allogenic cell. For example, T-cell genome engineering is useful for cancer immunotherapies, disease modulation such as HIV therapy (*e.g.,* receptor knock out, such as CXCR4 and CCR5) and immunodeficiency therapies. MHC receptors on B-cells can be targeted for immunotherapy. In some embodiments, gene modified host cells, *e.g.,* bone marrow stem cells, *e.g.,* $CD34^+$ cells, or induced pluripotent stem cells can be transplanted back into a patient for expression of a therapeutic protein.

B. Additional diseases for gene therapy:

[00667] In general, a ceDNA vector for expression of FVIII protein as disclosed herein can be used to deliver any FVIII protein in accordance with the description above to treat, prevent, or ameliorate the symptoms associated with hemophilia A related to an aberrant protein expression or gene expression in a subject.

[00668] In some embodiments, a ceDNA vector for expression of FVIII protein as disclosed herein can be used to deliver an FVIII protein to skeletal, cardiac or diaphragm muscle, for production of an FVIII protein for secretion and circulation in the blood or for systemic delivery to other tissues to treat, ameliorate, and/or prevent hemophilia A.

[00669] The a ceDNA vector for expression of FVIII protein as disclosed herein can be administered to the lungs of a subject by any suitable means, optionally by administering an aerosol suspension of respirable particles comprising the ceDNA vectors, which the subject inhales. The respirable particles can be liquid or solid. Aerosols of liquid particles comprising the ceDNA vectors may be produced by any suitable means, such as with a pressure-driven aerosol nebulizer or an ultrasonic nebulizer, as is known to those of skill in the art. See, *e.g.,* U.S. Pat. No. 4,501,729. Aerosols of solid particles comprising the ceDNA vectors may likewise be produced with any solid particulate medicament aerosol generator, by techniques known in the pharmaceutical art.

[00670] In some embodiments, a ceDNA vector for expression of FVIII protein as disclosed herein can be administered to tissues of the CNS (*e.g.,* brain, eye).

[00671] Ocular disorders that may be treated, ameliorated, or prevented with a ceDNA vector for expression of FVIII protein as disclosed herein include ophthalmic disorders involving the retina, posterior tract, and optic nerve (*e.g.,* retinitis pigmentosa, diabetic retinopathy and other retinal degenerative diseases, uveitis, age-related macular degeneration, glaucoma). Many ophthalmic diseases and disorders are associated with one or more of three types of indications: (1) angiogenesis, (2) inflammation, and (3) degeneration. In some embodiments, the ceDNA vector as disclosed herein can be employed to deliver anti-angiogenic factors; anti-inflammatory factors; factors that retard cell degeneration, promote cell sparing, or promote cell growth and combinations of the foregoing. Diabetic retinopathy, for example, is characterized by angiogenesis. Diabetic retinopathy can be treated by delivering one or more anti-angiogenic antibodies or fusion proteins either intraocularly (*e.g.,* in the vitreous) or periocularly (*e.g.,* in the sub-Tenon's region). Additional ocular diseases that may be treated, ameliorated, or prevented with the ceDNA vectors of the disclosure include

geographic atrophy, vascular or "wet" macular degeneration, PKU, Leber Congenital Amaurosis (LCA), Usher syndrome, pseudoxanthoma elasticum (PXE), x-linked retinitis pigmentosa (XLRP), x-linked retinoschisis (XLRS), Choroideremia, Leber hereditary optic neuropathy (LHON), Archomatopsia, cone-rod dystrophy, Fuchs endothelial corneal dystrophy, diabetic macular edema and ocular cancer and tumors.

[00672] In some embodiments, inflammatory ocular diseases or disorders (*e.g.,* uveitis) can be treated, ameliorated, or prevented by a ceDNA vector for expression of FVIII protein as disclosed herein. One or more anti-inflammatory antibodies or fusion proteins can be expressed by intraocular (*e.g.,* vitreous or anterior chamber) administration of the ceDNA vector as disclosed herein.

In some embodiments, a ceDNA vector for expression of FVIII protein as disclosed herein can encode an FVIII protein that is associated with transgene encoding a reporter polypeptide (*e.g.,* an enzyme such as Green Fluorescent Protein, or alkaline phosphatase). In some embodiments, a transgene that encodes a reporter protein useful for experimental or diagnostic purposes, is selected from any of: β-lactamase, β -galactosidase (LacZ), alkaline phosphatase, thymidine kinase, green fluorescent protein (GFP), chloramphenicol acetyltransferase (CAT), luciferase, and others well known in the art. In some aspects, ceDNA vectors expressing an FVIII protein linked to a reporter polypeptide may be used for diagnostic purposes, as well as to determine efficacy or as markers of the ceDNA vector's activity in the subject to which they are administered.

C. Testing for successful gene expression using a ceDNA vector

[00673] Assays well known in the art can be used to test the efficiency of gene delivery of an FVIII protein by a ceDNA vector can be performed in both *in vitro* and *in vivo* models. Levels of the expression of the FVIII protein by ceDNA can be assessed by one skilled in the art by measuring mRNA and protein levels of the FVIII protein (*e.g.,* reverse transcription PCR, western blot analysis, and enzyme-linked immunosorbent assay (ELISA)). In one embodiment, ceDNA comprises a reporter protein that can be used to assess the expression of the FVIII protein, for example by examining the expression of the reporter protein by fluorescence microscopy or a luminescence plate reader. For *in vivo* applications, protein function assays can be used to test the functionality of a given FVIII protein to determine if gene expression has successfully occurred. One skilled will be able to determine the best test for measuring functionality of an FVIII protein expressed by the ceDNA vector *in vitro* or *in vivo.*

[00674] It is contemplated herein that the effects of gene expression of an FVIII protein from the ceDNA vector in a cell or subject can last for at least 1 month, at least 2 months, at least 3 months, at least four months, at least 5 months, at least six months, at least 10 months, at least 12 months, at least 18 months, at least 2 years, at least 5 years, at least 10 years, at least 20 years, or can be permanent.

[00675] In some embodiments, an FVIII protein in the expression cassette, expression construct, or ceDNA vector described herein can be codon optimized for the host cell. As used herein, the term

"codon optimized" or "codon optimization" refers to the process of modifying a nucleic acid sequence for enhanced expression in the cells of the vertebrate of interest, *e.g.,* mouse or human (*e.g.,* humanized), by replacing at least one, more than one, or a significant number of codons of the native sequence (*e.g.,* a prokaryotic sequence) with codons that are more frequently or most frequently used in the genes of that vertebrate. Various species exhibit particular bias for certain codons of a particular amino acid. Typically, codon optimization does not alter the amino acid sequence of the original translated protein. Optimized codons can be determined using *e.g.,* Aptagen's Gene Forge® codon optimization and custom gene synthesis platform (Aptagen, Inc.) or another publicly available database.

D. Determining Efficacy by Assessing FVIII protein Expression from the ceDNA vector

[00676] Essentially any method known in the art for determining protein expression can be used to analyze expression of a FVIII protein from a ceDNA vector. Non-limiting examples of such methods/assays include enzyme-linked immunoassay (ELISA), affinity ELISA, ELISPOT, serial dilution, flow cytometry, surface plasmon resonance analysis, kinetic exclusion assay, mass spectrometry, Western blot, immunoprecipitation, and PCR.

[00677] For assessing FVIII protein expression *in vivo*, a biological sample can be obtained from a subject for analysis. Exemplary biological samples include a biofluid sample, a body fluid sample, blood (including whole blood), serum, plasma, urine, saliva, a biopsy and/or tissue sample etc. A biological sample or tissue sample can also refer to a sample of tissue or fluid isolated from an individual including, but not limited to, tumor biopsy, stool, spinal fluid, pleural fluid, nipple aspirates, lymph fluid, the external sections of the skin, respiratory, intestinal, and genitourinary tracts, tears, saliva, breast milk, cells (including, but not limited to, blood cells), tumors, organs, and also samples of *in vitro* cell culture constituent. The term also includes a mixture of the above-mentioned samples. The term "sample" also includes untreated or pretreated (or pre-processed) biological samples. In some embodiments, the sample used for the assays and methods described herein comprises a serum sample collected from a subject to be tested.

E. Determining Efficacy of the expressed FVIII protein by Clinical Parameters

[00678] The efficacy of a given FVIII protein expressed by a ceDNA vector for hemophilia A (*i.e.,* functional expression) can be determined by the skilled clinician. However, a treatment is considered "effective treatment," as the term is used herein, if any one or all of the signs or symptoms of hemophilia A is/are altered in a beneficial manner, or other clinically accepted symptoms or markers of disease are improved, or ameliorated, *e.g.,* by at least 10% following treatment with a ceDNA vector encoding a therapeutic FVIII protein as described herein. Efficacy can also be measured by failure of an individual to worsen as assessed by stabilization of hemophilia A, or the need for medical interventions (*i.e.,* progression of the disease is halted or at least slowed). Methods of measuring these indicators are known to those of skill in the art and/or described herein. Treatment includes any

treatment of a disease in an individual or an animal (some non-limiting examples include a human, or a mammal) and includes: (1) inhibiting hemophilia A, *e.g.,* arresting, or slowing progression of hemophilia A; or (2) relieving the hemophilia A, *e.g.,* causing regression of a hemophilia A symptom; and (3) preventing or reducing the likelihood of the development of the hemophilia A disease, or preventing secondary diseases/disorders associated with hemophilia A. An effective amount for the treatment of a disease means that amount which, when administered to a mammal in need thereof, is sufficient to result in effective treatment as that term is defined herein, for that disease. Efficacy of an agent can be determined by assessing physical indicators that are particular to hemophilia A disease. A physician can assess for any one or more of clinical symptoms of hemophilia A which include: unexplained and excessive bleeding from cuts or injuries, or after surgery or dental work; many large or deep bruises; unusual bleeding after vaccinations; pain, swelling or tightness in your joints; blood in your urine or stool; nosebleeds without a known cause; in infants, unexplained irritability.

XI. Various applications of ceDNA vectors expressing antibodies or fusion proteins

[00679] As disclosed herein, the compositions and ceDNA vectors for expression of FVIII protein as described herein can be used to express an FVIII protein for a range of purposes. In one embodiment, the ceDNA vector expressing an FVIII protein can be used to create a somatic transgenic animal model harboring the transgene, *e.g.,* to study the function or disease progression of hemophilia A. In some embodiments, a ceDNA vector expressing an FVIII protein is useful for the treatment, prevention, or amelioration of hemophilia A states or disorders in a mammalian subject.

[00680] In some embodiments the FVIII protein can be expressed from the ceDNA vector in a subject in a sufficient amount to treat a disease associated with increased expression, increased activity of the gene product, or inappropriate upregulation of a gene.

[00681] In some embodiments the FVIII protein can be expressed from the ceDNA vector in a subject in a sufficient amount to treat hemophilia A with a reduced expression, lack of expression or dysfunction of a protein.

[00682] It will be appreciated by one of ordinary skill in the art that the transgene may not be an open reading frame of a gene to be transcribed itself; instead it may be a promoter region or repressor region of a target gene, and the ceDNA vector may modify such region with the outcome of so modulating the expression of the FVIII gene.

[00683] The compositions and ceDNA vectors for expression of FVIII protein as disclosed herein can be used to deliver an FVIII protein for various purposes as described above.

[00684] In some embodiments, the transgene encodes one or more FVIII proteins which are useful for the treatment, amelioration, or prevention of hemophilia A states in a mammalian subject. The FVIII protein expressed by the ceDNA vector is administered to a patient in a sufficient amount to treat hemophilia A associated with an abnormal gene sequence, which can result in any one or more of

the following: increased protein expression, over activity of the protein, reduced expression, lack of expression or dysfunction of the target gene or protein.

[00685] In some embodiments, the ceDNA vectors for expression of FVIII protein as disclosed herein are envisioned for use in diagnostic and screening methods, whereby an FVIII protein is transiently or stably expressed in a cell culture system, or alternatively, a transgenic animal model.

[00686] Another aspect of the technology described herein provides a method of transducing a population of mammalian cells with a ceDNA vector for expression of FVIII protein as disclosed herein. In an overall and general sense, the method includes at least the step of introducing into one or more cells of the population, a composition that comprises an effective amount of one or more of the ceDNA vectors for expression of FVIII protein as disclosed herein.

[00687] Additionally, the present disclosure provides compositions, as well as therapeutic and/or diagnostic kits that include one or more of the disclosed ceDNA vectors for expression of FVIII protein as disclosed herein or ceDNA compositions, formulated with one or more additional ingredients, or prepared with one or more instructions for their use.

[00688] A cell to be administered a ceDNA vector for expression of FVIII protein as disclosed herein may be of any type, including but not limited to neural cells (including cells of the peripheral and central nervous systems, in particular, brain cells), lung cells, retinal cells, epithelial cells (*e.g.,* gut and respiratory epithelial cells), muscle cells, dendritic cells, pancreatic cells (including islet cells), hepatic cells, myocardial cells, bone cells (*e.g.,* bone marrow stem cells), hematopoietic stem cells, spleen cells, keratinocytes, fibroblasts, endothelial cells, prostate cells, germ cells, and the like. Alternatively, the cell may be any progenitor cell. As a further alternative, the cell can be a stem cell (*e.g.,* neural stem cell, liver stem cell). As still a further alternative, the cell may be a cancer or tumor cell. Moreover, the cells can be from any species of origin, as indicated above.

A. Production and Purification of ceDNA vectors expressing FVIII

[00689] The ceDNA vectors disclosed herein are to be used to produce FVIII protein either *in vitro* or *in vivo*. The FVIII proteins produced in this manner can be isolated, tested for a desired function, and purified for further use in research or as a therapeutic treatment. Each system of protein production has its own advantages/disadvantages. While proteins produced *in vitro* can be easily purified and can proteins in a short time, proteins produced *in vivo* can have post-translational modifications, such as glycosylation.

[00690] FVIII therapeutic protein produced using ceDNA vectors can be purified using any method known to those of skill in the art, for example, ion exchange chromatography, affinity chromatography, precipitation, or electrophoresis.

[00691] An FVIII therapeutic protein produced by the methods and compositions described herein can be tested for binding to the desired target protein.

[00692] The technology described herein is further illustrated by the following examples which in no way should be construed as being further limiting. It should be understood that this disclosure is not limited to the particular methodology, protocols, and reagents, etc., described herein and as such can vary. The terminology used herein is for the purpose of describing particular embodiments only and is not intended to limit the scope of the present disclosure, which is defined solely by the claims.

EXAMPLES

[00693] The following examples are provided by way of illustration not limitation. It will be appreciated by one of ordinary skill in the art that ceDNA vectors can be constructed from any of the wild-type or modified ITRs described herein, and that the following exemplary methods can be used to construct and assess the activity of such ceDNA vectors. While the methods are exemplified with certain ceDNA vectors, they are applicable to any ceDNA vector in keeping with the description.

EXAMPLE 1: Constructing ceDNA Vectors Using an Insect Cell-Based Method

[00694] Production of the ceDNA vectors using a polynucleotide construct template is described in Example 1 of PCT/US18/49996, which is incorporated herein in its entirety by reference. For example, a polynucleotide construct template used for generating the ceDNA vectors of the present disclosure can be a ceDNA-plasmid, a ceDNA-Bacmid, and/or a ceDNA-baculovirus. Without being limited to theory, in a permissive host cell, in the presence of *e.g.,* Rep, the polynucleotide construct template having two symmetric ITRs and an expression construct, where at least one of the ITRs is modified relative to a wild-type ITR sequence, replicates to produce ceDNA vectors. ceDNA vector production undergoes two steps: first, excision ("rescue") of template from the template backbone (*e.g.,* ceDNA-plasmid, ceDNA-bacmid, ceDNA-baculovirus genome etc.) via Rep proteins, and second, Rep mediated replication of the excised ceDNA vector.

[00695] *Production of ceDNA-bacmids:*

[00696] DH10Bac competent cells (MAX EFFICIENCY® DH10Bac™ Competent Cells, Thermo Fisher) were transformed with either test or control plasmids following a protocol according to the manufacturer's instructions. Recombination between the plasmid and a baculovirus shuttle vector in the DH10Bac cells were induced to generate recombinant ceDNA-bacmids. The recombinant bacmids were selected by screening a positive selection based on blue-white screening in *E. coli* (Φ80dlacZΔM15 marker provides α-complementation of the β-galactosidase gene from the bacmid vector) on a bacterial agar plate containing X-gal and IPTG with antibiotics to select for transformants and maintenance of the bacmid and transposase plasmids. White colonies caused by transposition that disrupts the β-galactoside indicator gene were picked and cultured in 10 ml of media.

[00697] The recombinant ceDNA-bacmids were isolated from the *E. coli* and transfected into Sf9 or Sf21 insect cells using FugeneHD to produce infectious baculovirus. The adherent Sf9 or Sf21 insect cells were cultured in 50 ml of media in T25 flasks at 25°C. Four days later, culture medium (containing the P0 virus) was removed from the cells, filtered through a 0.45 μm filter, separating the infectious baculovirus particles from cells or cell debris.

[00698] Optionally, the first generation of the baculovirus (P0) was amplified by infecting naïve Sf9 or Sf21 insect cells in 50 to 500 ml of media. Cells were maintained in suspension cultures in an orbital shaker incubator at 130 rpm at 25 °C, monitoring cell diameter and viability, until cells reach a diameter of 18-19 nm (from a naïve diameter of 14-15 nm), and a density of ~4.0E+6 cells/mL. Between 3 and 8 days post-infection, the P1 baculovirus particles in the medium were collected following centrifugation to remove cells and debris then filtration through a 0.45 μm filter.

[00699] The ceDNA-baculovirus comprising the test constructs were collected and the infectious activity, or titer, of the baculovirus was determined. Specifically, four x 20 ml Sf9 cell cultures at 2.5E+6 cells/ml were treated with P1 baculovirus at the following dilutions: 1/1000, 1/10,000, 1/50,000, 1/100,000, and incubated at 25-27°C. Infectivity was determined by the rate of cell diameter increase and cell cycle arrest, and change in cell viability every day for 4 to 5 days.

[00700] A "Rep-plasmid" as disclosed in FIG. 8A of PCT/US18/49996, which is incorporated herein in its entirety by reference, was produced in a pFASTBAC™-Dual expression vector (ThermoFisher) comprising both the Rep78 and Rep52 or Rep68 and Rep40. The Rep-plasmid was transformed into the DH10Bac competent cells (MAX EFFICIENCY® DH10Bac™ Competent Cells (Thermo Fisher) following a protocol provided by the manufacturer. Recombination between the Rep-plasmid and a baculovirus shuttle vector in the DH10Bac cells were induced to generate recombinant bacmids ("Rep-bacmids"). The recombinant bacmids were selected by a positive selection that included-blue-white screening in *E. coli* (Φ80dlacZΔM15 marker provides α-complementation of the β-galactosidase gene from the bacmid vector) on a bacterial agar plate containing X-gal and IPTG. Isolated white colonies were picked and inoculated in 10 ml of selection media (kanamycin, gentamicin, tetracycline in LB broth). The recombinant bacmids (Rep-bacmids) were isolated from the *E. coli* and the Rep-bacmids were transfected into Sf9 or Sf21 insect cells to produce infectious baculovirus.

[00701] The Sf9 or Sf21 insect cells were cultured in 50 ml of media for 4 days, and infectious recombinant baculovirus ("Rep-baculovirus") were isolated from the culture. Optionally, the first generation Rep-baculovirus (P0) were amplified by infecting naïve Sf9 or Sf21 insect cells and cultured in 50 to 500 ml of media. Between 3 and 8 days post-infection, the P1 baculovirus particles in the medium were collected either by separating cells by centrifugation or filtration or another fractionation process. The Rep-baculovirus were collected and the infectious activity of the baculovirus was determined. Specifically, four x 20 mL Sf9 cell cultures at $2.5x10^6$ cells/mL were treated with P1 baculovirus at the following dilutions, 1/1000, 1/10,000, 1/50,000, 1/100,000, and

incubated. Infectivity was determined by the rate of cell diameter increase and cell cycle arrest, and change in cell viability every day for 4 to 5 days.

[00702] *ceDNA vector generation and characterization*

[00703] With reference to **FIG. 3B**, Sf9 insect cell culture media containing either (1) a sample-containing a ceDNA-bacmid or a ceDNA-baculovirus, and (2) Rep-baculovirus described above were then added to a fresh culture of Sf9 cells (2.5E+6 cells/ml, 20ml) at a ratio of 1:1000 and 1:10,000, respectively. The cells were then cultured at 130 rpm at 25°C. 4-5 days after the co-infection, cell diameter and viability are detected. When cell diameters reached 18-20nm with a viability of ~70-80%, the cell cultures were centrifuged, the medium was removed, and the cell pellets were collected. The cell pellets are first resuspended in an adequate volume of aqueous medium, either water or buffer. The ceDNA vector was isolated and purified from the cells using Qiagen MIDI PLUS™ purification protocol (Qiagen, 0.2mg of cell pellet mass processed per column).

[00704] Yields of ceDNA vectors produced and purified from the Sf9 insect cells were initially determined based on UV absorbance at 260nm.

[00705] ceDNA vectors can be assessed by identified by agarose gel electrophoresis under native or denaturing conditions as illustrated in **FIG. 3D**, where (a) the presence of characteristic bands migrating at twice the size on denaturing gels versus native gels after restriction endonuclease cleavage and gel electrophoretic analysis and (b) the presence of monomer and dimer (2x) bands on denaturing gels for uncleaved material is characteristic of the presence of ceDNA vector.

[00706] Structures of the isolated ceDNA vectors were further analyzed by digesting the DNA obtained from co-infected Sf9 cells (as described herein) with restriction endonucleases selected for a) the presence of only a single cut site within the ceDNA vectors, and b) resulting fragments that were large enough to be seen clearly when fractionated on a 0.8% denaturing agarose gel (>800 bp). As illustrated in **FIGS. 3D and 3E**, linear DNA vectors with a non-continuous structure and ceDNA vector with the linear and continuous structure can be distinguished by sizes of their reaction products–for example, a DNA vector with a non-continuous structure is expected to produce 1kb and 2kb fragments, while a non-encapsidated vector with the continuous structure is expected to produce 2kb and 4kb fragments.

[00707] Therefore, to demonstrate in a qualitative fashion that isolated ceDNA vectors are covalently closed-ended as is required by definition, the samples were digested with a restriction endonuclease identified in the context of the specific DNA vector sequence as having a single restriction site, preferably resulting in two cleavage products of unequal size (*e.g.,* 1000 bp and 2000 bp). Following digestion and electrophoresis on a denaturing gel (which separates the two complementary DNA strands), a linear, non-covalently closed DNA will resolve at sizes 1000 bp and 2000 bp, while a covalently closed DNA (*i.e.,* a ceDNA vector) will resolve at 2x sizes (2000 bp and 4000 bp), as the two DNA strands are linked and are now unfolded and twice the length (though single stranded).

Furthermore, digestion of monomeric, dimeric, and *n*-meric forms of the DNA vectors will all resolve as the same size fragments due to the end-to-end linking of the multimeric DNA vectors (see **FIG. 3D**).

[00708] As used herein, the phrase "assay for the Identification of DNA vectors by agarose gel electrophoresis under native gel and denaturing conditions" refers to an assay to assess the close-endedness of the ceDNA by performing restriction endonuclease digestion followed by electrophoretic assessment of the digest products. One such exemplary assay follows, though one of ordinary skill in the art will appreciate that many art-known variations on this example are possible. The restriction endonuclease is selected to be a single cut enzyme for the ceDNA vector of interest that will generate products of approximately 1/3x and 2/3x of the DNA vector length. This resolves the bands on both native and denaturing gels. Before denaturation, it is important to remove the buffer from the sample. The Qiagen PCR clean-up kit or desalting "spin columns," *e.g.,* GE HEALTHCARE ILUSTRA™ MICROSPIN™ G-25 columns are some art-known options for the endonuclease digestion. The assay includes for example, i) digest DNA with appropriate restriction endonuclease(s), 2) apply to *e.g.,* a Qiagen PCR clean-up kit, elute with distilled water, iii) adding 10x denaturing solution (10x = 0.5 M NaOH, 10mM EDTA), add 10X dye, not buffered, and analyzing, together with DNA ladders prepared by adding 10X denaturing solution to 4x, on a 0.8 – 1.0 % gel previously incubated with 1mM EDTA and 200mM NaOH to ensure that the NaOH concentration is uniform in the gel and gel box, and running the gel in the presence of 1x denaturing solution (50 mM NaOH, 1mM EDTA). One of ordinary skill in the art will appreciate what voltage to use to run the electrophoresis based on size and desired timing of results. After electrophoresis, the gels are drained and neutralized in 1x TBE or TAE and transferred to distilled water or 1x TBE/TAE with 1x SYBR Gold. Bands can then be visualized with *e.g.,* Thermo Fisher, SYBR® Gold Nucleic Acid Gel Stain (10,000X Concentrate in DMSO) and epifluorescent light (blue) or UV (312nm).

[00709] The purity of the generated ceDNA vector can be assessed using any art-known method. As one exemplary and non-limiting method, contribution of ceDNA-plasmid to the overall UV absorbance of a sample can be estimated by comparing the fluorescent intensity of ceDNA vector to a standard. For example, if based on UV absorbance 4µg of ceDNA vector was loaded on the gel, and the ceDNA vector fluorescent intensity is equivalent to a 2kb band which is known to be 1µg, then there is 1µg of ceDNA vector, and the ceDNA vector is 25% of the total UV absorbing material. Band intensity on the gel is then plotted against the calculated input that band represents – for example, if the total ceDNA vector is 8kb, and the excised comparative band is 2kb, then the band intensity would be plotted as 25% of the total input, which in this case would be .25µg for 1.0µg input. Using the ceDNA vector plasmid titration to plot a standard curve, a regression line equation is then used to calculate the quantity of the ceDNA vector band, which can then be used to determine the percent of total input represented by the ceDNA vector, or percent purity.

[00710] For comparative purposes, Example 1 describes the production of ceDNA vectors using an insect cell based method and a polynucleotide construct template, and is also described in Example 1 of PCT/US18/49996, which is incorporated herein in its entirety by reference. For example, a polynucleotide construct template used for generating the ceDNA vectors of the present disclosure according to Example 1 can be a ceDNA-plasmid, a ceDNA-Bacmid, and/or a ceDNA-baculovirus. Without being limited to theory, in a permissive host cell, in the presence of *e.g.,* Rep, the polynucleotide construct template having two symmetric ITRs and an expression construct, where at least one of the ITRs is modified relative to a wild-type ITR sequence, replicates to produce ceDNA vectors. ceDNA vector production undergoes two steps: first, excision ("rescue") of template from the template backbone (*e.g.,* ceDNA-plasmid, ceDNA-bacmid, ceDNA-baculovirus genome etc.) via Rep proteins, and second, Rep mediated replication of the excised ceDNA vector.

[00711] An exemplary method to produce ceDNA vectors in a method using insect cell is from a ceDNA-plasmid as described herein.

EXAMPLE 2: Synthetic ceDNA production via excision from a double-stranded DNA molecule

[00712] Synthetic production of the ceDNA vectors is described in Examples 2-6 of International Application PCT/US19/14122, filed January 18, 2019, which is incorporated herein in its entirety by reference. One exemplary method of producing a ceDNA vector using a synthetic method that involves the excision of a double-stranded DNA molecule. In brief, a ceDNA vector can be generated using a double stranded DNA construct, *e.g.,* see FIGS. 7A-8E of PCT/US19/14122. In some embodiments, the double stranded DNA construct is a ceDNA plasmid, *e.g.,* see, *e.g.,* FIG. 6 in International patent application PCT/US2018/064242, filed December 6, 2018).

[00713] In some embodiments, a construct to make a ceDNA vector comprises a regulatory switch as described herein.

[00714] For illustrative purposes, Example 2 describes producing ceDNA vectors as exemplary closed-ended DNA vectors generated using this method. However, while ceDNA vectors are exemplified in this Example to illustrate *in vitro* synthetic production methods to generate a closed-ended DNA vector by excision of a double-stranded polynucleotide comprising the ITRs and expression cassette (*e.g.,* nucleic acid sequence, *e.g.,* heterologous nucleic acid sequence) followed by ligation of the free 3' and 5' ends as described herein, one of ordinary skill in the art is aware that one can, as illustrated above, modify the double stranded DNA polynucleotide molecule such that any desired closed-ended DNA vector is generated, including but not limited to, doggybone DNA, dumbbell DNA and the like. Exemplary ceDNA vectors for production of antibodies or fusion proteins that can be produced by the synthetic production method described in Example 2 are discussed in the sections entitled "III ceDNA vectors in general". Exemplary antibodies and fusion proteins expressed by the ceDNA vectors are described in the section entitled "IIC Exemplary antibodies and fusion proteins expressed by the ceDNA vectors".

[00715] The method involves (i) excising a sequence encoding the expression cassette from a double-stranded DNA construct and (ii) forming hairpin structures at one or more of the ITRs and (iii) joining the free 5' and 3' ends by ligation, *e.g.,* by T4 DNA ligase.

[00716] The double-stranded DNA construct comprises, in 5' to 3' order: a first restriction endonuclease site; an upstream ITR; an expression cassette; a downstream ITR; and a second restriction endonuclease site. The double-stranded DNA construct is then contacted with one or more restriction endonucleases to generate double-stranded breaks at both of the restriction endonuclease sites. One endonuclease can target both sites, or each site can be targeted by a different endonuclease as long as the restriction sites are not present in the ceDNA vector template. This excises the sequence between the restriction endonuclease sites from the rest of the double-stranded DNA construct (see Fig. 9 of PCT/US19/14122). Upon ligation a closed-ended DNA vector is formed.

[00717] One or both of the ITRs used in the method may be wild-type ITRs. Modified ITRs may also be used, where the modification can include deletion, insertion, or substitution of one or more nucleotides from the wild-type ITR in the sequences forming B and B' arm and/or C and C' arm (see, *e.g.,* Figs. 6-8 and 10 FIG. 11B of PCT/US19/14122), and may have two or more hairpin loops (see, *e.g.,* Figs. 6-8 FIG. 11B of PCT/US19/14122) or a single hairpin loop (see, *e.g.,* Fig. 10A-10B FIG. 11B of PCT/US19/14122). The hairpin loop modified ITR can be generated by genetic modification of an existing oligo or by de novo biological and/or chemical synthesis.

[00718] In a non-limiting example, ITR-6 Left and Right (SEQ ID NOS: 111 and 112), include 40 nucleotide deletions in the B-B' and C-C' arms from the wild-type ITR of AAV2. Nucleotides remaining in the modified ITR are predicted to form a single hairpin structure. Gibbs free energy of unfolding the structure is about -54.4 kcal/mol. Other modifications to the ITR may also be made, including optional deletion of a functional Rep binding site or a TRS site.

EXAMPLE 3: ceDNA Production via Oligonucleotide Construction

[00719] Another exemplary method of producing a ceDNA vector using a synthetic method that involves assembly of various oligonucleotides, is provided in Example 3 of PCT/US19/14122, where a ceDNA vector is produced by synthesizing a 5' oligonucleotide and a 3' ITR oligonucleotide and ligating the ITR oligonucleotides to a double-stranded polynucleotide comprising an expression cassette. FIG. 11B of PCT/US19/14122 shows an exemplary method of ligating a 5' ITR oligonucleotide and a 3' ITR oligonucleotide to a double stranded polynucleotide comprising an expression cassette.

[00720] As disclosed herein, the ITR oligonucleotides can comprise WT-ITRs (*e.g.,* see **FIG. 2A**, **FIG. 2C**), or modified ITRs (*e.g.,* see, **FIG. 2B** and **FIG. 2D**). (See also, *e.g.,* FIGS. 6A, 6B, 7A and 7B of PCT/US19/14122, which is incorporated herein in its entirety). Exemplary ITR oligonucleotides include, but are not limited to SEQ ID NOS: 134-145 (*e.g.,* see Table 7 in of PCT/US19/14122). Modified ITRs can include deletion, insertion, or substitution of one or more nucleotides from the

wild-type ITR in the sequences forming B and B' arm and/or C and C' arm. ITR oligonucleotides, comprising WT-ITRs or mod-ITRs as described herein, to be used in the cell-free synthesis, can be generated by genetic modification or biological and/or chemical synthesis. As discussed herein, the ITR oligonucleotides in Examples 2 and 3 can comprise WT-ITRs, or modified ITRs (mod-ITRs) in symmetrical or asymmetrical configurations, as discussed herein.

EXAMPLE 4: ceDNA Production via a Single-Stranded DNA Molecule

[00721] Another exemplary method of producing a ceDNA vector using a synthetic method is provided in Example 4 of PCT/US19/14122, and uses a single-stranded linear DNA comprising two sense ITRs which flank a sense expression cassette sequence and are attached covalently to two antisense ITRs which flank an antisense expression cassette, the ends of which single stranded linear DNA are then ligated to form a closed-ended single-stranded molecule. One non-limiting example comprises synthesizing and/or producing a single-stranded DNA molecule, annealing portions of the molecule to form a single linear DNA molecule which has one or more base-paired regions of secondary structure, and then ligating the free 5' and 3' ends to each other to form a closed single-stranded molecule.

[00722] An exemplary single-stranded DNA molecule for production of a ceDNA vector comprises, from 5' to 3': a sense first ITR; a sense expression cassette sequence; a sense second ITR; an antisense second ITR; an antisense expression cassette sequence; and an antisense first ITR.

[00723] A single-stranded DNA molecule for use in the exemplary method of Example 4 can be formed by any DNA synthesis methodology described herein, *e.g., in vitro* DNA synthesis, or provided by cleaving a DNA construct (*e.g.,* a plasmid) with nucleases and melting the resulting dsDNA fragments to provide ssDNA fragments.

[00724] Annealing can be accomplished by lowering the temperature below the calculated melting temperatures of the sense and antisense sequence pairs. The melting temperature is dependent upon the specific nucleotide base content and the characteristics of the solution being used, *e.g.,* the salt concentration. Melting temperatures for any given sequence and solution combination are readily calculated by one of ordinary skill in the art.

[00725] The free 5' and 3' ends of the annealed molecule can be ligated to each other, or ligated to a hairpin molecule to form the ceDNA vector. Suitable exemplary ligation methodologies and hairpin molecules are described in Examples 2 and 3.

EXAMPLE 5: Purifying and/or Confirming Production of ceDNA

[00726] Any of the DNA vector products produced by the methods described herein, *e.g.,* including the insect cell based production methods described in Example 1, or synthetic production methods described in Examples 2-4 can be purified, *e.g.,* to remove impurities, unused components, or byproducts using methods commonly known by a skilled artisan; and/or can be analyzed to confirm that DNA vector produced, (in this instance, a ceDNA vector) is the desired molecule. An exemplary

method for purification of the DNA vector, *e.g.,* ceDNA is using Qiagen Midi Plus purification protocol (Qiagen) and/or by gel purification.

[00727] The following is an exemplary method for confirming the identity of ceDNA vectors.

[00728] ceDNA vectors can be assessed by identified by agarose gel electrophoresis under native or denaturing conditions as illustrated in **FIG. 3D**, where (a) the presence of characteristic bands migrating at twice the size on denaturing gels versus native gels after restriction endonuclease cleavage and gel electrophoretic analysis and (b) the presence of monomer and dimer (2x) bands on denaturing gels for uncleaved material is characteristic of the presence of ceDNA vector.

[00729] Structures of the isolated ceDNA vectors were further analyzed by digesting the purified DNA with restriction endonucleases selected for a) the presence of only a single cut site within the ceDNA vectors, and b) resulting fragments that were large enough to be seen clearly when fractionated on a 0.8% denaturing agarose gel (>800 bp). As illustrated in **FIGS. 3C and 3D**, linear DNA vectors with a non-continuous structure and ceDNA vector with the linear and continuous structure can be distinguished by sizes of their reaction products– for example, a DNA vector with a non-continuous structure is expected to produce 1kb and 2kb fragments, while a ceDNA vector with the continuous structure is expected to produce 2kb and 4kb fragments.

[00730] Therefore, to demonstrate in a qualitative fashion that isolated ceDNA vectors are covalently closed-ended as is required by definition, the samples were digested with a restriction endonuclease identified in the context of the specific DNA vector sequence as having a single restriction site, preferably resulting in two cleavage products of unequal size (*e.g.,* 1000 bp and 2000 bp). Following digestion and electrophoresis on a denaturing gel (which separates the two complementary DNA strands), a linear, non-covalently closed DNA will resolve at sizes 1000 bp and 2000 bp, while a covalently closed DNA (*i.e.,* a ceDNA vector) will resolve at 2x sizes (2000 bp and 4000 bp), as the two DNA strands are linked and are now unfolded and twice the length (though single stranded). Furthermore, digestion of monomeric, dimeric, and *n*-meric forms of the DNA vectors will all resolve as the same size fragments due to the end-to-end linking of the multimeric DNA vectors (see **FIG. 3E**).

[00731] As used herein, the phrase "assay for the Identification of DNA vectors by agarose gel electrophoresis under native gel and denaturing conditions" refers to an assay to assess the close-endedness of the ceDNA by performing restriction endonuclease digestion followed by electrophoretic assessment of the digest products. One such exemplary assay follows, though one of ordinary skill in the art will appreciate that many art-known variations on this example are possible. The restriction endonuclease is selected to be a single cut enzyme for the ceDNA vector of interest that will generate products of approximately 1/3x and 2/3x of the DNA vector length. This resolves the bands on both native and denaturing gels. Before denaturation, it is important to remove the buffer from the sample. The Qiagen PCR clean-up kit or desalting "spin columns," *e.g.,* GE HEALTHCARE ILUSTRA™

MICROSPIN™ G-25 columns are some art-known options for the endonuclease digestion. The assay includes for example, i) digest DNA with appropriate restriction endonuclease(s), 2) apply to *e.g.,* a Qiagen PCR clean-up kit, elute with distilled water, iii) adding 10x denaturing solution (10x = 0.5 M NaOH, 10mM EDTA), add 10X dye, not buffered, and analyzing, together with DNA ladders prepared by adding 10X denaturing solution to 4x, on a 0.8 – 1.0 % gel previously incubated with 1mM EDTA and 200mM NaOH to ensure that the NaOH concentration is uniform in the gel and gel box, and running the gel in the presence of 1x denaturing solution (50 mM NaOH, 1mM EDTA). One of ordinary skill in the art will appreciate what voltage to use to run the electrophoresis based on size and desired timing of results. After electrophoresis, the gels are drained and neutralized in 1x TBE or TAE and transferred to distilled water or 1x TBE/TAE with 1x SYBR Gold. Bands can then be visualized with *e.g.,* Thermo Fisher, SYBR® Gold Nucleic Acid Gel Stain (10,000X Concentrate in DMSO) and epifluorescent light (blue) or UV (312nm). The foregoing gel-based method can be adapted to purification purposes by isolating the ceDNA vector from the gel band and permitting it to renature.

[00732] The purity of the generated ceDNA vector can be assessed using any art-known method. As one exemplary and non-limiting method, contribution of ceDNA-plasmid to the overall UV absorbance of a sample can be estimated by comparing the fluorescent intensity of ceDNA vector to a standard. For example, if based on UV absorbance 4µg of ceDNA vector was loaded on the gel, and the ceDNA vector fluorescent intensity is equivalent to a 2kb band which is known to be 1µg, then there is 1µg of ceDNA vector, and the ceDNA vector is 25% of the total UV absorbing material. Band intensity on the gel is then plotted against the calculated input that band represents – for example, if the total ceDNA vector is 8kb, and the excised comparative band is 2kb, then the band intensity would be plotted as 25% of the total input, which in this case would be .25µg for 1.0µg input. Using the ceDNA vector plasmid titration to plot a standard curve, a regression line equation is then used to calculate the quantity of the ceDNA vector band, which can then be used to determine the percent of total input represented by the ceDNA vector, or percent purity.

EXAMPLE 6: ceDNA FVIII Constructs and Methods

[00733] Full-length, unmodified FVIII poses a number of challenges in its application in gene therapy. FVIII does not perform well in heterologous systems, and shows poor expression compared to similarly sized proteins. It has been shown that inefficient secretion of FVIII can lead to cellular stress, both inactive and active forms of FVIII have a short half-life, and FVIII does not behave well in circulation. Further, FVIII has been shown to be highly immunogenic. A schematic of FVIII domains, as processed through active FVIIIa is shown in **FIG. 9**.

[00734] The following Examples describe preparation and testing of ceDNA FVIII constructs that show expression and activity following both hydrodynamic and lipid nanoparticle administration.

[00735] **FIG. 5** is an annotated schematic of the ceDNA 1638 construct. **FIG. 6** is an annotated schematic of the ceDNA 1652 construct. **FIG. 7** is an annotated schematic of the ceDNA 1923 construct. **FIG. 8** is an annotated schematic of the ceDNA 1373 intron.

[00736] The following ceDNA FVIII constructs were employed in the studies described in **Example 7 – Example 16**.

Table 20: ceDNA Construct Summary

ceDNA construct identifier	Description
692	B-domain deleted SQ codon optimized (Biomarin)
1362	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || hFVIII-Wt-Afstyla BDD || PacI_site || WPRE_3pUTR || bGH
1368	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309 || PacI_site || WPRE_3pUTR || bGH
1374	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || hFVIII-F309S-BD226seq124-Afstyla-BDD-F309 || PacI_site || WPRE_3pUTR || bGH
1918	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || FVIII-SC_0CpG_1_ORF || PacI_site || WPRE_3pUTR || bGH
1919	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || FVIII-SC_0CpG_6_ORF || PacI_site || WPRE_3pUTR || bGH
1920	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || FVIII-SC_0CpG_8_ORF || PacI_site || WPRE_3pUTR || bGH
1922	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || FVIII-SC_5k_wt3_3_ORF || PacI_site || WPRE_3pUTR || bGH
1923	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || FVIII-SC_5k_wt3_5_ORF || PacI_site || WPRE_3pUTR || bGH
1367	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309S || PacI_site || WPRE_3pUTR || bGH
1373	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || hFVIII-F309S-BD226seq124-Afstyla-BDD-F309S || PacI_site || WPRE_3pUTR || bGH
1632	CpGmin_hAAT_Promoter_Set || PmeI_site || Mod_Minimum_Consensus_Kozak || hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309S || PacI_site || HBBv3_3pUTR || SV40_polyA
1637	CpGmin_hAAT_Promoter_Set || PmeI_site || Mod_Minimum_Consensus_Kozak || hFVIII-F309S-BD226seq124-Afstyla-BDD-F309S || PacI_site || HBBv3_3pUTR || SV40_polyA
1638	CpGmin_hAAT_Promoter_Set || hFVIII-F309S-BD226seq124-Afstyla-BDD-F309S || PacI_site || HBBv3_3pUTR || SV40_polyA

1645	CpGmin_hAAT_Promoter_Set || PmeI_site || Mod_Minimum_Consensus_Kozak ||hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309S || PacI_site || SV40_polyA
1646	CpGmin_hAAT_Promoter_Set || PmeI_site || Mod_Minimum_Consensus_Kozak || hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309S || PacI_site || HBBv3_3pUTR
1648	3x hSerpEnh_VD _TTRe_PromoterSet || Consensus_Kozak || hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309S || PacI_site || WPRE_3pUTR || bGH
1657	3x hSerpEnh_VD _PromoterSet (5'UTR variant) || PmeI_site || Consensus_Kozak || hFVIII-F309S-BD226seq124-Afstyla-BDD || PacI_site || WPRE_3pUTR || bGH
1922	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || FVIII-SC_5k_wt3_3_ORF || PacI_site || WPRE_3pUTR || bGH
1923	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || FVIII-SC_5k_wt3_5_ORF || PacI_site || WPRE_3pUTR || bGH
1368	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309 || PacI_site || WPRE_3pUTR || bGH
1374	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || hFVIII-F309S-BD226seq124-Afstyla-BDD-F309 || PacI_site || WPRE_3pUTR || bGH
1649	3x SerpEnh VD _TTRe_PromoterSet || Consensus_Kozak || hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309 || PacI_site || WPRE_3pUTR || bGH
1651	3x SerpEnh VD _TTRe_PromoterSet || Consensus_Kozak || hFVIII-F309S-BD226seq124-Afstyla-BDD-F309 || PacI_site || WPRE_3pUTR || bGH
1652	3x SerpEnh VD _TTRe_PromoterSet (5'UTR variant) || Consensus_Kozak || hFVIII-F309S-BD226seq124-Afstyla-BDD-F309 || PacI_site || WPRE_3pUTR || bGH
1655	3x SerpEnh VD _PromoterSet || Consensus_Kozak || hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309 || PacI_site || WPRE_3pUTR || bGH
1668	3x SerpEnh VD _TTRe_PromoterSet_v2 || PmeI_site || Consensus_Kozak || hFVIII-F309S-BD226seq124-Afstyla-BDD-F309 || PacI_site || WPRE_3pUTR || bGH
1367	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309S || PacI_site || WPRE_3pUTR || bGH
1373	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || hFVIII-F309S-BD226seq124-Afstyla-BDD-F309S || PacI_site || WPRE_3pUTR || bGH
1700	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || ceDNA1367_ORF_exon1_33bpflanks_hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD || miniF8_50/100 || ceDNA1367_ORF_exon2-26_33bpflanks_hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD || PacI_site || WPRE_3pUTR || bGH
1701	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || ceDNA1367_ORF_exon1_33bpflanks_hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD || miniF8_200/200 || ceDNA1367_ORF_exon2-26_33bpflanks_hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD || PacI_site || WPRE_3pUTR || bGH

1708	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || ceDNA1373_ORF_exon1_ hFVIII-F309S-BD226seq124-Afstyla-BDD || miniF8_500/500 || ceDNA1373_ORF_exon2-26_ hFVIII-F309S-BD226seq124-Afstyla-BDD || PacI_site || WPRE_3pUTR || bGH
1712	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || ceDNA1373_ORF_exon1_ hFVIII-F309S-BD226seq124-Afstyla-BDD || miniF8_200_5p || miniF8_200_3p || ceDNA1373_ORF_exon2-26_ hFVIII-F309S-BD226seq124-Afstyla-BDD || PacI_site || WPRE_3pUTR || bGH
1725	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || ceDNA1373_ORF_exon1_33bpflanks_ hFVIII-F309S-BD226seq124-Afstyla-BDD || miniF8_50/100 || ceDNA1373_ORF_exon2-26_33bpflanks_ hFVIII-F309S-BD226seq124-Afstyla-BDD || PacI_site || WPRE_3pUTR || bGH
1368	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309 || PacI_site || WPRE_3pUTR || bGH
1374	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || hFVIII-F309S-BD226seq124-Afstyla-BDD-F309 || PacI_site || WPRE_3pUTR || bGH
1579	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || FVIII-1368ORF_ALB-SSv1 || PacI_site || WPRE_3pUTR || bGH
1582	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || FVIII-1368ORF_GAUS-SSv1 || PacI_site || WPRE_3pUTR || bGH
1585	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || FVIII-1368ORF_Secrecon-SSv1 || PacI_site || WPRE_3pUTR || bGH
1598	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || FVIII-1368ORF_Gaus-NS-CAI-v2 || PacI_site || WPRE_3pUTR || bGH
1611	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || FVIII-1374ORF_ALB-SSv1 || PacI_site || WPRE_3pUTR || bGH
1612	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || FVIII-1374ORF_CHY-SSv1 || PacI_site || WPRE_3pUTR || bGH
1615	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || FVIII-1374ORF_LONZ-SSv2 || PacI_site || WPRE_3pUTR || bGH
1616	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || FVIII-1374ORF_Secrecon-SSv1 || PacI_site || WPRE_3pUTR || bGH
1270	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || hFVIII-F309S-BD226seq124 || PacI_site || WPRE_3pUTR || bGH
1391	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || hFVIII-F309S-BD226-Codop-run4-seq102 || PacI_site || WPRE_3pUTR || bGH
1738	1x hSerpEnh_VD_PromoterSet || PmeI_site || 5pUTR-constant || 5pUTR-325243 || hFVIII-F309S-BD226seq124 || PacI_site || WPRE_3pUTR || bGH
1740	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || hFVIII-F309S-BD226-Codop-run4-seq102 || PacI_site || WPRE_3pUTR || bGH || 5'DTS_primer_pad || 5x_kB_mesika_DTS || 3'DTS_primer_pad
1741	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || hFVIII-F309S-BD226-Codop-run4-seq102 || PacI_site || WPRE_3pUTR || bGH || CpGfree20mer_1 ||

	SV40DNA_DTS_72bpTandemRepeat || CpGfree20mer_2 || SV40DNA_DTS_72bpTandemRepeat || CpGfree20mer_3 || SV40DNA_DTS_72bpTandemRepeat || CpGfree20mer_4 || SV40DNA_DTS_72bpTandemRepeat || CpGfree20mer_5 || SV40DNA_DTS_72bpTandemRepeat
1742	5'DTS_primer_pad || 5x_kB_mesika_DTS || 3'DTS_primer_pad || 1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || hFVIII-F309S-BD226-Codop-run4-seq102 || PacI_site || WPRE_3pUTR || bGH
1743	CpGfree20mer_1 || SV40DNA_DTS_72bpTandemRepeat || CpGfree20mer_2 || SV40DNA_DTS_72bpTandemRepeat || CpGfree20mer_3 || SV40DNA_DTS_72bpTandemRepeat || CpGfree20mer_4 || SV40DNA_DTS_72bpTandemRepeat || CpGfree20mer_5 || SV40DNA_DTS_72bpTandemRepeat || 1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || hFVIII-F309S-BD226-Codop-run4-seq102 || PacI_site || WPRE_3pUTR || bGH
1744	1x hSerpEnh_VD_PromoterSet || PmeI_site || 5pUTR-constant || 5pUTR-325243 || hFVIII-F309S-BD226seq124 || PacI_site || WPRE_3pUTR || bGH || AflII_site || CpGfree20mer_1, CBX3(674mut1) || 20mer_16
694	B domain deleted SQ condon optimized (Sangamo)
1572	CpGfree20mer_1 || 5xHNF1_ProEnh_10mer || 3x hSerpEnh_VD _TTRe_PromoterSet_v2 || PmeI_site || Consensus_Kozak || hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309S || PacI_site || WPRE_3pUTR || bGH
1664	3x hSerpEnh_VD _TTRe_PromoterSet_v2* || PmeI_site || Consensus_Kozak || hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309S || PacI_site || WPRE_3pUTR || bGH
1838	3x hSerpEnh_VD _TTRe_PromoterSet || 5pUTR-constant, 5pUTR-325243 || FVIII_SC_F309_codop_1b || PacI_site || WPRE_3pUTR || bGH
1840	3x hSerpEnh_VD _TTRe_PromoterSet || 5pUTR-constant, 5pUTR-325243 || FVIII_SC_F309_codop_3b || PacI_site || WPRE_3pUTR || bGH
1841	3x hSerpEnh_VD _TTRe_PromoterSet || 5pUTR-constant, 5pUTR-325243 || FVIII_SC_F309S_codop_3b || PacI_site || WPRE_3pUTR || bGH
1886	3x hSerpEnh_VD _TTRe_PromoterSet_v2 || PmeI_site || Consensus_Kozak || hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309S || PacI_site || WPRE_3pUTR || bGH
1921	1x hSerEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || FVIII-SC_5_ORF || PacI_site || WPRE_3pUTR || bGH
1922	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || FVIII-SC_5k_wt3_3_ORF || PacI_site || WPRE_3pUTR || bGH
1930	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || FVIII-SC_5_F309S || PacI_site || WPRE_3pUTR || bGH
1931	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || FVIII-SC_5k_wt3_3_F309S || PacI_site || WPRE_3pUTR || bGH

1574	Minimum_Consensus_Kozak, hFIX_Promoter, PmeI_site, Consensus_Kozak, hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD, PacI_site, WPRE_3pUTR, bGH
1628	CpGmin_hAAT_Promoter_Set, hFVIII-F309S-BD226seq124-Afstyla-BDD, PacI_site, WPRE_3pUTR, bGH,
1593	1x hSerpEnh_VD_PromoterSet, PmeI_site, Consensus_Kozak, FVIII-1368ORF_CD33-NS-struct-v2, PacI_site, WPRE_3pUTR, bGH,
1602	1x hSerpEnh_VD_PromoterSet, PmeI_site, Consensus_Kozak, FVIII_1368ORF_Lonz-NS-CAI-v2, PacI_site, WPRE_3pUTR, bGH,
1367	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309S || PacI_site || WPRE_3pUTR || bGH
1620	CpGmin_hAAT_Promoter_Set || PmeI_site || Consensus_Kozak || hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309S || PacI_site || WPRE_3pUTR || bGH
1622	CpGmin_hAAT_Promoter_Set || PmeI_site || Mod_Minimum_Consensus_Kozak || hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD || PacI_site || WPRE_3pUTR || bGH
1627	CpGmin_hAAT_Promoter_Set || PmeI_site || Mod_Minimum_Consensus_Kozak || hFVIII-F309S-BD226seq124-Afstyla-BDD || PacI_site || WPRE_3pUTR || bGH
1636	CpGmin_hAAT_Promoter_Set || PmeI_site || Mod_Minimum_Consensus_Kozak || hFVIII-F309S-BD226seq124-Afstyla-BDD || PacI_site || HBBv3_3pUTR || SV40_polyA
1648	3x SerpEnh VD _TTRe_PromoterSet || Consensus_Kozak || hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309S || PacI_site || WPRE_3pUTR || bGH
1695	CRM8_SERP_enhancer || TTR_liver_specific_Promoter || MVM_intron_post_splice || PmeI_site || Consensus_Kozak || ceDNA1367_ORF_exon1_hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD || HBB_intron1 || ceDNA1367_ORF_exon2-26_hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD || PacI_site || WPRE_3pUTR || bGH
1696	CRM8_SERP_enhancer || TTR_liver_specific_Promoter || MVM_intron_post_splice || PmeI_site || Consensus_Kozak || ceDNA1367_ORF_exon1_hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD || F8_intron8 || ceDNA1367_ORF_exon2-26_hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD || PacI_site || WPRE_3pUTR || bGH
1710	1x hSerpEnh_VD_PromoterSet || PmeI_site || Consensus_Kozak || ceDNA1373_ORF_exon1_ hFVIII-F309S-BD226seq124-Afstyla-BDD || miniF8_200_5p || Minimum_Consensus_Kozak || miniF8_200_3p || ceDNA1373_ORF_exon2-26_ hFVIII-F309S-BD226seq124-Afstyla-BDD || PacI_site || WPRE_3pUTR || bGH

[00737] **FIG. 10** is a schematic detailing a method of insertion of introns into FVIII ORF. Chimeric FVIII intron a with functional splice donor and acceptor sites is inserted at native position of intron 1 into codon optimized FVIII ORF. Intron flanking regions (33bp) derived from FVIII Wt cDNA sequence were substituted for codon optimized sequence in FVIII CDS.

[00738] **FIG. 11** is a schematic detailing a method of insertion of introns into FVIII ORF ceDNA 1373. Chimeric FVIII intron with functional splice donor and acceptor sites inserted at native position of intron 1 into codon optimized FVIII ORF. Enhancer element inserted in between 5p and 3p regions of chimeric intron.

[00739] **FIGs. 12A-12B** are schematics detailing method of substituting heterologous secretion signal sequences for the native FVIII signal sequence. **FIG. 12A** shows substitution of native FVIII signal sequence for signal sequence from chymotrypsinogen (CHY-SSv1) ORF. FVIII mature peptide is shown. **FIG. 12B** shows the sequence of FVIII N-terminus showing signal sequence and mature peptide cleavage site.

[00740] Screening was carried out to determine FVIII activity using the following assays:

In vitro screening assay:

[00741] Using Lipofectamine p3000 (Thermofisher cat no: L3000001), 24 hours prior to transfection: HepG2 cells were plated in a 96 well collagen coated plate at a density of 20,000 cells/well (100uL in each well = 200,000 cells/mL). On the day of transfection, the media was changed in all cell-containing wells.

[00742] Lipofectamine dilution was prepared as follows: 0.3uL lipofectamine3000 reagent+10uL Opti-MEM (per well)

[00743] P3000 dilutions was prepared as follows: 10uL Opti-MEM + 0.4uL p3000 (per well)

[00744] 800ng DNA was plated into single wells of a 96 well prep-plate.

[00745] 21uL of the L3000 dilution and 21uL of the P3000 dilutions were added to each DNA-containing well, and gently mixed, followed by incubation at room temperature (RT) for 15 minutes. 10uL/well of the L3000 and P3000 mixture was added to the cells in triplicate, followed by incubation for 72 hours at 37C, 5% CO2, humidified air.

[00746] 72 hours after transfection, supernatant media was collected into equal volume aliquots into 2X 96W storage plates and either frozen immediated or used in the Chromogenix FVIII Activity assay.

Chromogenix FVIII Activity assay:

[00747] The chromogenic assay to determine FVIII activity was carried out as follows: Kit components were allowed to acclimiate to room temperature from 4C before use. The lyophilized kit components were reconstituted with sterile water: 3mL per Factor Reagent (green cap) and 6mL to the S-2765 + I-2581 Reagent (white cap). The Technoclone Coagulation Reference was reconstituted with 1mL sterile water and allow to mix slowly on a rocker for 15min before use. Samples are diluted as follows: 5uL sample + 400uL buffer in a 96W block.

[00748] Standard (coagulation reference) was prepared in the 96W block as seen on "extended curve" tab. Samples and standards were plated on the 384W plate using the 125uL Voyager Pipette: 10uL each. Reconstituted Factor Reagent (green cap) and S-2765 + I-2581 (white cap) was prewarmed from the kit at 37C. Plate was uncubated at 37°C for 4min.

[00749] Next, 10uL Factor Reagent was added to each well, using the 50uL pipette, leaving 10 seconds between row additions to maintain incubation times. Plate was incubated at 37C for 4min. 10uL S-2765 + I-2581 was added to each well, using the 50uL pipette, leaving 10 seconds between row additions to maintain incubation times. Plate was incubated at 37C for 10min. 10uL acetic acid (20%) was added as a stop solution to each well, using the 50uL pipette, leaving 10 seconds between row additions to maintain incubation times. Plate absorbance was read on M3 at 405nm.

[00750] Analysis was performed as follows: After reading on M3, data was exported to Excel for processing. All raw absorbance values were normalized to the 0 IU/mL standard wells (average the 2 wells, then subtract that averaged value from each other well value). Normalized values were plugged into GraphPad Prism, XY table format. Values of IU/mL for FVIII standards were added with their normalized absorbance value. Unknowns were added-the sample name and the normalized absorbance values.

[00751] Data was processed as follows: "transform concentrations (x)" à transform to log (log(10)); XY analysis à "nonlinear regression (curve fit)" à "asymmetric 5 parameter, X is log(concentration); go to "interpolated X mean values" tab à analyze à transform à standard functions and "transform Y values using: Y=10^Y" AND check off "create new graph of the results"; this gives the processed and interpolated IU/mL values for the unknowns (samples).

EXAMPLE 7: Study to Determine FVIII Expression after Hydrodynamic ceDNA Delivery in Male FVIII Knockout Mice

[00752] A well-known method of introducing nucleic acid to the liver in rodents is by hydrodynamic tail vein injection. In this system, the pressurized injection in a large volume of non-encapsulated nucleic acid results in a transient increase in cell permeability and delivery directly into tissues and cells. This provides an experimental mechanism to bypass many of the host immune systems, such as macrophage delivery, providing the opportunity to observe delivery and expression in the absence of such activity

[00753] A hydrodynamic delivery system was used to test the effect of various ceDNA vectors expressing FVIII on serum FVIII levels, where detection of FVIII in the serum indicated that the ceDNA vector was able to express FVIII after injection.

[00754] ceDNA vectors as described in Example 6 were employed. SEQ ID NOs for the ceDNA constructs are shown in **Table 18**, and a description of the constructs is provided in **Table 20**. Test materials for each study are shown in the **Tables 21-23**, below

Table 21: Study 1

Group No.	Animals per Group	Treatment	Dose Level (µg)	Dose Volume	Treatment Regimen, IV	Terminal Time Point
1	4	PBS	1.0		Once on	Day 7

2	4	ceDNA1362		90 – 100 ml/kg (set volume)	Day 0 by IV Hydrodynamic	
3	4	ceDNA1367				
4	4	ceDNA1368				
5	4	ceDNA1373				
6	4	ceDNA1374				
7	4	ceDNA1361				
8	4	ceDNA1265				
9	4	ceDNA1270				
10	4	ceDNA 692				

Table 22: Study 2

Group No.	Animals per Group	Treatment	Dose Level (µg)	Dose Volume	Treatment Regimen, IV	Terminal Time Point
1	4	PBS	1.0	90 – 100 ml/kg (set volume)	Once on Day 0 by IV Hydrodynamic	Day 7
2	4	ceDNA694				
3	4	ceDNA1320				
4	4	ceDNA704				
5	4	ceDNA1260				
6	4	ceDNA1258				
7	4	ceDNA933				
8	4	ceDNA1259				
9	4	ceDNA1270				
10	4	ceDNA1257				

Table 23: Study 3

Group No.	No. of Animals	Strain	Test Material	Dose Levels (µg/an)	Dose Volume (mL/kg)	Dosing Regimen ROA	Terminal Time Point
1	4	CD-1	PBS	NA	90 – 100 ml/kg (set volume)	Once on Day 0 by IV Hydrodynamic	Day 3
2	4		ceDNA692	5			
3	4		ceDNA1362	5			
4	4		ceDNA1368	5			
5	4		ceDNA1374	5			
6	4		ceDNA1918	5			
7	4		ceDNA1919	5			
8	4		ceDNA1920	5			
9	4		ceDNA1922	5			

10	4		ceDNA1923	5			

[00755] Test articles were supplied in a concentrated stock, and stored at nominally 4°C. Formulations were not vortexed or centrifuged. Groups were housed in clear polycarbonate cages with contact bedding on a ventilated rack in a procedure room. Food and filtered tap water acidified with 1N HCl to a targeted pH of 2.5 - 3.0 were provided to the animals ad libitum. Blood was collected at interim and terminal time points as set forth below in **Tables 24-26**.

Table 24: Study 1 and 2 Interim and terminal collection

Group Number	**Sample Collection Times**	
	Whole Blood (orbital)	**Terminal Whole Blood** (cardiac)
	Sodium Citrate Plasma	
1 - 10	Day 3 72 hours ± 50% post dose	Day 7
Volume / Portion	120 µL whole blood	MOV
Processing / Storage	120 µL whole blood was added to tube pre-coated with 13.33 µL of 3.2% sodium citrate and kept ambient until processed One (1) aliquot of plasma Frozen at nominally -70°C	600 µL whole blood was added to tube pre-coated with 66.6 µL of 3.2% sodium citrate and kept ambient until processed Three (3) aliquots of plasma Frozen at nominally -70°C

Table 25: Study 3 Blood Collection (Interim): All animals in Groups 1 – 10 hadinterim blood collected on Day 1; 24 hours post Test Material dose (±5%)

Group Number	**Sample Collection Times**
	Whole Blood (orbital only)
	Sodium Citrate Plasma
1 – 10	Day 1 24 hours post Test Material dose (±5%)
Volume / Portion	120 µL whole blood
Processing / Storage	120 µL whole blood was added to tube pre-coated with 13.33 µL of 3.2% sodium citrate and kept ambient until processed Two (2) aliquots of plasma Frozen at nominally -70°C

Table 26: Study 3 Terminal Collection

Group Number	Sample Collection Times Terminal Whole Blood (cardiac) Sodium Citrate Plasma
1 – 10	Day 3
Volume / Portion	MOV
Processing	600 µL whole blood was added to tube pre-coated with 66.6 µL of 3.2% sodium citrate and kept ambient until processed Four (4) aliquots processed plasma
Storage	Frozen samples at nominally -70°C

[00756] MOV = maximum obtainable volume**Study details are provided as follows:**

[00757] *Species (number, sex, age):* FVIII KO (B6;129S-F8<tm1Kaz>/J) mice (N = 40 + 4 spare, male, ~4 weeks of age at arrival) from Jackson Laboratories.

[00758] *Cage Side Observations:* Cage side observations were performed daily.

[00759] *Clinical Observations:* Clinical observations were performed ~1, ~5-6 and ~24 hours post the Day 0 Test Material dose.

[00760] *Body Weights:* Body weight for all animals was recorded on Days 0, 3 and 7, including prior to euthanasia.

[00761] *Dose Formulation:* Test articles were supplied in a concentration stock. Stock was diluted with PBS immediately prior to use. Prepared materials were stored at ~4°C (or on wet ice) if dosing was not performed immediately.

[00762] *Dose Administration:* Test Materials were dosed on Day 0 by hydrodynamic IV administration, at a set volume per animal, 90 – 100 ml / kg (dependent on the lightest animal in the group) via lateral tail vein (dosed within 5 seconds). *After each collection, animals received 0.5 – 1.0 mL lactated Ringer's, subcutaneously*. For plasma collections, whole blood was collected into non-coated Eppendorf style tubes via orbital sinus puncture under anesthesia per facility SOPS. Immediately 120µL was withdrawn and placed into tubes containing 13.33 µL of 3.2% sodium citrate. Blood was gently mixed and maintained ambient until processed. Whole blood samples were centrifuged at 2,000g for 15 minutes under ambient conditions (20-25°C). Plasma samples were withdrawn avoiding the cell pack. One (1) aliquot was made and any clotting in the whole blood sample or hemolysis in the plasma was noted. Samples were stored at nominally -70°C until analysis.

[00763] *Anesthesia Recovery:* Animals were monitored continuously while under anesthesia, during recovery and until mobile.

[00764] *Euthanasia & Terminal Blood Collection:* On Day 7, animals were euthanized by CO_2 asphyxiation followed by thoracotomy and exsanguination. Maximum obtainable blood volume was collected by cardiac puncture and processed to plasma. No other tissues were be collected.

[00765] For plasma collections, whole blood was collected by syringe and 600 µL placed immediately tubes containing 66.66 µL of 3.2% sodium citrate. Blood was gently mixed and maintained ambient until processed. Whole blood samples were centrifuged at 2,000g for 15 minutes under ambient conditions (20-25°C). Plasma samples were withdrawn avoiding the cell pack and three (3) aliquots made. Any clotting in the whole blood sample or hemolysis in the plasma were noted. All plasma samples were stored at nominally -70°C shipped until analysis.

[00766] **Results: FIG. 13** shows a schematic of B-domain selection for the constructs described herein. Briefly, selection of FVIII protein sequences in clinical use was prioritized to minimize immunogenicity risk. Through ELISA and chromagenic assay analysis (data not shown), FVIII-SC was found to be a favored FVIII protein sequence. In FVIII-SC, the heavy and light chains are covalently linked, and this construct shows increased affinity to von Willbrand factor (VFW), which reduces binding to antigen presenting cells (APCs), improving stability and immunogenicity *in vitro*.

[00767] A comparison was done between the ELISA assay method and the chromogenic assay method to determine if one method produced more reliable results than another in determining FVIII activity. In particular, it was found that the ELISA used to measure plasma human FVIII in WT mice underpredicted FVIII activity for constructs with a short or deleted B domain (SQ and SC - 1373[SC/F309S]). However, good concordance was found between the ELISA and activity only v226 constructs (1270 [v226/F309S]). Therefore, it was concluded that comparisons can only be made between constructs with the same B-domain in studies that used the ELISA assay (hydrodynamic studies in CD-1 or C57bl/6 mice), but among the constructs with different type of B-domain or different optimized sequence. The chromogenic assay assay appeared to provide more consistent results. Exemplary results are shown in **FIG. 14.**

EXAMPLE 8: Study to Determine FVIII Expression after Hydrodynamic ceDNA delivery in Male CD-1 and FVIII KO Mice

[00768] A hydrodynamic delivery system was used to test the effect of various ceDNA vectors expressing FVIII on serum FVIII levels, where detection of FVIII in the serum indicated that the ceDNA vector was able to express FVIII after injection.

[00769] ceDNA vectors as described in Example 6 were employed. SEQ ID NOs for the ceDNA constructs are shown in **Table 18**, and a description of the constructs is provided in **Table 20**. Test materials for the study are shown in **Table 27** below.

Table 27: Study 4

Group No.	No. of Animals	Strain	Test Material	Dose Levels (µg/an)	Dose Volume (mL/kg)	Dosing Regimen ROA	Terminal Time Point
1	3	CD-1	PBS	NA	90 – 100 ml/kg (set volume)	Once on Day 0 by IV Hydrodynamic	Day 3
2	3		ceDNA1373	0.01			
3	3		ceDNA1373	0.1			

4	3		ceDNA1373	1.0			
5	3		ceDNA1374	0.01			
6	3		ceDNA1374	0.1			
7	3		ceDNA1374	1.0			
8	3	FVIII KO	PBS	NA			
9	3		ceDNA1270	0.01			
10	3		ceDNA1270	0.1			
11	3		ceDNA1270	1.0			
12	3		ceDNA1373	0.01			
13	3		ceDNA1373	0.1			
14	3		ceDNA1373	1.0			
15	**1**	**CD-1**	**ceDNA1373**	**1.0**			**Day 1**

No. = Number; IV = intravenous; ROA = route of administration; min = minute; hr = hour

[00770] Test articles were supplied in a concentrated stock, and stored at nominally 4°C. Formulations were not vortexed or centrifuged. Groups were housed in clear polycarbonate cages with contact bedding on a ventilated rack in a procedure room. Food and filtered tap water acidified with 1N HCl to a targeted pH of 2.5 - 3.0 were provided to the animals ad libitum. Blood was collected at interim and terminal time points as set forth in **Table 28** below.

Table 28: Study 4 Terminal collection

Group Number	Sample Collection Times		
	Terminal Whole Blood (cardiac)	Liver	**Liver**
	Sodium Citrate Plasma		
1 – 14	Day 3		
15			**Day 1**
Volume / Portion	MOV	Whole organ, weighed then divided 4 x ~20-30 mg pieces weighed	**Whole organ**
Processing	600 µL whole blood was added to tube pre-coated with 66.6 µL of 3.2% sodium citrate and kept ambient until processed Three (3) aliquots processed plasma	Snap frozen individually (Lake Pharma)	**Placed in cold PBS** **No processing**
Storage	Frozen samples at nominally -70°C		**Send Same Day on WET ICE**

MOV = maximum obtainable volume

[00771] Study details are provided as follows:

[00772] *Species (number, sex, age):* FVIII KO (B6;129S-F8<tm1Kaz>/J) mice (N = 40 + 4 spare, male, ~4 weeks of age at arrival) from Jackson Laboratories. CD-1, 22 plus 1 spare. 4 weeks at age of arrival.

[00773] *Cage Side Observations:* Cage side observations were performed daily.

[00774] *Dose Formulation:* Test articles were supplied in a concentration stock. Stock was diluted with PBS immediately prior to use. Prepared materials were stored at ~4°C (or on wet ice) if dosing was not performed immediately.

[00775] *Dose Administration:* Test Materials were dosed on Day 0 by hydrodynamic IV administration, at a set volume per animal, 90 – 100 ml / kg (dependent on the lightest animal in the group) via lateral tail vein (dosed within 5 seconds).

[00776] Whole blood from Groups 1 – 14 only was collected by syringe and 600 µL placed immediately tubes containing 66.66 µL of 3.2% sodium citrate. Blood was gently mixed and maintained ambient until processed. Whole blood samples were centrifuged at 2,000g for 15 minutes under ambient conditions (20-25°C). Plasma samples were withdrawn avoiding the cell pack and three (3) aliquots made. Any clotting in the whole blood sample or hemolysis in the plasma was noted.

[00777] All plasma samples were stored at nominally -70°C until analysis.

[00778] *Perfusion:* Following exsanguination, all animals (including Group 15) underwent cardiac perfusion with saline. In brief, whole body intracardiac perfusion was performed by inserting 23/21-gauge needle attached to 10 mL syringe containing saline into the lumen of the left ventricle for perfusion. The right atrium was incised to provide a drainage outlet for perfusate. Gentle and steady pressure was applied to the plunger to perfuse the animal after the needle has been positioned in the heart. Adequate flow of the flushing solution was ensured until the exiting perfusate flows clear (free of visible blood) indicating that the flushing solution has saturated the body and the procedure was complete.

[00779] *Tissue Collection:* After euthanasia, exsanguination and perfusion, the liver was harvested and whole organ weights were recorded. No whole organ weight was needed for Group 15.

[00780] Groups 1 – 14 Tissue specifications - From the liver: 4 x ~20-30 mg sections (≤ 30 mg) were collected and weighed. Any remaining liver was discarded. Weighed sections were snap frozen individually, stored at nominally -70°C until shipped.

[00781] Group 15 Tissue specifications: The whole liver was placed in cold PBS. Sample were stored on wet ice.

[00782] Similar experiments were conducted and repeated using the protocol shown above. Test articles were B domain (SQ) deleted like 692, 693, 694; or v226/F309S like 1270 and 1391; single chain F309S like 1367 and 1373; and single chain FVIII like 1368 and 1374, as described in Tables 18 and 20.

[00783] Result: Various B-domain and secretion mutant combinations of FVIII ceDNA constructs were tested for their ability to express functional FVIII protein. As shown in **FIG. 15**, FVIII constructs having SC optionally with F309S showed consistently high expression *in vitro* and *in vivo* (see, *e.g.*, ceDNA1368, ceDNA1373 and ceDNA1374).

EXAMPLE 9: A Study to Determine FVIII Expression after Hydrodynamic ceDNA delivery in Male CD-1 Mice

[00784] A hydrodynamic delivery system was used to determine FVIII expression after ceDNA devlivery using FVIII ceDNA constructs with various elements (e.g., testing different 3'UTRs, promoter-enhancer combinations, introns for effect on FVIII expression). ceDNA vectors as described in Example 6 were employed. SEQ ID NOs for the ceDNA constructs are shown in **Table 18**, and a description of the constructs is provided in **Table 20**. Test materials for the study are shown in **Tables 29-30** below.

Table 29: Study 5

Group No.	No. of Animals	Strain	Test Material	Dose Levels (µg/an)	Dose Volume (mL/kg)	Dosing Regimen ROA	Terminal Time Point
1	4	CD-1	PBS	NA	90 – 100 ml/kg (set volume)	Once on Day 0 by IV Hydrodynamic	Day 7
2	4		ceDNA1367	5			
3	4		ceDNA1373	5			
4	4		ceDNA1632	5			
5	4		ceDNA1637	5			
6	4		ceDNA1638	5			
7	4		ceDNA1645	5			
8	4		ceDNA1646	5			
9	4		ceDNA1648	5			
10	4		ceDNA1657	5			

Table 30: Study 6

Group No.	No. of Animals	Strain	Test Material	Dose Levels (µg/an)	Dose Volume (mL/kg)	Dosing Regimen ROA	Terminal Time Point
1	4	CD-1	PBS	NA	90 – 100 ml/kg (set volume)	Once on Day 0 by IV Hydrodynamic	Day 1 24 hours post dose ±5%
2	4		ceDNA1270	5			
3	4		ceDNA1375	5			
4	4		ceDNA1377	5			
5	4		ceDNA1378	5			
6	4		ceDNA1381	5			
7	4		ceDNA1387	5			
8	4		ceDNA1391	5			
9	4		ceDNA1647	5			
10	4		ceDNA1374	5			

[00785] *Species (number, sex, age):* CD-1,40 plus 4 spares. 4 weeks at age of arrival.

Remaining study details are similar to those provided in Examples 8 and 9 above. Similar experiments were conducted and repeated to test the effect of the various combinations of promoters-enhancers sets, introns, 3'-UTR on FVIII protein expression. The elements that were tested were as follows:

FIG. 20 shows the results of FVIII expression from ceDNA having the following promoter sets:

1x hSerpEnh_VD_PromoterSet (1x SerpEnh)

SC: 1362, 1368, 1374, 1918, 1919, 1920, 1921, 1922, 1923, 1593, 1602

SC/Leader: 1579, 1582, 1585, 1598, 1611, 1612, 1615, 1616

SC/F309S: 1367, 1373, 1700, 1701, 1708, 1712, 1725, 1930, 1931, 1710

v226/F309S: 1270, 1391, 1740, 1741, 1742, 1743, 1744

3x hSerpEnh_VD _PromoterSet

SC: 1655

v226/F309S: 1375, 1381

3x hSerpEnh_VD _PromoterSet (5'UTR variant)

SC: 1652

SC/F309S: 1657

3x hSerpEnh_VD _TTRe_PromoterSet

SC: 1649, 1651, 1838, 1840, 1841

SC/F309S: 1648

v226/F309S: 1647

3x hSerpEnh_VD _TTRe_PromoterSet_v2

SC: 1668

SC/F309S: 1886

3x SerpEnh_VD _TTRe_PromoterSet_v2*

SC/F309S: 1664

CpGmin_hAAT_Promoter_Set

SC/F309S: 1632, 1637, 1638, 1645, 1646, 1620, 1622, 1627, 1636, 1628

3xSerpEnh-TTRm

v226/F309S: 1377, 1378

hAAT(979)_PromoterSet

v226/F309S: 1387

TTR_liver_specific_Promoter

SC/F309S: 1695, 1696

hFIX_Promoter

SC/F309S: 1574

CpGfree20mer_1, 5xHNF1_ProEnh_10mer, 3x hSerpEnh_VD_TTRe_PromoterSet_v2

SC/F309S: 1572

FIG. 21 shows the results of FVIII expression from ceDNA having the following introns:

miniF8_50/100: 1700, 1725

miniF8_200/200: 1701, 1712

miniF8_500/500: 1708

HBB_intron1: 1695

FIG. 19 shows the results of FVIII expression from ceDNA having the following 3'UTRs:

WPRE_3pUTR, bGH: all constructs tested

HBBv3_3pUTR, SV40_polyA:

CpGmin_hAAT, SC/F309S: 1632 (1622), 1636 (1627), 1637 (1627), 1638 (1628)

hAAT(979), v226/F309S: 1387 (none)

SV40_polyA:

CpGmin_hAAT, SC/F309S: 1645 (1622)

HBBv3_3pUTR:

CpGmin_hAAT, SC/F309S: 1646 (1622)

3xSerpEnh-TTRm_MVM_intron, v226/F309S: 1377 (none)

bGH:

3x hSerpEnh_VD, v226/F309S: 1375 (1381)

HBBv2_3pUTR, bGH:

3xSerpEnh-TTRm_MVM_intron, v226/F309S: 1378

FIG. 24 shows the results of FVIII expression from ceDNA having the following leader sequences (and their locations):

Albumin: 1611, 1579

Gaussia: 1598, 1582

Secrecon: 1616, 1585

Chymotrypsinogen: 1612

Lonza: 1615, 1602

CD33: 1593

DTS

5'DTS_primer_pad || 5x_kB_mesika_DTS || 3'DTS_primer_pad:

After 3pUTR: 1740

Before promotor: 1742

CpGfree20mer_1 || SV40DNA_DTS_72bpTandemRepeat || CpGfree20mer_2 || SV40DNA_DTS_72bpTandemRepeat || CpGfree20mer_3 || SV40DNA_DTS_72bpTandemRepeat || CpGfree20mer_4 || SV40DNA_DTS_72bpTandemRepeat || CpGfree20mer_5 || SV40DNA_DTS_72bpTandemRepeat:

After 3pUTR: 1741

Before promotor: 1743

CpGfree20mer_1, CBX3(674mut1) || 20mer_16

After 3pUTR: 1744 (also has 5pUTR – 1738 is comparator)

[00786] Results: FIG. 19 shows the results of FVIII expression from ceDNA having various 3pUTRs and the effects on plasma FVIII concentration (IU/ml) in 1622, 1632, 1645, 1646, 1627, 1636, 1637, 1628, 1638, 1382, 1375, 1377, 1378, and 1387. The studies described above were also carried out, in part, to test various promoter and enhancer combinations, and their effects on plasma FVIII concentration. **FIG. 20** describes various promoters and promoter/enhancer combinations employed and tested. **FIG. 21** shows the results of intron combination in 1367, 1700, 1701, 1695, 1373, 1708, 1725, 1712 *in vitro* and *in vivo*. **FIG. 23** shows the results of the effect on FVIII expression from ceDNA having DNA nuclear targeting sequences (DTS) on FVIII expression. **FIG. 24** shows the results of the impact of having a leader sequence variation on FVIII expression.

EXAMPLE 10: A Study to Evaluate ceDNA Formulations via IV Delivery in Male C57Bl/6 Mice

[00787] The following study was carried out to determine protein expression after IV injection of LNP formulated ceDNA. ceDNA1270 was formulated in two different LNPs compositions (LNP formulation1: Ionizable lipid: DSPC : Cholesterol : PEG-Lipid + DSPE-PEG-GalNAc4 (47.5 : 10.0 : 39.2 : 3.3) (designated "DP#1"); and LNP formulation 2: Ionizable lipid : DSPC : Cholesterol : PEG-Lipid + DSPE-PEG2000-GalNAc4 (47.3 : 10.0 : 40.5 : 2.3) (designated "DP#2"). Doses of test material were administered on Day 0 by intravenous dosing into the lateral tail vein. Doses were administered at a dose volume of 5 mL/kg. Doses were rounded to the nearest 0.01 mL. Test materials for the study are shown in Tables 31 and 32 below. ceDNA expressing Factor IX (ceDNA-FIX) was used as an independent control.

[00788] Table 31

Group No.	No. of Animals	Test Material	Dose Levels (mg/kg)	Dose Volume (mL/kg)	Dosing Regimen ROA	Terminal Time Point
1	5	PBS	NA	5	Once on Day 0 by IV	Day 14
2	5	ceDNA1270	0.5			
3	5	ceDNA1270	2.0			
4	5	ceDNA-FIX	2.0			
5	5	ceDNA-FIX	2.0			
6	5	ceDNA-FIX	2.0			

Table 32

Group No.	No. of Animals	Test Material	Dose Levels (mg/kg)	Dose Volume (mL/kg)	Dosing Regimen ROA	Terminal Time Point

1	4	PBS	NA	5	Once on Day 0 by IV	N = 2 on Day 10 **and N = 2 on Day 57**
2	4	ceDNA1270	1.0			Day 10
3	4	ceDNA1270	0.3			Day 10
4	4		1.0			Day 10
5	4		3.0			Day 10
6	4	ceDNA-FIX	2.0			**Day 57**

[00789] *Species (number, sex, age)*: C57Bl/6,30 plus 3 spares. 6 weeks at age of arrival. Remaining study details are similar to those provided in Examples 8 and 9 above.

[00790] Clinical observations were performed on Day 0: 60 – 120 minutes post dose and at the end of the work day (3 – 6 hours post) and on Day 1: 22 - 26 hours post the Day 0 Test Material dose. Additional observations were made per exception.

[00791] Results: Mice were administered 1 mg/kg ceDNA1270 in LNP formulation 1 (Ionizable lipid: DSPC : Cholesterol : PEG-Lipid + DSPE-PEG-GalNAc4 (47.5 : 10.0 : 39.2 : 3.3) (DP#1) or 2 mg/kg ceDNA 1270 in LNP formulation 2 (Ionizable lipid : DSPC : Cholesterol : PEG-Lipid + DSPE-PEG2000-GalNAc4 (47.3 : 10.0 : 40.5 : 2.3) (DP#2). **FIG. 25** shows that mice treated with ceDNA1270 LNP formulations exhibited increased plasma FVIII as compared to mice treated with vehicles, indicating that ceDNA LNP was successfully targeted to the liver and integrated into cells, resulting in succesful expression of FVIII protein.

EXAMPLE 11: A Study to Evaluate ceDNA Hydrodynamically Administered via IV Delivery in Male FVIII KO Mice

[00792] The following studies were carried out to determine protein expression after IV injection of naked ceDNA constructs. SEQ ID NOs for the ceDNA constructs are shown in **Table 18**, and a description of the constructs is provided in **Table 20.** Doses of test material were administered on Day 0 by intravenous dosing into the lateral tail vein. Doses were administered at a dose volume of 5 mL/kg. Doses were rounded to the nearest 0.01 mL. Test materials for the study are shown in Tables 33 and 34, below.

Table 33

Group No.	No. of Animals	Strain	Test Material	Dose Levels (mg/kg)	Dose Volume (mL/kg)	Dosing Regimen ROA	Terminal Time Point
1	2	FVIII KO	PBS	NA	5	Once on Day 0 by IV	Day 10
2	5			0.3			

3	5		ceDNA1270	1.0			
4	5			2.0			
5	5		ceDNA1368	1.0			
6	5			2.0			
7	5			3.0			
8	5		ceDNA1923	0.3			
9	5			1.0			
10	5			2.0			
11	5		ceDNA1651	0.3			
12	5			1.0			
13	5			2.0			

No. = Number; IV = intravenous; ROA = route of administration; min = minute; hr = hour

[00793] *Species (number, sex, age)*: FVIII KO (B6;129S-F8<tm1Kaz>/J), 62 plus 3 spares. 4-8 weeks at age of arrival. Remaining study details are similar to those provided in Examples 8 and 9 above.

[00794] Clinical observations were performed on Day 0: 60 – 120 minutes post dose and at the end of the work day (3 – 6 hours post) and on Day 1: 22 - 26 hours post the Day 0 Test Material dose.

[00795] **Results:** As shown in **FIG. 26**, after 10 days, mice administered ceDNA1270, ceDNA1368, ceDNA1923 or ceDNA1651 constructs at all of the doses tested exhibited increased plasma FVIII concentration. Overall, the increased FVIII plasma concentration was dose dependent. These ceDNA constructs showed a dramatic increase in plasma FVIII concentration ranging from the 0.5 mg/kg dose to the 2.0 mg/kg dose.

EXAMPLE 12: A Study to Determine FVIII Transgene Expression after IV LNP:ceDNA delivery in Male CD-1 and FVIII KO Mice

[00796] The objective of this Study was to determine transgene expression after IV administration of formulated ceDNA. SEQ ID NOs for the ceDNA constructs are shown in **Table 18**, and a description of the constructs is provided in **Table 20**. Test materials for the study are shown in the **Tables 34-37** below.

Table 34: Kinase Inhibitor Administration

Group No.	No. of Animals	Strain	Inhibitor	Dose Levels (mg/kg)	Dose Volume (mL/kg)	Dosing Regimen ROA
1	4	CD-1	Ruxolitinib	300	10	Day 0 30 min. pre-dose & 5 hrs post-dose of Test Material by PO
2	4					
3	4					
4	4					
5	4					

6	4	FVIII KO				
7	4					
8	4					

Table 35: Test Material Administration

Group No.	No. of Animals	Strain	Test Material	Dose Levels (µg/an)	Dose Volume (mL/kg)	Dosing Regimen ROA	Terminal Time Point
1	4	CD-1	PBS	NA	5	Once on Day 0 by IV	Day 14
2	4		LNP ceDNA691	2			
3	4		LNP ceDNA933				
4	4		LNP ceDNA1270				
5	4	FVIII KO	LNP ceDNA0933				
6	4		LNP ceDNA1270				
7	4		LNP ceDNA1367				
8	4		LNP ceDNA1368.				

No. = Number; IV = intravenous; ROA = route of administration; min = minute; hr = hour

Table 36: Blood Collection (Interim):

Group Number	**Sample Collection Times**
	Whole Blood (orbital only)
	Sodium Citrate Plasma
1 – 8	Days 4, 7, 10
Volume / Portion	120 µL whole blood
Processing	120 µL whole blood was added to tube pre-coated with 13.33 µL of 3.2% sodium citrate and kept ambient until processed Two (2) aliquots processed plasma
Storage	Frozen at nominally -70°C

Table 37: Blood Collection (Terminal)

Group Number	**Sample Collection Times**
	Terminal Whole Blood (cardiac)
	Sodium Citrate Plasma
1 – 8	Day 14
Volume / Portion	MOV

Group Number	Sample Collection Times
	Terminal Whole Blood (cardiac)
	Sodium Citrate Plasma
Processing	600 µL whole blood was added to tube pre-coated with 66.6 µL of 3.2% sodium citrate and kept ambient until processed Three (3) aliquots processed plasma
Storage	Frozen at nominally -70°C

MOV = maximum obtainable volume

[00797] Study details are provided as follows:

[00798] *Species (number, sex, age):* FVIII KO (B6;129S-F8<tm1Kaz>/J) mice (N = 16 + 2 spare, male, ~4 weeks of age at arrival) from Jackson Laboratories. CD1. 16 + 2 spares, male. 4 weeks at time of arrival.

[00799] *Cage Side Observations:* Cage side observations were performed daily.

[00800] *Clinical Observations:* Clinical observations were performed ~1, ~5-6 and ~24 hours post the Day 0 Test Material dose, as applicable for remaining groups.

[00801] *Body Weights:* Body weights for all animals were recorded on Days 0, 1, 2, 4, 7 & 14. Weights were rounded to the nearest 0.1 g. Additional weights were recorded as requested.

[00802] *Dose Formulation:* Test articles (ceDNA) were supplied in a concentration stock, at 1.0 mg/mL. Stock was warmed to room temperature and diluted with the provided PBS immediately prior to use. Prepared materials may be stored at ~4°C if dosing was not performed immediately.

[00803] Inhibitor was supplied in daily ready to dose aliquots; formulated in 0.5% methylcellulose. Formulations were mixed (pipetting) and/or sonicated prior to administration to distribute particulates of oral gavage suspension.

[00804] *Dose Administration:* Inhibitor was dosed on Day 0 per Table 1 above, by PO administration (oral gavage) at 10 mL/kg. Inhibitor was dosed 30 minutes (± 5 minutes) prior to, and 5 hours (± 10 minutes) post the Day 0 ceDNA administration. Doses of test material was administered on Day 0 by intravenous dosing into the lateral tail vein. Doses were administered at a dose volume of 5 mL/kg. Doses were rounded to the nearest 0.01 mL.

[00805] *Blood collection:* All animals in Groups 1 – 8, had interim blood collected on Days 4, 7 & 10.

[00806] For plasma collections, whole blood was collected into non-coated Eppendorf style tubes *via* orbital sinus puncture under anesthesia per facility SOPS. Immediately 120µL was withdrawn and placed into tubes containing 13.33 µL of 3.2% sodium citrate. Blood was gently mixed and maintained ambient until processed. Whole blood samples were centrifuged at 2,000g for 15 minutes under ambient conditions (20-25°C). Plasma samples were withdrawn avoiding the cell pack. Two (2) aliquots were made and any clotting in the whole blood sample or hemolysis in the plasma was noted.

[00807] *Anesthesia Recovery:* Animals were monitored continuously while under anesthesia, during recovery and until mobile.

[00808] *Euthanasia & Terminal Blood Collection:* On Day 14, animals were euthanized by CO_2 asphyxiation followed by thoracotomy and exsanguination..

[00809] Whole blood was collected by syringe and 600 µL placed immediately tubes containing 66.66 µL of 3.2% sodium citrate. Blood was gently mixed and maintained ambient until processed. Whole blood samples were centrifuged at 2,000g for 15 minutes under ambient conditions (20-25°C). Plasma samples were withdrawn avoiding the cell pack and three (3) aliquots made. Any clotting in the whole blood sample or hemolysis in the plasma was noted.

[00810] All plasma samples were stored at nominally -70ºC for analysis.

[00811] **Results: FIGs. 16 and 22** show plasma FVIII concentration (IU/mL) at 11 days after administration of the LNP:ceDNAFVIII-vector test articles, as indicated. As shown, FVIII was detected at a much greater level in plasma samples from mice treated with the LNP:ceDNAFVIII-vector 1270, 1367, 1368 test article, compared to the first generation vector 993. FVIII was not observed in mice treated with vehicle only (not shown).

EXAMPLE 13: A Study to Determine FVIII Expression after Hydrodynamic ceDNA delivery in Male FVIII KO Mice

[00812] A hydrodynamic delivery system was used to determine FVIII expression and activity after hydrodynamic injection of ceDNA. ceDNA vectors as described in Example 6 were employed. SEQ ID NOs for the ceDNA constructs are shown in **Table 18**, and a description of the constructs is provided in **Table 20**. Test materials for the study are shown in **Table 35**, below.

Table 38

Group No.	No. of Animals	Strain	Test Material	Dose Levels (µg/an)	Dose Volume (mL/kg)	Dosing Regimen ROA	Terminal Time Point
1	4	FVIII KO	PBS	NA	90 – 100 ml/kg (set volume)	Once on Day 0 by IV Hydrodynamic	Day 3
2	4		ceDNA1368	5			
3	4		ceDNA1374	5			
4	4		ceDNA1652	5			
5	4		ceDNA1838	5			
6	4		ceDNA1840	5			
7	4		ceDNA1922	5			
8	4		ceDNA1923	5			

No. = Number; IV = intravenous; ROA = route of administration; min = minute; hr = hour

[00813] *Species (number, sex, age):* FVIII KO (B6;129S-F8<tm1Kaz>/J). 4-8 weeks at age of arrival.

[00814] Remaining study details are similar to those provided in Examples 7 and 8 above.

[00815] Results: FIGS. 17 and 18 show that codon optimized constructs *without* F309S mutation: *i.e.*, 1368 and its variants such as 1923, 1823, 1840, provides improvements in single chain version of FVIII ("SC") protein expression. Among these constructs, 1923 demonstrated consistently higher expression over other condon optimized SC FVIII ceDNA constructs.

EXAMPLE 14: Evaluation of LNP in Non-Human Primate Tolerability Study

[00816] The objective of this study wass to evaluate the tolerability of a 70-minute intravenous infusion of LNP formulated ceDNA to male *Cynomolgus* monkeys. SEQ ID NOs for the ceDNA constructs are shown in **Table 18**, and a description of the constructs is provided in Table 20. Test materials for the study are shown in **Table 39** below. ceDNA containing a Factor IX expression cassette was used as an independent control.

[00817] Table 39

Group No.	No. of Males	Pretreatments	Test Material	Dose Level (mg/kg)	Cocn. (mg/mL)	Necropsy Timepoint	Dose Volume (mL)	Dose Route/ Regimen
1	1	Dexamethasone and Diphenhydramine 30 minutes prior to dosing	ceDNA1270	0.3	0.06	24 hr	5	70 min IV infusion on Day 1 Infusion rate for first 15 minutes: 0.42 mL/kg Infusion rate for the remaining 55 minutes: 4.59 mL/kg
2	2			1.0	0.2	Day 14		
3	1		ceDNA1270	0.3	0.06	24 hr		
4	2			1.0	0.2	Day 14		
5	1		ceDNA-FIX	0.3	0.06	24 hr		
6	2			1.0	0.2	Day 15		
7	2			2.0	0.4	Day 15		
8	1		Saline Control	0	NA	Day 15		

[00818] The following study details are provided:

[00819] *Animals:* Species: Macaca fascicularis; Strain: Cynomolgus macaque; Number of Males: 12; Age: Adult; Research Status: Non-naïve; Weight: ~2-5 kg; Source: Testing Facility Colony.

[00820] *Dose Administration*

[00821] Pre-treatment: All animals in Groups 1-8 were administered diphenhydramine (5 mg/kg, IV or IM) and dexamethasone (1 mg/kg, IV or IM) 30 minutes (± 3 minutes) prior to the start of dosing.

[00822] *Test Article Administration:* the test materials were administered by IV infusion to restrained animals in Groups 1-8 over an approximate 70-minute period. Doses were administered through either the saphenous or cephalic vein with a temporary IV catheter. The catheter was flushed with with 0.5 mL of saline at the end of dosing. Dose volumes were calculated based on the most recent body weight and rounded to the nearest 0.1 mL. The end time of IV administration was used to determine target times for blood sample and necropsy collection time points. Injection site, dosing start and finish times were recorded in the raw data. The injection site was marked with indelible ink.

[00823] *In-Life Observations and Measurements*

[00824] Animal Health Checks: animal health checks were performed at least twice daily, in which all animals were checked for general health.

[00825] Clinical Observations: clinical observations were performed at least once before dosing (Day-1 or 1) and at least once daily thereafter for the duration of the study.

[00826] Body Weights: body weights were recorded prior to dosing on Day-1 and weekly thereafter. Weights were rounded to the nearest 0.1 kg.

[00827] Body Temperature: body temperature was recorded for all animals at predose and at 1, 2, 4, and 6 hours post dose.

[00828] Sample Collection: blood samples were collected from an appropriate peripheral vein (not the vein used for dosing) as indicated in Table 40 below.

Table 40

Group No.	Cytokines	Complement Analysis	Liver Enzymes (AST, ALT, CK)	Coagulation	Whole Blood for qPCR	FVIII and FIX Expression
1, 3, 5	Pretest, 15 min, 6 hr, 24 hr	Pretest, 15 min, 6 hr, 24 hr	Pretest, 24 hr	Pretest, 24 hr	Pretest, 24 hr	N/A
2, 4, 6-8	Pretest, 15 min, 6 hr, 24 hr	Pretest, 15 min, 6 hr, 24 hr	Pretest, 24 hr, Day 14 or 15	Pretest, 24 hr, Day 14 or 15	Pretest, 24 hr, Day 14 or 15	Day 5, 7 and 14 or 15
Additive	SST	K2EDTA	SST	Sodium Citrate	K2EDTA	Sodium Citrate
~Volume of Whole Blood	0.2 mL	0.2 mL	0.2 mL	1.8 mL	1 mL	1.8 mL
Aliquots	75 µL	100 µL	80 µL	700 µL	2 ~0.5 mL	FVIII: 6 aliquots of 150uL plasma FIX: Remainder in 2 aliquots

[00829] ***Blood Collection for FVIII Expression***

[00830] Whole blood samples were collected from a peripheral vein via direct needle puncture into sodium citrate tubes. Blood was gently mixed and maintained ambient until processed. Whole blood samples were centrifuged as soon as practical at 2,000g for 15 minutes under ambient conditions (20-25°C). Plasma samples were withdrawn avoiding the cell pack. Any clotting in the whole blood sample or hemolysis in the plasma was noted. Plasma samples were stored at nominally -80°C until shipment to the Sponsor for analysis.

[00831] ***Blood Collection for FIX Expression***

[00832] Whole blood samples were collected from a peripheral vein via direct needle puncture into sodium citrate tubes. Blood was gently mixed and maintained ambient until processed. Whole blood samples were centrifuged as soon as practical at 2,000g for 15 minutes under ambient conditions (20-25°C). Plasma samples were withdrawn avoiding the cell pack.

[00833] Any clotting in the whole blood sample or hemolysis in the plasma was noted. Plasma samples were stored at nominally -80°C until shipment for analysis.

[00834] ***Cytokine Analysis***

[00835] Whole blood samples were collected from a peripheral vein via direct needle puncture into SST tubes and were processed for serum according to Testing Facility SOP. Serum samples were stored at -80°C until shipment for analysis.

[00836] ***Complement Analysis***

[00837] Whole blood samples were collected from a peripheral vein via direct needle puncture into K2EDTA tubes were processed for plasma according to Testing Facility SOP. Plasma samples were be stored at -80°C until shipment for analysis.

[00838] ***Liver Enzyme Analysis***

[00839] Whole blood samples were collected from a peripheral vein via direct needle puncture into SST tubes and were processed for serum according to Testing Facility SOP. Serum samples were analyzed by the Testing Facility laboratory for the liver enzymes ALT, AST and CK using an Alfa Wassermann Ace Axcel.

[00840] ***Coagulation Analysis***

[00841] Whole blood samples were collected from a peripheral vein via direct needle puncture into sodium citrate tubes and were processed for plasma according to Testing Facility SOP. Samples were transferred on wet ice if shipped same day or were stored at -80°C until transferred to IDEXX Corp for analysis of PTT, aPTT and fibrinogen.

[00842] **Whole Blood for qPCR**

[00843] Whole blood samples were collected from a peripheral vein via direct needle puncture into K2EDTA tubes and were stored at 4°C until shipment to LakePharma. Day 14 samples were collected, but not processed unless directed by amendment.

[00844] Necropsy and Tissue Collection

[00845] *qPCR:*

[00846] Two sets of six samples (12 samples total per tissue) of the following tissues were collected from all animals for qPCR (collection sites outlined below). Only the 24 hr samples were evaluated for qPCR, the Day 14 samples were collected, but not processed.

[00847] Samples weighed at a minimum 25 mg (50 mg preferred, weights to be recorded) each and were snap frozen in liquid nitrogen and stored at nominally -80°C until analysis.

[00848] Heart: Samples collected from left ventricle.

[00849] Kidney: Both the right and the left kidneys were each be bisected and half was used for histology and the other half were snap frozen for qPCR samples.

[00850] Liver: Samples were collected from a consistent area across animals.

[00851] Lung: The left lobe was processed for histology and the right lobe was snap frozen for qPCR samples.

[00852] Spleen: Samples were collected from a consistent area across animals.

[00853] *ISH*

[00854] For all animals, the remainder of the liver and spleen were collected and were placed into individually labeled cassettes (size-appropriate to fit cassette), then placed into 10% NBF. Only the 24-hour samples wereevaluated for ISH, the Day 14 samples were collected, but not processed.

[00855] Histopathology Tissue Processing

[00856] For animals euthanized at Day 14 only, the remainder of the liver and spleen were processed to the slide stage for paraffin embedded, H&E staining. Slides were processed and then shipped for ISH staining and microscopic evaluation.

EXAMPLE 15: A 14-Day Single Dose Intravenous Infusion Toxicity Study of a Lipid Nano Particle Formulation in Cynomolgus Monkeys

[00857] The objective of this study was to determine the toxicity effects of a single IV dose of a lipid nanoparticle ceDNA transgene expression after IV administration of LNP formulated ceDNA to male Cynomolgus monkeys. SEQ ID NOs for the ceDNA constructs are shown in **Table 18**, and a description of the constructs is provided in Table 20. Test materials for the study are shown in Table 41 below. Dosing was by intravenous infusion (70 minutes± 10 minutes) to the saphenous vein (cephalic or tail vein was used, if necessary) dosed at 0.42 mL/kg/hr for 15 min and then escalating to 4.59 mL/kg/hr for 55 min. Prolonged infusion with escalating dosing rate design was necessary to prevent/mitigate infusion reactions. The first day of dosing was designated as day 1. Dosing was performed once on day 1 and was carried out for 15 days.

[00858] Prior to the start of infusion, the catheters were flushed with approximately 2 mL of sterile saline. Next the dosing formulations were administered at 0.42 mL/kg/hr for the first 15 minutes (target time). The infusion pump was stopped, reprogrammed to infuse the remaining dose for an

infusion rate of 4.59 mL/kg/hr, for the remaining 55 minutes (target time) of the infusion. An approximate 1.0 mL flush of sterile salinewas administered via the catheter after dose administration.

Table 41.

Group No.	Test Material	Dose Level (mg/kg/dose	Dose Volume[a] (mL/kg)	Dose Concentration (mg/mL)	No. of Animals
					Males[b]
1	Vehicle	0	4.31	0	2
2	ceDNA-FIX	2.0	4.31	0.46	2
3	ceDNA-FIX	2.0	4.31	0.46	2
4	ceDNA-FIX	2.0	4.31	0.46	2
5	ceDNA1270	1.0	4.31	0.23	1
6	ceDNA1270	2.0	4.31	0.46	2

[a] Based on the most recent body weight measurement. The first day of dosing was be based on Day 1 body weights.

To mitigate potential infusion reactions, all animals were pretreated approximately 30 ± 5 minutes prior to start of infusion with diphenhydramine and dexamethasone. In addition, all animals received a second dose of diphenhydramine and dexamethasone approximately 4 hours ± 10 minutes post infusion. Diphenhydramine was administered as an intramuscular injection at a dose volume of 0.1 ml/kg to achieve a dose level of 5 mg/kg/dose. Dexamethasone was administered as an intramuscular injection at a dose volume of 0.25 ml/kg to achieve a dose level of 1 mg/kg/dose.

[00859] : **FIG. 25** shows the results from *in vivo* studies in mice and non-human primates (NHP) using various ceDNA vectors disclosed herein to express FVIII protein as described in Examples 10, 15 and 16. Non human primates (NHPs) were administered with 1 mg/kg ceDNA 1270 in LNP formulation 1 (Ionizable lipid: DSPC : Cholesterol : PEG-Lipid + DSPE-PEG-GalNAc4 (47.5 : 10.0 : 39.2 : 3.3) (DP#1) or 2mg/kg ceDNA 1270 in LNP formulation 2 (Ionizable lipid : DSPC : Cholesterol : PEG-Lipid + DSPE-PEG2000-GalNAc4 (47.3 : 10.0 : 40.5 : 2.3) (DP#2). As shown in **FIG. 25**, it was observed that plasma FVIII concentration (IU/ml) was increased in NHP in the studies described in both Examples 14 (for DP#1) and 15 (for DP#2) as compared to control (vehicle), suggesting that the LNP formulated FVIII ceDNA constructs disclosed herein could be effectively delivered and expressed to increase plasma FVIII protein levels even in non-human primates that may exhibit heightened levels of neutralizing antibodies responses against human FVIII.

[00860] **FIG. 27** depicts a chart showing FVIII expression levels using various spacer variants of 3x hSerpEnh (2-mer and 11-mer) and Serpin enhancer sequence variants (*e.g.*, bushbaby Serpin enahancer and Chinese tree shrew Serpin enhancer) as compared to 3x human serpin enhancer as in ceDNA construct 1651 whose FVIII expression is driven be 3x VD promoter set). These constructs are identical except the spacers for hSerpEnh (spacer variants) or the SerpEnh sequence (SerpEnh variants from bushbaby and Chinese tree shrew). One dose of 50ng plasmid containing FVIII ceDNA sequence was hydrodynamically injected into the tail vein of Rag2 mice on day 0 with a single blood collection performed at day 3 (~72hr post dose) followed by FVIII activity measurements. As shown in FIG. 27, FVIII construct having 3x hSerpin enhancers with a spacer of two nucleotides ("2mer" spacers) placed inbetween each hSerpEnh showed higher FVIII expression levels as compared the

control construct having 3x VD promoter set with a single nucleotide spacer. Surprisingly, the construct having an 11-nucleotide spacer (3xhSerpEnh_11mer_spacers_v3) exhibited an increased level of FVIII expression as compared to 1 nucleotide spacers or 5 nucleotide spacers (data not shown). Further, three repeats of the bushbaby serpin enhancer sequence as well as Chinese tree shrew Serpin enhancer sequence drove higher levels of FVIII expression as compared to 3x human Serpin enhancer (*i.e.*, 3x VD promoter set), suggesting that these conserved homologous enhancer sequences may have a positive impact on FVIII transcription in the liver.

[00861] **FIG. 28** depicts a chart showing the results from an *in vivo* study in which C57BL/6J mice were hydrodynamically injected with synthetically made FVIII-ceDNA molecules, and FVIII activity was measured at Day 3 in the serum of the treated mice. The ceDNA constructs were: (1) ceDNA construct 10 (wild-type left ITR : left ITR spacer : 3x hSerpEnh VD promoter set : Mouse TTR 5'UTR : MVM Intron: hFVIII-F309S_BD226seq124-BDD-F309 ORF which is identical to the ORF sequence of ceDNA 1651 : WPRE_3pUTR : bGH : right ITR Spacer : wild-type right ITR; (2) ceDNA construct 60 which is essentially identical to ceDNA construct 10 except that it constains 3x_hSerpEnh-2mer spacer v17; (3) ceDNA construct 61 which is essentially identical to ceDNA construct 10 except that it contains 3x_SerpEnh_11-mer_spacers_v3; (4) ceDNA construct 62 which is essentially identical to ceDNA construct 10 except it has 3x_Bushbaby SerpEnh with adenine (A) spacers ("Aspacers"); and (5) ceDNA construct 39 which is essentially identicalto ceDNA construct 10 except that it contains a truncated right ITR. Consistent with the observations in **FIG. 27**, ceDNA constructs having the 3x human serpin enhancer with 11mer spacers (3xhSerpEnh_11mer_spacers_v3) and 3x bushbaby serpin enhancers (3xBushbaby_ Aspacers) exhibited equivalent or superior expression profiles in the ceDNA platform as compared to that of 3x VD driving FVIII expression (*see*, **FIG. 27**).

[00862] ceDNA construct 10 contains wild-type left ITR : left ITR spacer : 3x hSerpEnh VD promoter set : mouse TTR 5'UTR : MVM Intron: hFVIII-F309S_BD226seq124-BDD-F309 ORF identical to the ORF sequence of ceDNA 1651) :

(1) **WPRE_3pUTR : bGH : right ITR Spacer : wild-type right ITR** as shown below:

TGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCCTTGTAG
TTAATGATTAACCCGCCATGCTACTTATCGCGGCCGCGGGGGAGGCTGCTGGTGAATATT
AACCAAGGTCACCCCAGTTATCGGAGGAGCAAACAGGGGCTAAGTCCACCGGGGGAGGC
TGCTGGTGAATATTAACCAAGGTCACCCCAGTTATCGGAGGAGCAAACAGGGGCTAAGTC
CACCGGGGGAGGCTGCTGGTGAATATTAACCAAGGTCACCCCAGTTATCGGAGGAGCAA
ACAGGGGCTAAGTCCACGGTACCCACTGGGAGGATGTTGAGTAAGATGGAAAACTACTG
ATGACCCTTGCAGAGACAGAGTATTAGGACATGTTTGAACAGGGGCCGGGCGATCAGCA
GGTAGCTCTAGAGGATCCCCGTCTGTCTGCACATTTCGTAGAGCGAGTGTTCCGATACTCT
AATCTCCCTAGGCAAGGTTCATATTTGTGTAGGTTACTTATTCTCCTTTTGTTGACTAAGTC

AATAATCAGAATCAGCAGGTTTGGAGTCAGCTTGGCAGGGATCAGCAGCCTGGGTTGGA
AGGAGGGGGTATAAAAGCCCCTTCACCAGGAGAAGCCGTCACACAGATCCACAAGCTCC
TGAAGAGGTAAGGGTTTAAGGGATGGTTGGTTGGTGGGGTATTAATGTTTAATTACCTGG
AGCACCTGCCTGAAATCACTTTTTTTCAGGTTGGTTTAAACGCCGCCACCATGCAGATTGA
GCTGAGCACCTGCTTCTTCCTGTGCCTGCTGAGGTTCTGCTTCTCTGCCACCAGGAGATAC
TACCTGGGGGCTGTGGAGCTGAGCTGGGACTACATGCAGTCTGACCTGGGGGAGCTGCCT
GTGGATGCCAGGTTCCCCCCCAGAGTGCCCAAGAGCTTCCCCTTCAACACCTCTGTGGTGT
ACAAGAAGACCCTGTTTGTGGAGTTCACTGACCACCTGTTCAACATTGCCAAGCCCAGGC
CCCCCTGGATGGGCCTGCTGGGCCCCACCATCCAGGCTGAGGTGTATGACACTGTGGTGA
TCACCCTGAAGAACATGGCCAGCCACCCTGTGAGCCTGCATGCTGTGGGGGTGAGCTACT
GGAAGGCCTCTGAGGGGGCTGAGTATGATGACCAGACCAGCCAGAGGGAGAAGGAGGAT
GACAAGGTGTTCCCTGGGGGCAGCCACACCTATGTGTGGCAGGTGCTGAAGGAGAATGG
CCCCATGGCCTCTGACCCCCTGTGCCTGACCTACAGCTACCTGAGCCATGTGGACCTGGTG
AAGGACCTGAACTCTGGCCTGATTGGGGCCCTGCTGGTGTGCAGGGAGGGCAGCCTGGCC
AAGGAGAAGACCCAGACCCTGCACAAGTTCATCCTGCTGTTTGCTGTGTTTGATGAGGGC
AAGAGCTGGCACTCTGAAACCAAGAACAGCCTGATGCAGGACAGGGATGCTGCCTCTGC
CAGGGCCTGGCCCAAGATGCACACTGTGAATGGCTATGTGAACAGGAGCCTGCCTGGCCT
GATTGGCTGCCACAGGAAGTCTGTGTACTGGCATGTGATTGGCATGGGCACCACCCCTGA
GGTGCACAGCATCTTCCTGGAGGGCCACACCTTCCTGGTCAGGAACCACAGGCAGGCCAG
CCTGGAGATCAGCCCCATCACCTTCCTGACTGCCCAGACCCTGCTGATGGACCTGGGCCA
GTTCCTGCTGTTCTGCCACATCAGCAGCCACCAGCATGATGGCATGGAGGCCTATGTGAA
GGTGGACAGCTGCCCTGAGGAGCCCCAGCTGAGGATGAAGAACAATGAGGAGGCTGAGG
ACTATGATGATGACCTGACTGACTCTGAGATGGATGTGGTGAGGTTTGATGATGACAACA
GCCCCAGCTTCATCCAGATCAGGTCTGTGGCCAAGAAGCACCCCAAGACCTGGGTGCACT
ACATTGCTGCTGAGGAGGAGGACTGGGACTATGCCCCCCTGGTGCTGGCCCCTGATGACA
GGAGCTACAAGAGCCAGTACCTGAACAATGGCCCCCAGAGGATTGGCAGGAAGTACAAG
AAGGTCAGGTTCATGGCCTACACTGATGAAACCTTCAAGACCAGGGAGGCCATCCAGCAT
GAGTCTGGCATCCTGGGCCCCCTGCTGTATGGGGAGGTGGGGGACACCCTGCTGATCATC
TTCAAGAACCAGGCCAGCAGGCCCTACAACATCTACCCCCATGGCATCACTGATGTGAGG
CCCCTGTACAGCAGGAGGCTGCCCAAGGGGGTGAAGCACCTGAAGGACTTCCCCATCCTG
CCTGGGGAGATCTTCAAGTACAAGTGGACTGTGACTGTGGAGGATGGCCCCACCAAGTCT
GACCCCAGGTGCCTGACCAGATACTACAGCAGCTTTGTGAACATGGAGAGGGACCTGGCC
TCTGGCCTGATTGGCCCCCTGCTGATCTGCTACAAGGAGTCTGTGGACCAGAGGGGCAAC
CAGATCATGTCTGACAAGAGGAATGTGATCCTGTTCTCTGTGTTTGATGAGAACAGGAGC
TGGTACCTGACTGAGAACATCCAGAGGTTCCTGCCCAACCCTGCTGGGGTGCAGCTGGAG
GACCCTGAGTTCCAGGCCAGCAACATCATGCACAGCATCAATGGCTATGTGTTTGACAGC

CTGCAGCTGTCTGTGTGCCTGCATGAGGTGGCCTACTGGTACATCCTGAGCATTGGGGCC
CAGACTGACTTCCTGTCTGTGTTCTTCTCTGGCTACACCTTCAAGCACAAGATGGTGTATG
AGGACACCCTGACCCTGTTCCCCTTCTCTGGGGAGACTGTGTTCATGAGCATGGAGAACC
CTGGCCTGTGGATTCTGGGCTGCCACAACTCTGACTTCAGGAACAGGGGCATGACTGCCC
TGCTGAAAGTCTCCAGCTGTGACAAGAACACTGGGGACTACTATGAGGACAGCTATGAG
GACATCTCTGCCTACCTGCTGAGCAAGAACAATGCCATTGAGCCCAGGAGCTTCAGCCAG
AATAGCAGGCACCCCAGCACCAGGCAGAAGCAGTTCAATGCCACCACCATCCCAGAGAA
TACCACCCTGCAGTCTGACCAGGAGGAGATTGACTATGATGACACCATCTCTGTGGAGAT
GAAGAAGGAGGACTTTGACATCTACGACGAGGACGAGAACCAGAGCCCCAGGAGCTTCC
AGAAGAAGACCAGGCACTACTTCATTGCTGCTGTGGAGAGGCTGTGGGACTATGGCATGA
GCAGCAGCCCCCATGTGCTGAGGAACAGGGCCCAGTCTGGCTCTGTGCCCCAGTTCAAGA
AGGTGGTGTTCCAGGAGTTCACTGATGGCAGCTTCACCCAGCCCCTGTACAGAGGGGAGC
TGAATGAGCACCTGGGCCTGCTGGGCCCCTACATCAGGGCTGAGGTGGAGGACAACATC
ATGGTGACCTTCAGGAACCAGGCCAGCAGGCCCTACAGCTTCTACAGCAGCCTGATCAGC
TATGAGGAGGACCAGAGGCAGGGGGCTGAGCCCAGGAAGAACTTTGTGAAGCCCAATGA
AACCAAGACCTACTTCTGGAAGGTGCAGCACCACATGGCCCCCACCAAGGATGAGTTTGA
CTGCAAGGCCTGGGCCTACTTCTCTGATGTGGACCTGGAGAAGGATGTGCACTCTGGCCT
GATTGGCCCCCTGCTGGTGTGCCACACCAACACCCTGAACCCTGCCCATGGCAGGCAGGT
GACTGTGCAGGAGTTTGCCCTGTTCTTCACCATCTTTGATGAAACCAAGAGCTGGTACTTC
ACTGAGAACATGGAGAGGAACTGCAGGGCCCCCTGCAACATCCAGATGGAGGACCCCAC
CTTCAAGGAGAACTACAGGTTCCATGCCATCAATGGCTACATCATGGACACCCTGCCTGG
CCTGGTGATGGCCCAGGACCAGAGGATCAGGTGGTACCTGCTGAGCATGGGCAGCAATG
AGAACATCCACAGCATCCACTTCTCTGGCCATGTGTTCACTGTGAGGAAGAAGGAGGAGT
ACAAGATGGCCCTGTACAACCTGTACCCTGGGGTGTTTGAGACTGTGGAGATGCTGCCCA
GCAAGGCTGGCATCTGGAGGGTGGAGTGCCTGATTGGGGAGCACCTGCATGCTGGCATG
AGCACCCTGTTCCTGGTGTACAGCAACAAGTGCCAGACCCCCCTGGGCATGGCCTCTGGC
CACATCAGGGACTTCCAGATCACTGCCTCTGGCCAGTATGGCCAGTGGGCCCCCAAGCTG
GCCAGGCTGCACTACTCTGGCAGCATCAATGCCTGGAGCACCAAGGAGCCCTTCAGCTGG
ATCAAGGTGGACCTGCTGGCCCCCATGATCATCCATGGCATCAAGACCCAGGGGGCCAGG
CAGAAGTTCAGCAGCCTGTACATCAGCCAGTTCATCATCATGTACAGCCTGGATGGCAAG
AAGTGGCAGACCTACAGGGGCAACAGCACTGGCACCCTGATGGTGTTCTTTGGCAATGTG
GACAGCTCTGGCATCAAGCACAACATCTTCAACCCCCCCATCATTGCCAGATACATCAGG
CTGCACCCCACCCACTACAGCATCAGGAGCACCCTGAGGATGGAGCTGATGGGCTGTGAC
CTGAACAGCTGCAGCATGCCCCTGGGCATGGAGAGCAAGGCCATCTCTGATGCCCAGATC
ACTGCCAGCAGCTACTTCACCAACATGTTTGCCACCTGGAGCCCCAGCAAGGCCAGGCTG
CACCTGCAGGGCAGGAGCAATGCCTGGAGGCCCCAGGTCAACAACCCCAAGGAGTGGCT

GCAGGTGGACTTCCAGAAGACCATGAAGGTGACTGGGGTGACCACCCAGGGGGTGAAGA
GCCTGCTGACCAGCATGTATGTGAAGGAGTTCCTGATCAGCAGCAGCCAGGATGGCCACC
AGTGGACCCTGTTCTTCCAGAATGGCAAGGTGAAGGTGTTCCAGGGCAACCAGGACAGCT
TCACCCCTGTGGTGAACAGCCTGGACCCCCCCCTGCTGACCAGATACCTGAGGATTCACC
CCCAGAGCTGGGTGCACCAGATTGCCCTGAGGATGGAGGTGCTGGGCTGTGAGGCCCAG
GACCTGTACTGATTAATTAAGAGCATCTTACCGCCATTTATTCCCATATTTGTTCTGTTTTT
CTTGATTTGGGTATACATTTAAATGTTAATAAAACAAAATGGTGGGGCAATCATTTACATT
TTTAGGGATATGTAATTACTAGTTCAGGTGTATTGCCACAAGACAAACATGTTAAGAAAC
TTTCCCGTTATTTACGCTCTGTTCCTGTTAATCAACCTCTGGATTACAAAATTTGTGAAAG
ATTGACTGATATTCTTAACTATGTTGCTCCTTTTACGCTGTGTGGATATGCTGCTTTATAGC
CTCTGTATCTAGCTATTGCTTCCCGTACGGCTTTCGTTTTCTCCTCCTTGTATAAATCCTGG
TTGCTGTCTCTTTTAGAGGAGTTGTGGCCCGTTGTCCGTCAACGTGGCGTGGTGTGCTCTG
TGTTTGCTGACGCAACCCCCACTGGCTGGGGCATTGCCACCACCTGTCAACTCCTTTCTGG
GACTTTCGCTTTCCCCCTCCCGATCGCCACGGCAGAACTCATCGCCGCCTGCCTTGCCCGC
TGCTGGACAGGGGCTAGGTTGCTGGGCACTGATAATTCCGTGGTGTTGTCTGTGCCTTCTA
GTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCAC
TCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCAT
TCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAG
CAGGCATGCTGGGGATGCGGTGGGCTCTATGGCTCTAGAGCATGGCTACGTAGATAAGTA
GCATGGCGGGTTAATCATTAACTACACCTGCAGGAGGAACCCCTAGTGATGGAGTTGGCC
ACTCCCTCTCTGCGCGCTCGCTCGCTCA

(SEQ ID NO: 642)

(2) ceDNA construct 60 constains 3x_hSerpEnh-2mer spacer v17

[00863] TGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCC
TTGTAGTTAATGATTAACCCGCCATGCTACTTATCGCGGCCGCGGGGGAGGCTGCTGGTG
AATATTAACCAAGGTCACCCCAGTTATCGGAGGAGCAAACAGGGGCTAAGTCCACCTGG
GGGAGGCTGCTGGTGAATATTAACCAAGGTCACCCCAGTTATCGGAGGAGCAAACAGGG
GCTAAGTCCACAAGGGGGAGGCTGCTGGTGAATATTAACCAAGGTCACCCCAGTTATCGG
AGGAGCAAACAGGGGCTAAGTCCACGGTACCCACTGGGAGGATGTTGAGTAAGATGGAA
AACTACTGATGACCCTTGCAGAGACAGAGTATTAGGACATGTTTGAACAGGGGCCGGGC
GATCAGCAGGTAGCTCTAGAGGATCCCCGTCTGTCTGCACATTTCGTAGAGCGAGTGTTC
CGATACTCTAATCTCCCTAGGCAAGGTTCATATTTGTGTAGGTTACTTATTCTCCTTTTGTT
GACTAAGTCAATAATCAGAATCAGCAGGTTTGGAGTCAGCTTGGCAGGGATCAGCAGCCT
GGGTTGGAAGGAGGGGGTATAAAAGCCCCTTCACCAGGAGAAGCCGTCACACAGATCCA
CAAGCTCCTGAAGAGGTAAGGGTTTAAGGGATGGTTGGTTGGTGGGGTATTAATGTTTAA

TTACCTGGAGCACCTGCCTGAAATCACTTTTTTTCAGGTTGGTTTAAACGCCGCCACCATG
CAGATAGAGCTCAGCACCTGCTTCTTCCTGTGCCTCCTCAGGTTCTGCTTCTCTGCCACCA
GGAGATACTACCTGGGGGCTGTGGAGCTGAGCTGGGACTACATGCAGTCTGACCTGGGG
GAGCTGCCTGTGGACGCCAGGTTCCCCCCCAGAGTGCCCAAGAGCTTCCCCTTCAACACC
TCTGTGGTGTACAAGAAGACCCTGTTTGTGGAGTTCACTGACCACCTGTTCAACATTGCCA
AGCCCAGGCCCCCCTGGATGGGCCTGCTGGGCCCCACCATCCAGGCTGAGGTGTATGACA
CTGTGGTGATCACCCTGAAGAACATGGCCAGCCACCCTGTGAGCCTGCATGCTGTGGGGG
TGAGCTACTGGAAGGCCTCTGAGGGGGCTGAGTATGATGACCAGACCAGCCAGAGGGAG
AAGGAGGATGACAAGGTGTTCCCTGGGGGCAGCCACACCTATGTGTGGCAGGTGCTGAA
GGAGAATGGCCCCATGGCCTCTGACCCCCTGTGCCTGACCTACAGCTACCTGAGCCATGT
GGACCTGGTGAAGGACCTGAACTCTGGCCTGATTGGGGCCCTGCTGGTGTGCAGGGAGGG
CAGCCTGGCCAAGGAGAAGACCCAGACCCTGCACAAGTTCATCCTGCTGTTTGCTGTGTT
TGATGAGGGCAAGAGCTGGCACTCTGAAACCAAGAACAGCCTGATGCAGGACAGGGATG
CTGCCTCTGCCAGGGCCTGGCCCAAGATGCACACTGTGAATGGCTATGTGAACAGGAGCC
TGCCTGGCCTGATTGGCTGCCACAGGAAGTCTGTGTACTGGCATGTGATTGGCATGGGCA
CCACCCCTGAGGTGCACAGCATCTTCCTGGAGGGCCACACCTTCCTGGTCAGGAACCACA
GGCAGGCCAGCCTGGAGATCAGCCCCATCACCTTCCTGACTGCCCAGACCCTGCTGATGG
ACCTGGGCCAGTTCCTGCTGTTCTGCCACATCAGCAGCCACCAGCATGATGGCATGGAGG
CCTATGTGAAGGTGGACAGCTGCCCTGAGGAGCCCCAGCTGAGGATGAAGAACAATGAG
GAGGCTGAGGACTATGATGATGACCTGACTGACTCTGAGATGGATGTGGTGAGGTTTGAT
GATGACAACAGCCCCAGCTTCATCCAGATCAGGTCTGTGGCCAAGAAGCACCCCAAGACC
TGGGTGCACTACATTGCTGCTGAGGAGGAGGACTGGGACTATGCCCCCCTGGTGCTGGCC
CCTGATGACAGGAGCTACAAGAGCCAGTACCTGAACAATGGCCCCCAGAGGATTGGCAG
GAAGTACAAGAAGGTCAGGTTCATGGCCTACACTGATGAAACCTTCAAGACCAGGGAGG
CCATCCAGCATGAGTCTGGCATCCTGGGCCCCCTGCTGTATGGGGAGGTGGGGGACACCC
TGCTGATCATCTTCAAGAACCAGGCCAGCAGGCCCTACAACATCTACCCCCACGGCATCA
CTGATGTGAGGCCCCTGTACAGCAGGAGGCTGCCCAAGGGGGTGAAGCACCTGAAGGAC
TTCCCCATCCTGCCTGGGGAGATCTTCAAGTACAAGTGGACTGTGACTGTGGAGGATGGC
CCCACCAAGTCTGACCCCAGGTGCCTGACCAGATACTACAGCAGCTTTGTGAACATGGAG
AGGGACCTGGCCTCTGGCCTGATTGGCCCCCTGCTGATCTGCTACAAGGAGTCTGTGGAC
CAGAGGGGCAACCAGATCATGTCTGACAAGAGGAATGTGATCCTGTTCTCTGTGTTTGAT
GAGAACAGGAGCTGGTACCTGACTGAGAACATCCAGAGGTTCCTGCCCAACCCTGCTGGG
GTGCAGCTGGAGGACCCTGAGTTCCAGGCCAGCAACATCATGCACAGCATCAATGGCTAT
GTGTTTGACAGCCTGCAGCTGTCTGTGTGCCTGCATGAGGTGGCCTACTGGTACATCCTGA
GCATTGGGGCCCAGACTGACTTCCTGTCTGTGTTCTTCTCTGGCTACACCTTCAAGCACAA
GATGGTGTATGAGGACACCCTGACCCTGTTCCCCTTCTCTGGGGAGACTGTGTTCATGAGC

ATGGAGAACCCTGGCCTGTGGATTCTGGGCTGCCACAACTCTGACTTCAGGAACAGGGGC
ATGACTGCCCTGCTGAAAGTCTCCAGCTGTGACAAGAACACTGGGGACTACTACGAGGAC
AGCTATGAGGACATCTCTGCCTACCTGCTGAGCAAGAACAATGCCATTGAGCCCAGGAGC
TTCAGCCAGAATAGCAGGCACCCCAGCACCAGGCAGAAGCAGTTCAATGCCACCACCAT
CCCAGAGAATACCACCCTGCAGTCTGACCAGGAGGAGATTGACTATGATGACACCATCTC
TGTGGAGATGAAGAAGGAGGACTTTGACATCTACGACGAGGACGAGAACCAGAGCCCCA
GGAGCTTCCAGAAGAAGACCAGGCACTACTTCATTGCTGCTGTGGAGAGGCTGTGGGACT
ATGGCATGAGCAGCAGCCCCCATGTGCTGAGGAACAGGGCCCAGTCTGGCTCTGTGCCCC
AGTTCAAGAAGGTGGTGTTCCAGGAGTTCACTGATGGCAGCTTCACCCAGCCCCTGTACA
GAGGGGAGCTGAATGAGCACCTGGGCCTGCTGGGCCCCTACATCAGGGCTGAGGTGGAG
GACAACATCATGGTGACCTTCAGGAACCAGGCCAGCAGGCCCTACAGCTTCTACAGCAGC
CTGATCAGCTATGAGGAGGACCAGAGGCAGGGGGCTGAGCCCAGGAAGAACTTTGTGAA
GCCCAATGAAACCAAGACCTACTTCTGGAAGGTGCAGCACCACATGGCCCCCACCAAGG
ATGAGTTTGACTGCAAGGCCTGGGCCTACTTCTCTGATGTGGACCTGGAGAAGGATGTGC
ACTCTGGCCTGATTGGCCCCCTGCTGGTGTGCCACACCAACACCCTGAACCCTGCCCATG
GCAGGCAGGTGACTGTGCAGGAGTTTGCCCTGTTCTTCACCATCTTTGATGAAACCAAGA
GCTGGTACTTCACTGAGAACATGGAGAGGAACTGCAGGGCCCCCTGCAACATCCAGATG
GAGGACCCCACCTTCAAGGAGAACTACAGGTTCCATGCCATCAATGGCTACATCATGGAC
ACCCTGCCTGGCCTGGTGATGGCCCAGGACCAGAGGATCAGGTGGTACCTGCTGAGCATG
GGCAGCAATGAGAACATCCACAGCATCCACTTCTCTGGCCATGTGTTCACTGTGAGGAAG
AAGGAGGAGTACAAGATGGCCCTGTACAACCTGTACCCTGGGGTGTTTGAGACTGTGGAG
ATGCTGCCCAGCAAGGCTGGCATCTGGAGGGTGGAGTGCCTGATTGGGGAGCACCTGCAT
GCTGGCATGAGCACCCTGTTCCTGGTGTACAGCAACAAGTGCCAGACCCCCCTGGGCATG
GCCTCTGGCCACATCAGGGACTTCCAGATCACTGCCTCTGGCCAGTATGGCCAGTGGGCC
CCCAAGCTGGCCAGGCTGCACTACTCTGGCAGCATCAATGCCTGGAGCACCAAGGAGCCC
TTCAGCTGGATCAAGGTGGACCTGCTGGCCCCCATGATCATCCATGGCATCAAGACCCAG
GGGGCCAGGCAGAAGTTCAGCAGCCTGTACATCAGCCAGTTCATCATCATGTACAGCCTG
GATGGCAAGAAGTGGCAGACCTACAGGGGCAACAGCACTGGCACCCTGATGGTGTTCTTT
GGCAATGTGGACAGCTCTGGCATCAAGCACAACATCTTCAACCCCCCCATCATTGCCAGA
TACATCAGGCTGCACCCCACCCACTACAGCATCAGGAGCACCCTGAGGATGGAGCTGATG
GGCTGTGACCTGAACAGCTGCAGCATGCCCCTGGGCATGGAGAGCAAGGCCATCTCTGAT
GCCCAGATCACTGCCAGCAGCTACTTCACCAACATGTTTGCCACCTGGAGCCCCAGCAAG
GCCAGGCTGCACCTGCAGGGCAGGAGCAATGCCTGGAGGCCCCAGGTCAACAACCCCAA
GGAGTGGCTGCAGGTGGACTTCCAGAAGACCATGAAGGTGACTGGGGTGACCACCCAGG
GGGTGAAGAGCCTGCTGACCAGCATGTATGTGAAGGAGTTCCTGATCAGCAGCAGCCAG
GATGGCCACCAGTGGACCCTGTTCTTCCAGAATGGCAAGGTGAAGGTGTTCCAGGGCAAC

CAGGACAGCTTCACCCCTGTGGTGAACAGCCTGGACCCCCCCCTGCTGACCAGATACCTG
AGGATTCACCCCCAGAGCTGGGTGCACCAGATTGCCCTGAGGATGGAGGTGCTGGGCTGT
GAGGCCCAGGACCTGTACTGATTAATTAAGAGCATCTTACCGCCATTTATTCCCATATTTG
TTCTGTTTTTCTTGATTTGGGTATACATTTAAATGTTAATAAAACAAAATGGTGGGGCAAT
CATTTACATTTTTAGGGATATGTAATTACTAGTTCAGGTGTATTGCCACAAGACAAACATG
TTAAGAAACTTTCCCGTTATTTACGCTCTGTTCCTGTTAATCAACCTCTGGATTACAAAAT
TTGTGAAAGATTGACTGATATTCTTAACTATGTTGCTCCTTTTACGCTGTGTGGATATGCT
GCTTTATAGCCTCTGTATCTAGCTATTGCTTCCCGTACGGCTTTCGTTTTCTCCTCCTTGTA
TAAATCCTGGTTGCTGTCTCTTTTAGAGGAGTTGTGGCCCGTTGTCCGTCAACGTGGCGTG
GTGTGCTCTGTGTTTGCTGACGCAACCCCCACTGGCTGGGGCATTGCCACCACCTGTCAAC
TCCTTTCTGGGACTTTCGCTTTCCCCCTCCCGATCGCCACGGCAGAACTCATCGCCGCCTG
CCTTGCCCGCTGCTGGACAGGGGCTAGGTTGCTGGGCACTGATAATTCCGTGGTGTTGTCT
GTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGA
AGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGT
AGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGA
AGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGCTCTAGAGCATGGCTACGT
AGATAAGTAGCATGGCGGGTTAATCATTAACTACACCTGCAGGAGGAACCCCTAGTGATG
GAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCA

(SEQ ID NO: 643)

(3) ceDNA construct 61 contains 3x_SerpEnh_11-mer_spacers_v3

[00864] TGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCC
TTGTAGTTAATGATTAACCCGCCATGCTACTTATCGCGGCCGCGGGGGAGGCTGCTGGTG
AATATTAACCAAGGTCACCCCAGTTATCGGAGGAGCAAACAGGGGCTAAGTCCACTGCA
AAGTCCTGGGGGAGGCTGCTGGTGAATATTAACCAAGGTCACCCCAGTTATCGGAGGAGC
AAACAGGGGCTAAGTCCACAGTGTTTACAAGGGGGAGGCTGCTGGTGAATATTAACCAA
GGTCACCCCAGTTATCGGAGGAGCAAACAGGGGCTAAGTCCACGGTACCCACTGGGAGG
ATGTTGAGTAAGATGGAAAACTACTGATGACCCTTGCAGAGACAGAGTATTAGGACATGT
TTGAACAGGGGCCGGGCGATCAGCAGGTAGCTCTAGAGGATCCCCGTCTGTCTGCACATT
TCGTAGAGCGAGTGTTCCGATACTCTAATCTCCCTAGGCAAGGTTCATATTTGTGTAGGTT
ACTTATTCTCCTTTTGTTGACTAAGTCAATAATCAGAATCAGCAGGTTTGGAGTCAGCTTG
GCAGGGATCAGCAGCCTGGGTTGGAAGGAGGGGGTATAAAAGCCCCTTCACCAGGAGAA
GCCGTCACACAGATCCACAAGCTCCTGAAGAGGTAAGGGTTTAAGGGATGGTTGGTTGGT
GGGGTATTAATGTTTAATTACCTGGAGCACCTGCCTGAAATCACTTTTTTTCAGGTTGGTT
TAAACGCCGCCACCATGCAGATAGAGCTCAGCACCTGCTTCTTCCTGTGCCTCCTCAGGTT
CTGCTTCTCTGCCACCAGGAGATACTACCTGGGGGCTGTGGAGCTGAGCTGGGACTACAT

GCAGTCTGACCTGGGGGAGCTGCCTGTGGACGCCAGGTTCCCCCCCAGAGTGCCCAAGAG
CTTCCCCTTCAACACCTCTGTGGTGTACAAGAAGACCCTGTTTGTGGAGTTCACTGACCAC
CTGTTCAACATTGCCAAGCCCAGGCCCCCCTGGATGGGCCTGCTGGGCCCCACCATCCAG
GCTGAGGTGTATGACACTGTGGTGATCACCCTGAAGAACATGGCCAGCCACCCTGTGAGC
CTGCATGCTGTGGGGGTGAGCTACTGGAAGGCCTCTGAGGGGGCTGAGTATGATGACCAG
ACCAGCCAGAGGGAGAAGGAGGATGACAAGGTGTTCCCTGGGGGCAGCCACACCTATGT
GTGGCAGGTGCTGAAGGAGAATGGCCCCATGGCCTCTGACCCCCTGTGCCTGACCTACAG
CTACCTGAGCCATGTGGACCTGGTGAAGGACCTGAACTCTGGCCTGATTGGGGCCCTGCT
GGTGTGCAGGGAGGGCAGCCTGGCCAAGGAGAAGACCCAGACCCTGCACAAGTTCATCC
TGCTGTTTGCTGTGTTTGATGAGGGCAAGAGCTGGCACTCTGAAACCAAGAACAGCCTGA
TGCAGGACAGGGATGCTGCCTCTGCCAGGGCCTGGCCCAAGATGCACACTGTGAATGGCT
ATGTGAACAGGAGCCTGCCTGGCCTGATTGGCTGCCACAGGAAGTCTGTGTACTGGCATG
TGATTGGCATGGGCACCACCCCTGAGGTGCACAGCATCTTCCTGGAGGGCCACACCTTCC
TGGTCAGGAACCACAGGCAGGCCAGCCTGGAGATCAGCCCCATCACCTTCCTGACTGCCC
AGACCCTGCTGATGGACCTGGGCCAGTTCCTGCTGTTCTGCCACATCAGCAGCCACCAGC
ATGATGGCATGGAGGCCTATGTGAAGGTGGACAGCTGCCCTGAGGAGCCCCAGCTGAGG
ATGAAGAACAATGAGGAGGCTGAGGACTATGATGATGACCTGACTGACTCTGAGATGGA
TGTGGTGAGGTTTGATGATGACAACAGCCCCAGCTTCATCCAGATCAGGTCTGTGGCCAA
GAAGCACCCCAAGACCTGGGTGCACTACATTGCTGCTGAGGAGGAGGACTGGGACTATG
CCCCCCTGGTGCTGGCCCCTGATGACAGGAGCTACAAGAGCCAGTACCTGAACAATGGCC
CCCAGAGGATTGGCAGGAAGTACAAGAAGGTCAGGTTCATGGCCTACACTGATGAAACC
TTCAAGACCAGGGAGGCCATCCAGCATGAGTCTGGCATCCTGGGCCCCCTGCTGTATGGG
GAGGTGGGGGACACCCTGCTGATCATCTTCAAGAACCAGGCCAGCAGGCCCTACAACATC
TACCCCCACGGCATCACTGATGTGAGGCCCCTGTACAGCAGGAGGCTGCCCAAGGGGGTG
AAGCACCTGAAGGACTTCCCCATCCTGCCTGGGGAGATCTTCAAGTACAAGTGGACTGTG
ACTGTGGAGGATGGCCCCACCAAGTCTGACCCCAGGTGCCTGACCAGATACTACAGCAGC
TTTGTGAACATGGAGAGGGACCTGGCCTCTGGCCTGATTGGCCCCCTGCTGATCTGCTAC
AAGGAGTCTGTGGACCAGAGGGGCAACCAGATCATGTCTGACAAGAGGAATGTGATCCT
GTTCTCTGTGTTTGATGAGAACAGGAGCTGGTACCTGACTGAGAACATCCAGAGGTTCCT
GCCCAACCCTGCTGGGGTGCAGCTGGAGGACCCTGAGTTCCAGGCCAGCAACATCATGCA
CAGCATCAATGGCTATGTGTTTGACAGCCTGCAGCTGTCTGTGTGCCTGCATGAGGTGGC
CTACTGGTACATCCTGAGCATTGGGGCCCAGACTGACTTCCTGTCTGTGTTCTTCTCTGGC
TACACCTTCAAGCACAAGATGGTGTATGAGGACACCCTGACCCTGTTCCCCTTCTCTGGG
GAGACTGTGTTCATGAGCATGGAGAACCCTGGCCTGTGGATTCTGGGCTGCCACAACTCT
GACTTCAGGAACAGGGGCATGACTGCCCTGCTGAAAGTCTCCAGCTGTGACAAGAACACT
GGGGACTACTACGAGGACAGCTATGAGGACATCTCTGCCTACCTGCTGAGCAAGAACAAT

GCCATTGAGCCCAGGAGCTTCAGCCAGAATAGCAGGCACCCCAGCACCAGGCAGAAGCA
GTTCAATGCCACCACCATCCCAGAGAATACCACCCTGCAGTCTGACCAGGAGGAGATTGA
CTATGATGACACCATCTCTGTGGAGATGAAGAAGGAGGACTTTGACATCTACGACGAGGA
CGAGAACCAGAGCCCCAGGAGCTTCCAGAAGAAGACCAGGCACTACTTCATTGCTGCTGT
GGAGAGGCTGTGGGACTATGGCATGAGCAGCAGCCCCCATGTGCTGAGGAACAGGGCCC
AGTCTGGCTCTGTGCCCCAGTTCAAGAAGGTGGTGTTCCAGGAGTTCACTGATGGCAGCT
TCACCCAGCCCCTGTACAGAGGGGAGCTGAATGAGCACCTGGGCCTGCTGGGCCCCTACA
TCAGGGCTGAGGTGGAGGACAACATCATGGTGACCTTCAGGAACCAGGCCAGCAGGCCC
TACAGCTTCTACAGCAGCCTGATCAGCTATGAGGAGGACCAGAGGCAGGGGGCTGAGCC
CAGGAAGAACTTTGTGAAGCCCAATGAAACCAAGACCTACTTCTGGAAGGTGCAGCACC
ACATGGCCCCCACCAAGGATGAGTTTGACTGCAAGGCCTGGGCCTACTTCTCTGATGTGG
ACCTGGAGAAGGATGTGCACTCTGGCCTGATTGGCCCCCTGCTGGTGTGCCACACCAACA
CCCTGAACCCTGCCCATGGCAGGCAGGTGACTGTGCAGGAGTTTGCCCTGTTCTTCACCAT
CTTTGATGAAACCAAGAGCTGGTACTTCACTGAGAACATGGAGAGGAACTGCAGGGCCC
CCTGCAACATCCAGATGGAGGACCCCACCTTCAAGGAGAACTACAGGTTCCATGCCATCA
ATGGCTACATCATGGACACCCTGCCTGGCCTGGTGATGGCCCAGGACCAGAGGATCAGGT
GGTACCTGCTGAGCATGGGCAGCAATGAGAACATCCACAGCATCCACTTCTCTGGCCATG
TGTTCACTGTGAGGAAGAAGGAGGAGTACAAGATGGCCCTGTACAACCTGTACCCTGGG
GTGTTTGAGACTGTGGAGATGCTGCCCAGCAAGGCTGGCATCTGGAGGGTGGAGTGCCTG
ATTGGGGAGCACCTGCATGCTGGCATGAGCACCCTGTTCCTGGTGTACAGCAACAAGTGC
CAGACCCCCCTGGGCATGGCCTCTGGCCACATCAGGGACTTCCAGATCACTGCCTCTGGC
CAGTATGGCCAGTGGGCCCCCAAGCTGGCCAGGCTGCACTACTCTGGCAGCATCAATGCC
TGGAGCACCAAGGAGCCCTTCAGCTGGATCAAGGTGGACCTGCTGGCCCCCATGATCATC
CATGGCATCAAGACCCAGGGGGCCAGGCAGAAGTTCAGCAGCCTGTACATCAGCCAGTT
CATCATCATGTACAGCCTGGATGGCAAGAAGTGGCAGACCTACAGGGGCAACAGCACTG
GCACCCTGATGGTGTTCTTTGGCAATGTGGACAGCTCTGGCATCAAGCACAACATCTTCA
ACCCCCCCATCATTGCCAGATACATCAGGCTGCACCCCACCCACTACAGCATCAGGAGCA
CCCTGAGGATGGAGCTGATGGGCTGTGACCTGAACAGCTGCAGCATGCCCCTGGGCATGG
AGAGCAAGGCCATCTCTGATGCCCAGATCACTGCCAGCAGCTACTTCACCAACATGTTTG
CCACCTGGAGCCCCAGCAAGGCCAGGCTGCACCTGCAGGGCAGGAGCAATGCCTGGAGG
CCCCAGGTCAACAACCCCAAGGAGTGGCTGCAGGTGGACTTCCAGAAGACCATGAAGGT
GACTGGGGTGACCACCCAGGGGGTGAAGAGCCTGCTGACCAGCATGTATGTGAAGGAGT
TCCTGATCAGCAGCAGCCAGGATGGCCACCAGTGGACCCTGTTCTTCCAGAATGGCAAGG
TGAAGGTGTTCCAGGGCAACCAGGACAGCTTCACCCCTGTGGTGAACAGCCTGGACCCCC
CCCTGCTGACCAGATACCTGAGGATTCACCCCCAGAGCTGGGTGCACCAGATTGCCCTGA
GGATGGAGGTGCTGGGCTGTGAGGCCCAGGACCTGTACTGATTAATTAAGAGCATCTTAC

CGCCATTTATTCCCATATTTGTTCTGTTTTTCTTGATTTGGGTATACATTTAAATGTTAATA
AAACAAAATGGTGGGGCAATCATTTACATTTTTAGGGATATGTAATTACTAGTTCAGGTG
TATTGCCACAAGACAAACATGTTAAGAAACTTTCCCGTTATTTACGCTCTGTTCCTGTTAA
TCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGATATTCTTAACTATGTTGCTCCT
TTTACGCTGTGTGGATATGCTGCTTTATAGCCTCTGTATCTAGCTATTGCTTCCCGTACGGC
TTTCGTTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTCTTTTAGAGGAGTTGTGGCCCG
TTGTCCGTCAACGTGGCGTGGTGTGCTCTGTGTTTGCTGACGCAACCCCCACTGGCTGGGG
CATTGCCACCACCTGTCAACTCCTTTCTGGGACTTTCGCTTTCCCCCTCCCGATCGCCACG
GCAGAACTCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCTAGGTTGCTGGGCACT
GATAATTCCGTGGTGTTGTCTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCC
CGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAA
ATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGAC
AGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTAT
GGCTCTAGAGCATGGCTACGTAGATAAGTAGCATGGCGGGTTAATCATTAACTACACCTG
CAGGAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCA

(SEQ ID NO: 644)

(4) ceDNA construct 62 contains 3x_Bushbaby SerpEnh with adenine (A) spacers ("3xBushbaby_ Aspacers").

TGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCCTTGTAG
TTAATGATTAACCCGCCATGCTACTTATCGCGGCCGCAGGGGAAGCTACTGGTGAATATT
AACCAAGGTCACCCAGTTATCAGGGAGCAAACAGGAGCTAAGTCCATAGGGGGAAGCTA
CTGGTGAATATTAACCAAGGTCACCCAGTTATCAGGGAGCAAACAGGAGCTAAGTCCATA
GGGGGAAGCTACTGGTGAATATTAACCAAGGTCACCCAGTTATCAGGGAGCAAACAGGA
GCTAAGTCCATGGTACCCACTGGGAGGATGTTGAGTAAGATGGAAAACTACTGATGACCC
TTGCAGAGACAGAGTATTAGGACATGTTTGAACAGGGGCCGGGCGATCAGCAGGTAGCT
CTAGAGGATCCCCGTCTGTCTGCACATTTCGTAGAGCGAGTGTTCCGATACTCTAATCTCC
CTAGGCAAGGTTCATATTTGTGTAGGTTACTTATTCTCCTTTTGTTGACTAAGTCAATAAT
CAGAATCAGCAGGTTTGGAGTCAGCTTGGCAGGGATCAGCAGCCTGGGTTGGAAGGAGG
GGGTATAAAAGCCCCTTCACCAGGAGAAGCCGTCACACAGATCCACAAGCTCCTGAAGA
GGTAAGGGTTTAAGGGATGGTTGGTTGGTGGGGTATTAATGTTTAATTACCTGGAGCACC
TGCCTGAAATCACTTTTTTTCAGGTTGGTTTAAACGCCGCCACCATGCAGATAGAGCTCAG
CACCTGCTTCTTCCTGTGCCTCCTCAGGTTCTGCTTCTCTGCCACCAGGAGATACTACCTG
GGGGCTGTGGAGCTGAGCTGGGACTACATGCAGTCTGACCTGGGGGAGCTGCCTGTGGAC
GCCAGGTTCCCCCCCAGAGTGCCCAAGAGCTTCCCCTTCAACACCTCTGTGGTGTACAAG
AAGACCCTGTTTGTGGAGTTCACTGACCACCTGTTCAACATTGCCAAGCCCAGGCCCCCCT

GGATGGGCCTGCTGGGCCCCACCATCCAGGCTGAGGTGTATGACACTGTGGTGATCACCC
TGAAGAACATGGCCAGCCACCCTGTGAGCCTGCATGCTGTGGGGGTGAGCTACTGGAAG
GCCTCTGAGGGGGCTGAGTATGATGACCAGACCAGCCAGAGGGAGAAGGAGGATGACAA
GGTGTTCCCTGGGGGCAGCCACACCTATGTGTGGCAGGTGCTGAAGGAGAATGGCCCCAT
GGCCTCTGACCCCCTGTGCCTGACCTACAGCTACCTGAGCCATGTGGACCTGGTGAAGGA
CCTGAACTCTGGCCTGATTGGGGCCCTGCTGGTGTGCAGGGAGGGCAGCCTGGCCAAGGA
GAAGACCCAGACCCTGCACAAGTTCATCCTGCTGTTTGCTGTGTTTGATGAGGGCAAGAG
CTGGCACTCTGAAACCAAGAACAGCCTGATGCAGGACAGGGATGCTGCCTCTGCCAGGG
CCTGGCCCAAGATGCACACTGTGAATGGCTATGTGAACAGGAGCCTGCCTGGCCTGATTG
GCTGCCACAGGAAGTCTGTGTACTGGCATGTGATTGGCATGGGCACCACCCCTGAGGTGC
ACAGCATCTTCCTGGAGGGCCACACCTTCCTGGTCAGGAACCACAGGCAGGCCAGCCTGG
AGATCAGCCCCATCACCTTCCTGACTGCCCAGACCCTGCTGATGGACCTGGGCCAGTTCCT
GCTGTTCTGCCACATCAGCAGCCACCAGCATGATGGCATGGAGGCCTATGTGAAGGTGGA
CAGCTGCCCTGAGGAGCCCCAGCTGAGGATGAAGAACAATGAGGAGGCTGAGGACTATG
ATGATGACCTGACTGACTCTGAGATGGATGTGGTGAGGTTTGATGATGACAACAGCCCCA
GCTTCATCCAGATCAGGTCTGTGGCCAAGAAGCACCCCAAGACCTGGGTGCACTACATTG
CTGCTGAGGAGGAGGACTGGGACTATGCCCCCCTGGTGCTGGCCCCTGATGACAGGAGCT
ACAAGAGCCAGTACCTGAACAATGGCCCCCAGAGGATTGGCAGGAAGTACAAGAAGGTC
AGGTTCATGGCCTACACTGATGAAACCTTCAAGACCAGGGAGGCCATCCAGCATGAGTCT
GGCATCCTGGGCCCCCTGCTGTATGGGGAGGTGGGGGACACCCTGCTGATCATCTTCAAG
AACCAGGCCAGCAGGCCCTACAACATCTACCCCCACGGCATCACTGATGTGAGGCCCCTG
TACAGCAGGAGGCTGCCCAAGGGGGTGAAGCACCTGAAGGACTTCCCCATCCTGCCTGG
GGAGATCTTCAAGTACAAGTGGACTGTGACTGTGGAGGATGGCCCCACCAAGTCTGACCC
CAGGTGCCTGACCAGATACTACAGCAGCTTTGTGAACATGGAGAGGGACCTGGCCTCTGG
CCTGATTGGCCCCCTGCTGATCTGCTACAAGGAGTCTGTGGACCAGAGGGGCAACCAGAT
CATGTCTGACAAGAGGAATGTGATCCTGTTCTCTGTGTTTGATGAGAACAGGAGCTGGTA
CCTGACTGAGAACATCCAGAGGTTCCTGCCCAACCCTGCTGGGGTGCAGCTGGAGGACCC
TGAGTTCCAGGCCAGCAACATCATGCACAGCATCAATGGCTATGTGTTTGACAGCCTGCA
GCTGTCTGTGTGCCTGCATGAGGTGGCCTACTGGTACATCCTGAGCATTGGGGCCCAGAC
TGACTTCCTGTCTGTGTTCTTCTCTGGCTACACCTTCAAGCACAAGATGGTGTATGAGGAC
ACCCTGACCCTGTTCCCCTTCTCTGGGGAGACTGTGTTCATGAGCATGGAGAACCCTGGCC
TGTGGATTCTGGGCTGCCACAACTCTGACTTCAGGAACAGGGGCATGACTGCCCTGCTGA
AAGTCTCCAGCTGTGACAAGAACACTGGGGACTACTACGAGGACAGCTATGAGGACATC
TCTGCCTACCTGCTGAGCAAGAACAATGCCATTGAGCCCAGGAGCTTCAGCCAGAATAGC
AGGCACCCCAGCACCAGGCAGAAGCAGTTCAATGCCACCACCATCCCAGAGAATACCAC
CCTGCAGTCTGACCAGGAGGAGATTGACTATGATGACACCATCTCTGTGGAGATGAAGAA

GGAGGACTTTGACATCTACGACGAGGACGAGAACCAGAGCCCCAGGAGCTTCCAGAAGA
AGACCAGGCACTACTTCATTGCTGCTGTGGAGAGGCTGTGGGACTATGGCATGAGCAGCA
GCCCCCATGTGCTGAGGAACAGGGCCCAGTCTGGCTCTGTGCCCCAGTTCAAGAAGGTGG
TGTTCCAGGAGTTCACTGATGGCAGCTTCACCCAGCCCCTGTACAGAGGGGAGCTGAATG
AGCACCTGGGCCTGCTGGGCCCCTACATCAGGGCTGAGGTGGAGGACAACATCATGGTG
ACCTTCAGGAACCAGGCCAGCAGGCCCTACAGCTTCTACAGCAGCCTGATCAGCTATGAG
GAGGACCAGAGGCAGGGGGCTGAGCCCAGGAAGAACTTTGTGAAGCCCAATGAAACCAA
GACCTACTTCTGGAAGGTGCAGCACCACATGGCCCCCACCAAGGATGAGTTTGACTGCAA
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CCCCCTGCTGGTGTGCCACACCAACACCCTGAACCCTGCCCATGGCAGGCAGGTGACTGT
GCAGGAGTTTGCCCTGTTCTTCACCATCTTTGATGAAACCAAGAGCTGGTACTTCACTGAG
AACATGGAGAGGAACTGCAGGGCCCCCTGCAACATCCAGATGGAGGACCCCACCTTCAA
GGAGAACTACAGGTTCCATGCCATCAATGGCTACATCATGGACACCCTGCCTGGCCTGGT
GATGGCCCAGGACCAGAGGATCAGGTGGTACCTGCTGAGCATGGGCAGCAATGAGAACA
TCCACAGCATCCACTTCTCTGGCCATGTGTTCACTGTGAGGAAGAAGGAGGAGTACAAGA
TGGCCCTGTACAACCTGTACCCTGGGGTGTTTGAGACTGTGGAGATGCTGCCCAGCAAGG
CTGGCATCTGGAGGGTGGAGTGCCTGATTGGGGAGCACCTGCATGCTGGCATGAGCACCC
TGTTCCTGGTGTACAGCAACAAGTGCCAGACCCCCCTGGGCATGGCCTCTGGCCACATCA
GGGACTTCCAGATCACTGCCTCTGGCCAGTATGGCCAGTGGGCCCCCAAGCTGGCCAGGC
TGCACTACTCTGGCAGCATCAATGCCTGGAGCACCAAGGAGCCCTTCAGCTGGATCAAGG
TGGACCTGCTGGCCCCCATGATCATCCATGGCATCAAGACCCAGGGGGCCAGGCAGAAGT
TCAGCAGCCTGTACATCAGCCAGTTCATCATCATGTACAGCCTGGATGGCAAGAAGTGGC
AGACCTACAGGGGCAACAGCACTGGCACCCTGATGGTGTTCTTTGGCAATGTGGACAGCT
CTGGCATCAAGCACAACATCTTCAACCCCCCCATCATTGCCAGATACATCAGGCTGCACC
CCACCCACTACAGCATCAGGAGCACCCTGAGGATGGAGCTGATGGGCTGTGACCTGAAC
AGCTGCAGCATGCCCCTGGGCATGGAGAGCAAGGCCATCTCTGATGCCCAGATCACTGCC
AGCAGCTACTTCACCAACATGTTTGCCACCTGGAGCCCCAGCAAGGCCAGGCTGCACCTG
CAGGGCAGGAGCAATGCCTGGAGGCCCCAGGTCAACAACCCCAAGGAGTGGCTGCAGGT
GGACTTCCAGAAGACCATGAAGGTGACTGGGGTGACCACCCAGGGGGTGAAGAGCCTGC
TGACCAGCATGTATGTGAAGGAGTTCCTGATCAGCAGCAGCCAGGATGGCCACCAGTGG
ACCCTGTTCTTCCAGAATGGCAAGGTGAAGGTGTTCCAGGGCAACCAGGACAGCTTCACC
CCTGTGGTGAACAGCCTGGACCCCCCCCTGCTGACCAGATACCTGAGGATTCACCCCCAG
AGCTGGGTGCACCAGATTGCCCTGAGGATGGAGGTGCTGGGCTGTGAGGCCCAGGACCT
GTACTGATTAATTAAGAGCATCTTACCGCCATTTATTCCCATATTTGTTCTGTTTTTCTTGA
TTTGGGTATACATTTAAATGTTAATAAAACAAAATGGTGGGGCAATCATTTACATTTTTAG
GGATATGTAATTACTAGTTCAGGTGTATTGCCACAAGACAAACATGTTAAGAAACTTTCC

CGTTATTTACGCTCTGTTCCTGTTAATCAACCTCTGGATTACAAAATTTGTGAAAGATTGA
CTGATATTCTTAACTATGTTGCTCCTTTTACGCTGTGTGGATATGCTGCTTTATAGCCTCTG
TATCTAGCTATTGCTTCCCGTACGGCTTTCGTTTTCTCCTCCTTGTATAAATCCTGGTTGCT
GTCTCTTTTAGAGGAGTTGTGGCCCGTTGTCCGTCAACGTGGCGTGGTGTGCTCTGTGTTT
GCTGACGCAACCCCCACTGGCTGGGGCATTGCCACCACCTGTCAACTCCTTTCTGGGACTT
TCGCTTTCCCCCTCCCGATCGCCACGGCAGAACTCATCGCCGCCTGCCTTGCCCGCTGCTG
GACAGGGGCTAGGTTGCTGGGCACTGATAATTCCGTGGTGTTGTCTGTGCCTTCTAGTTGC
CAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCA
CTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTAT
TCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGG
CATGCTGGGGATGCGGTGGGCTCTATGGCTCTAGAGCATGGCTACGTAGATAAGTAGCAT
GGCGGGTTAATCATTAACTACACCTGCAGGAGGAACCCCTAGTGATGGAGTTGGCCACTC
CCTCTCTGCGCGCTCGCTCGCTCACTCA

(SEQ ID NO: 645)

(5) ceDNA construct 39 which has the essentially identical sequence to ceDNA construct 10 except that it contains a truncated right ITR.

TGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCCTTGTAG
TTAATGATTAACCCACCATGCTACTTATGGCCTGCAGGGGGGGAGGCTGCTGGTGAATAT
TAACCAAGGTCACCCCAGTTATCGGAGGAGCAAACAGGGGCTAAGTCCACCGGGGGAGG
CTGCTGGTGAATATTAACCAAGGTCACCCCAGTTATCGGAGGAGCAAACAGGGGCTAAGT
CCACCGGGGGAGGCTGCTGGTGAATATTAACCAAGGTCACCCCAGTTATCGGAGGAGCA
AACAGGGGCTAAGTCCACGGTACCCACTGGGAGGATGTTGAGTAAGATGGAAAACTACT
GATGACCCTTGCAGAGACAGAGTATTAGGACATGTTTGAACAGGGGCCGGGCGATCAGC
AGGTAGCTCTAGAGGATCCCCGTCTGTCTGCACATTTCGTAGAGCGAGTGTTCCGATACTC
TAATCTCCCTAGGCAAGGTTCATATTTGTGTAGGTTACTTATTCTCCTTTTGTTGACTAAGT
CAATAATCAGAATCAGCAGGTTTGGAGTCAGCTTGGCAGGGATCAGCAGCCTGGGTTGGA
AGGAGGGGGTATAAAAGCCCCTTCACCAGGAGAAGCCGTCACACAGATCCACAAGCTCC
TGAAGAGGTAAGGGTTTAAGGGATGGTTGGTTGGTGGGGTATTAATGTTTAATTACCTGG
AGCACCTGCCTGAAATCACTTTTTTTCAGGTTGGTTTAAACGCCGCCACCATGCAGATTGA
GCTGAGCACCTGCTTCTTCCTGTGCCTGCTGAGGTTCTGCTTCTCTGCCACCAGGAGATAC
TACCTGGGGGCTGTGGAGCTGAGCTGGGACTACATGCAGTCTGACCTGGGGGAGCTGCCT
GTGGATGCCAGGTTCCCCCCCAGAGTGCCCAAGAGCTTCCCCTTCAACACCTCTGTGGTGT
ACAAGAAGACCCTGTTTGTGGAGTTCACTGACCACCTGTTCAACATTGCCAAGCCCAGGC
CCCCCTGGATGGGCCTGCTGGGCCCCACCATCCAGGCTGAGGTGTATGACACTGTGGTGA
TCACCCTGAAGAACATGGCCAGCCACCCTGTGAGCCTGCATGCTGTGGGGGTGAGCTACT

GGAAGGCCTCTGAGGGGGCTGAGTATGATGACCAGACCAGCCAGAGGGAGAAGGAGGAT
GACAAGGTGTTCCCTGGGGGCAGCCACACCTATGTGTGGCAGGTGCTGAAGGAGAATGG
CCCCATGGCCTCTGACCCCCTGTGCCTGACCTACAGCTACCTGAGCCATGTGGACCTGGTG
AAGGACCTGAACTCTGGCCTGATTGGGGCCCTGCTGGTGTGCAGGGAGGGCAGCCTGGCC
AAGGAGAAGACCCAGACCCTGCACAAGTTCATCCTGCTGTTTGCTGTGTTTGATGAGGGC
AAGAGCTGGCACTCTGAAACCAAGAACAGCCTGATGCAGGACAGGGATGCTGCCTCTGC
CAGGGCCTGGCCCAAGATGCACACTGTGAATGGCTATGTGAACAGGAGCCTGCCTGGCCT
GATTGGCTGCCACAGGAAGTCTGTGTACTGGCATGTGATTGGCATGGGCACCACCCCTGA
GGTGCACAGCATCTTCCTGGAGGGCCACACCTTCCTGGTCAGGAACCACAGGCAGGCCAG
CCTGGAGATCAGCCCCATCACCTTCCTGACTGCCCAGACCCTGCTGATGGACCTGGGCCA
GTTCCTGCTGTTCTGCCACATCAGCAGCCACCAGCATGATGGCATGGAGGCCTATGTGAA
GGTGGACAGCTGCCCTGAGGAGCCCCAGCTGAGGATGAAGAACAATGAGGAGGCTGAGG
ACTATGATGATGACCTGACTGACTCTGAGATGGATGTGGTGAGGTTTGATGATGACAACA
GCCCCAGCTTCATCCAGATCAGGTCTGTGGCCAAGAAGCACCCCAAGACCTGGGTGCACT
ACATTGCTGCTGAGGAGGAGGACTGGGACTATGCCCCCCTGGTGCTGGCCCCTGATGACA
GGAGCTACAAGAGCCAGTACCTGAACAATGGCCCCCAGAGGATTGGCAGGAAGTACAAG
AAGGTCAGGTTCATGGCCTACACTGATGAAACCTTCAAGACCAGGGAGGCCATCCAGCAT
GAGTCTGGCATCCTGGGCCCCCTGCTGTATGGGGAGGTGGGGGACACCCTGCTGATCATC
TTCAAGAACCAGGCCAGCAGGCCCTACAACATCTACCCCCATGGCATCACTGATGTGAGG
CCCCTGTACAGCAGGAGGCTGCCCAAGGGGGTGAAGCACCTGAAGGACTTCCCCATCCTG
CCTGGGGAGATCTTCAAGTACAAGTGGACTGTGACTGTGGAGGATGGCCCCACCAAGTCT
GACCCCAGGTGCCTGACCAGATACTACAGCAGCTTTGTGAACATGGAGAGGGACCTGGCC
TCTGGCCTGATTGGCCCCCTGCTGATCTGCTACAAGGAGTCTGTGGACCAGAGGGGCAAC
CAGATCATGTCTGACAAGAGGAATGTGATCCTGTTCTCTGTGTTTGATGAGAACAGGAGC
TGGTACCTGACTGAGAACATCCAGAGGTTCCTGCCCAACCCTGCTGGGGTGCAGCTGGAG
GACCCTGAGTTCCAGGCCAGCAACATCATGCACAGCATCAATGGCTATGTGTTTGACAGC
CTGCAGCTGTCTGTGTGCCTGCATGAGGTGGCCTACTGGTACATCCTGAGCATTGGGGCC
CAGACTGACTTCCTGTCTGTGTTCTTCTCTGGCTACACCTTCAAGCACAAGATGGTGTATG
AGGACACCCTGACCCTGTTCCCCTTCTCTGGGGAGACTGTGTTCATGAGCATGGAGAACC
CTGGCCTGTGGATTCTGGGCTGCCACAACTCTGACTTCAGGAACAGGGGCATGACTGCCC
TGCTGAAAGTCTCCAGCTGTGACAAGAACACTGGGGACTACTATGAGGACAGCTATGAG
GACATCTCTGCCTACCTGCTGAGCAAGAACAATGCCATTGAGCCCAGGAGCTTCAGCCAG
AATAGCAGGCACCCCAGCACCAGGCAGAAGCAGTTCAATGCCACCACCATCCCAGAGAA
TACCACCCTGCAGTCTGACCAGGAGGAGATTGACTATGATGACACCATCTCTGTGGAGAT
GAAGAAGGAGGACTTTGACATCTACGACGAGGACGAGAACCAGAGCCCCAGGAGCTTCC
AGAAGAAGACCAGGCACTACTTCATTGCTGCTGTGGAGAGGCTGTGGGACTATGGCATGA

GCAGCAGCCCCCATGTGCTGAGGAACAGGGCCCAGTCTGGCTCTGTGCCCCAGTTCAAGA
AGGTGGTGTTCCAGGAGTTCACTGATGGCAGCTTCACCCAGCCCCTGTACAGAGGGGAGC
TGAATGAGCACCTGGGCCTGCTGGGCCCCTACATCAGGGCTGAGGTGGAGGACAACATC
ATGGTGACCTTCAGGAACCAGGCCAGCAGGCCCTACAGCTTCTACAGCAGCCTGATCAGC
TATGAGGAGGACCAGAGGCAGGGGGCTGAGCCCAGGAAGAACTTTGTGAAGCCCAATGA
AACCAAGACCTACTTCTGGAAGGTGCAGCACCACATGGCCCCCACCAAGGATGAGTTTGA
CTGCAAGGCCTGGGCCTACTTCTCTGATGTGGACCTGGAGAAGGATGTGCACTCTGGCCT
GATTGGCCCCCTGCTGGTGTGCCACACCAACACCCTGAACCCTGCCCATGGCAGGCAGGT
GACTGTGCAGGAGTTTGCCCTGTTCTTCACCATCTTTGATGAAACCAAGAGCTGGTACTTC
ACTGAGAACATGGAGAGGAACTGCAGGGCCCCCTGCAACATCCAGATGGAGGACCCCAC
CTTCAAGGAGAACTACAGGTTCCATGCCATCAATGGCTACATCATGGACACCCTGCCTGG
CCTGGTGATGGCCCAGGACCAGAGGATCAGGTGGTACCTGCTGAGCATGGGCAGCAATG
AGAACATCCACAGCATCCACTTCTCTGGCCATGTGTTCACTGTGAGGAAGAAGGAGGAGT
ACAAGATGGCCCTGTACAACCTGTACCCTGGGGTGTTTGAGACTGTGGAGATGCTGCCCA
GCAAGGCTGGCATCTGGAGGGTGGAGTGCCTGATTGGGGAGCACCTGCATGCTGGCATG
AGCACCCTGTTCCTGGTGTACAGCAACAAGTGCCAGACCCCCCTGGGCATGGCCTCTGGC
CACATCAGGGACTTCCAGATCACTGCCTCTGGCCAGTATGGCCAGTGGGCCCCCAAGCTG
GCCAGGCTGCACTACTCTGGCAGCATCAATGCCTGGAGCACCAAGGAGCCCTTCAGCTGG
ATCAAGGTGGACCTGCTGGCCCCCATGATCATCCATGGCATCAAGACCCAGGGGGCCAGG
CAGAAGTTCAGCAGCCTGTACATCAGCCAGTTCATCATCATGTACAGCCTGGATGGCAAG
AAGTGGCAGACCTACAGGGGCAACAGCACTGGCACCCTGATGGTGTTCTTTGGCAATGTG
GACAGCTCTGGCATCAAGCACAACATCTTCAACCCCCCCATCATTGCCAGATACATCAGG
CTGCACCCCACCCACTACAGCATCAGGAGCACCCTGAGGATGGAGCTGATGGGCTGTGAC
CTGAACAGCTGCAGCATGCCCCTGGGCATGGAGAGCAAGGCCATCTCTGATGCCCAGATC
ACTGCCAGCAGCTACTTCACCAACATGTTTGCCACCTGGAGCCCCAGCAAGGCCAGGCTG
CACCTGCAGGGCAGGAGCAATGCCTGGAGGCCCCAGGTCAACAACCCCAAGGAGTGGCT
GCAGGTGGACTTCCAGAAGACCATGAAGGTGACTGGGGTGACCACCCAGGGGGTGAAGA
GCCTGCTGACCAGCATGTATGTGAAGGAGTTCCTGATCAGCAGCAGCCAGGATGGCCACC
AGTGGACCCTGTTCTTCCAGAATGGCAAGGTGAAGGTGTTCCAGGGCAACCAGGACAGCT
TCACCCCTGTGGTGAACAGCCTGGACCCCCCCCTGCTGACCAGATACCTGAGGATTCACC
CCCAGAGCTGGGTGCACCAGATTGCCCTGAGGATGGAGGTGCTGGGCTGTGAGGCCCAG
GACCTGTACTGATTAATTAAGAGCATCTTACCGCCATTTATTCCCATATTTGTTCTGTTTTT
CTTGATTTGGGTATACATTTAAATGTTAATAAAACAAAATGGTGGGGCAATCATTTACATT
TTTAGGGATATGTAATTACTAGTTCAGGTGTATTGCCACAAGACAAACATGTTAAGAAAC
TTTCCCGTTATTTACGCTCTGTTCCTGTTAATCAACCTCTGGATTACAAAATTTGTGAAAG
ATTGACTGATATTCTTAACTATGTTGCTCCTTTTACGCTGTGTGGATATGCTGCTTTATAGC

CTCTGTATCTAGCTATTGCTTCCCGTACGGCTTTCGTTTTCTCCTCCTTGTATAAATCCTGG
TTGCTGTCTCTTTTAGAGGAGTTGTGGCCCGTTGTCCGTCAACGTGGCGTGGTGTGCTCTG
TGTTTGCTGACGCAACCCCCACTGGCTGGGGCATTGCCACCACCTGTCAACTCCTTTCTGG
GACTTTCGCTTTCCCCCTCCCGATCGCCACGGCAGAACTCATCGCCGCCTGCCTTGCCCGC
TGCTGGACAGGGGCTAGGTTGCTGGGCACTGATAATTCCGTGGTGTTGTCTGTGCCTTCTA
GTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCAC
TCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCAT
TCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAG
CAGGCATGCTGGGGATGCGGTGGGCTCTATGGCGCTAGCCCACAATCTGCCTCCCAGTAG
TACATGACATTAGTTTATTAATAGCCTAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTC
TCTGCGCGCTCGCTCGCTCA

(SEQ ID NO: 646)

REFERENCES

[00865] All publications and references, including but not limited to patents and patent applications, cited in this specification and Examples herein are incorporated by reference in their entirety as if each individual publication or reference were specifically and individually indicated to be incorporated by reference herein as being fully set forth. Any patent application to which this application claims priority is also incorporated by reference herein in the manner described above for publications and references.

CLAIMS

1. A capsid-free close-ended DNA (ceDNA) vector comprising:

at least one nucleic acid sequence between flanking inverted terminal repeats (ITRs), wherein at least one nucleic acid sequence encodes at least one FVIII protein, wherein the at least one nucleic acid sequence that encodes at least one FVIII protein is selected from a sequence having at least 85% identity to any nucleic acid sequence set forth in **Table 1A** (SEQ ID NOs: 71-183, 556 and 626-633).

2. The ceDNA vector of claim 1, wherein the ceDNA vector comprises a promoter or promoter set operatively linked to the least one nucleic acid sequence that encodes at least one FVIII protein.

3. The ceDNA vector of claim 2, wherein the promoter is selected from the group consisting of: human a1 antitrypsin (hAAT) promoter, minimal transthyretin promoter (TTRm), hAAT_core_C06, hAAT_core_C07, hAAT_core_08, hAAT_core_C09, hAAT_core_C10, and hAAT_core_truncated.

4. The ceDNA vector of claim 2, wherein the promoter is selected from a nucleic acid sequence having at least 85% identity to any one of SEQ ID NOs: 210-217.

5. The ceDNA vector of claim 2, wherein the promoter set comprises a synthetic liver specific promoter set including enhancers and core promoter, without 5pUTR.

6. The ceDNA vector of claim 2, wherein the promoter set is selected from a nucleic acid sequence having at least 85 % identity to any one of SEQ ID NOs: 184-197, 400, 401, and 484.

7. The ceDNA vector of any of claims 1-6, wherein the ceDNA vector comprises an enhancer.

8. The ceDNA vector of claim 7, wherein the enhancer is selected from the group consisting of: a Serpin enhancer (SerpEnh), the transthyretin (TTRe) gene enhancer (TTRe), the Hepatic Nuclear Factor 1 binding site (HNF1), Hepatic Nuclear Factor 4 binding site (HNF4), Human apolipoprotein E/C-I liver specific enhancer (ApoE_Enh), the enhancer region from Pro-albumin gene (ProEnh), a CpG minimized version of the ApoE_Enh (Human apolipoprotein E/C-I liver specific enhancer) (ApoE_Enh_C03, ApoE_Enh_C04, ApoE_Enh_C09, and ApoE_Enh_C10), and Hepatic nuclear factor enhancer array embedded in GE-856 (Embedded_enhancer_HNF_array).

9. The ceDNA vector of claim 8, wherein the Serpin enhancer comprises a nucleic acid sequence at least 85% identical to SEQ ID NO: 198.

10. The ceDNA vector of claim 7, wherein the enhancer is selected from a nucleic acid sequence having at least 85 % identity to any one of SEQ ID NOs: 198-209, 485 and 557-616.

11. The ceDNA vector of claim 1, wherein the ceDNA vector comprises a 5' UTR sequence.

12. The ceDNA vector of claim 11, wherein the 5' UTR sequence is selected from a

sequence having at least 85% identity to any sequence in **Table 10**.

13. The ceDNA vector of claim 1, wherein the ceDNA vector comprises an intron sequence.

14. The ceDNA vector of claim 13, wherein the intron sequence is selected from a sequence having at least 85% identity to any sequence in **Table 11**.

15. The ceDNA vector of claim 1, wherein the ceDNA vector comprises an exon sequence.

16. The ceDNA vector of claim 15, wherein the exon sequence is selected from a sequence having at least 85% identity to any sequence in **Table 12**.

17. The ceDNA vector of any of claims 1, wherein the ceDNA vector comprises a 3' UTR sequence.

18. The ceDNA vector of claim 17, wherein the exon sequence is selected from a sequence having at least 85% identity to any sequence in **Table 13**.

19. The ceDNA vector of claim 1, wherein the ceDNA vector comprises at least one poly A sequence.

20. The ceDNA vector of claim 1, wherein the ceDNA vector comprises one or more DNA nuclear targeting sequences (DTS).

21. The ceDNA vector of claim 20, wherein the DTS is selected from a sequence having at least 85% identity to any sequence in **Table 14**.

22. The ceDNA vector of claim 1, wherein the ceDNA vector comprises one or more of the following: Ubiquitous Chromatin-opening Elements (UCOEs), Kozak sequences, spacer sequences or leader sequences.

23. The ceDNA vector of any one of claims 1-22, wherein at least one nucleic acid sequence is cDNA.

24. The ceDNA vector of any one of claims 1-22, wherein at least one ITR comprises a functional terminal resolution site and a Rep binding site.

25. The ceDNA vector of any one of claims 1-24, wherein one or both of the ITRs are from a virus selected from a parvovirus, a dependovirus, and an adeno-associated virus (AAV).

26. The ceDNA vector of any one of claims 1-22, wherein the flanking ITRs are symmetric or asymmetric.

27. The ceDNA vector of claim 26, wherein the flanking ITRs are symmetrical or substantially symmetrical.

28. The ceDNA vector of claim 26, wherein the flanking ITRs are asymmetric.

29. The ceDNA vector of any one of claims 1-28, wherein one or both of the ITRs are wild-type, or wherein both of the ITRs are wild-type.

30. The ceDNA vector of any one of claims 1-29, wherein the flanking ITRs are from different viral serotypes.

31. The ceDNA vector of any one of claims 1-29, wherein the flanking ITRs are from the same

viral serotypes.

32. The ceDNA vector of any one of claims 1-31, wherein one or both of the ITRs comprises a sequence selected from the sequences in **Table 2, Table 4A, Table 4B,** and **Table 5**.

33. The ceDNA vector of any one of claims 1-32, wherein at least one of the ITRs is altered from a wild-type AAV ITR sequence by a deletion, addition, or substitution that affects the overall three-dimensional conformation of the ITR.

34. The ceDNA vector of any one of claims 1-33, wherein one or both of the ITRs are derived from an AAV serotype selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, and AAV12.

35. The ceDNA vector of any one of claims 1-34, wherein one or both of the ITRs are synthetic.

36. The ceDNA vector of any one of claims 1-35, wherein one or both of the ITRs is not a wild-type ITR, or wherein both of the ITRs are not wild-type.

37. The ceDNA vector of any one of claims 1-36, wherein one or both of the ITRs is modified by a deletion, insertion, and/or substitution in at least one of the ITR regions selected from A, A', B, B', C, C', D, and D'.

38. The ceDNA vector of claim 37, wherein the deletion, insertion, and/or substitution results in the deletion of all or part of a stem-loop structure normally formed by the A, A', B, B' C, or C' regions.

39. The ceDNA vector of any one of claims 1-37, wherein one or both of the ITRs are modified by a deletion, insertion, and/or substitution that results in the deletion of all or part of a stem-loop structure normally formed by the B and B' regions.

40. The ceDNA vector of any one of claims 1-37, wherein one or both of the ITRs are modified by a deletion, insertion, and/or substitution that results in the deletion of all or part of a stem-loop structure normally formed by the C and C' regions.

41. The ceDNA vector of any one of claims 1-37, wherein one or both of the ITRs are modified by a deletion, insertion, and/or substitution that results in the deletion of part of a stem-loop structure normally formed by the B and B' regions and/or part of a stem-loop structure normally formed by the C and C' regions.

42. The ceDNA vector of any one of claims 1-41, wherein one or both of the ITRs comprise a single stem-loop structure in the region that normally comprises a first stem-loop structure formed by the B and B' regions and a second stem-loop structure formed by the C and C' regions.

43. The ceDNA vector of any one of claims 1-42, wherein one or both of the ITRs comprise a single stem and two loops in the region that normally comprises a first stem-loop structure formed by the B and B' regions and a second stem-loop structure formed by the C and C' regions.

44. The ceDNA vector of any one of claims 1-43, wherein one or both of the ITRs comprise a single stem and a single loop in the region that normally comprises a first stem-loop structure formed

by the B and B' regions and a second stem-loop structure formed by the C and C' regions.

45. The ceDNA vector of any one of claims 1-44, wherein both ITRs are altered in a manner that results in an overall three-dimensional symmetry when the ITRs are inverted relative to each other.

46. The ceDNA vector of any one of claims 1-45, wherein one or both of the ITRs comprises a sequence selected from the sequences in **Tables 2, Table 4A, Table 4B**, and **Table 5**.

47. The ceDNA vector of claim 1, wherein the ceDNA vector comprises a nucleic acid sequence selected from a sequence having at least 85% identity with a sequence in **Table 18**.

48. A method of expressing an FVIII protein in a cell comprising contacting the cell with the ceDNA vector of any one of claims 1-47 or 72-79.

49. The method of claim 48, wherein the cell is a photoreceptor or a RPE cell.

50. The method of claim 48 or 49, wherein the cell in *in vitro* or *in vivo*.

51. The method of any one of claims 48-50, wherein the at least one nucleic acid sequence is codon optimized for expression in the eukaryotic cell.

52. The method of any one of claims 48-51, wherein the at least one nucleic acid sequence is a sequence having at least 85% identity to any one of the sequences set forth in **Table 1A** (SEQ ID NOs: 71-183, 556 and 626-633).

53. A method of treating a subject with hemophilia A, comprising administering to the subject a ceDNA vector of any one of claims 1-47 or 72-79, wherein at least one nucleic acid sequence encodes at least one FVIII protein.

54. A method of treating a subject with hemophilia A, comprising administering to the subject a nucleic acid sequence selected from a sequence having at least 85% identity with a sequence in **Table 18**.

55. The method of claim 53 or claim 54, wherein a level of FVIII in the plasma of the subject is increased in the subject after administration.

56. The method of claim 55, wherein the level of FVIII in the plasma of the subject is increased by at least about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, 5-fold, 10-fold, 15-fold, 20-fold, 25-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, 200-fold, 300-fold, 400-fold, 500-fold, 600-fold, 700-fold, 800-fold, 900-fold or 1,000-fold after administration.

57. The method of claim 53 or 54, wherein a level of FVIII in the serum of the subject is increased the subject administered the ceDNA vector as compared to a control.

58. The method of claim 57, wherein the increase in the level of FVIII in the serum of the subject is greater than about 40%, 50%, 60%, 70%, 80%, 90%, 100%, 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, 5-fold, 10-fold, 15-fold, 20-fold, 25-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, 200-fold, 300-fold, 400-fold, 500-fold, 600-fold, 700-fold, 800-fold, 900-

fold or 1,000-fold compared to the control.

59. The method of any one of claims 57-58, wherein the control is a level of FVIII in the serum of the subject prior to administration, wherein the control is a level of FVIII in the serum of a subject having hemophilia A who did not receive the administration or wherein the control is a level of FVIII in a subject not having hemophilia A.

60. The method of any one of claims 53-59, wherein the administration restores a plasma level of FVIII in the subject to at least about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% of a FVIII plasma level of a healthy individual not affected by hemophilia A.

61. The method of any one of claims 53-60, wherein the ceDNA vector is administered at a dose of about 0.1 mg/kg , 0.2 mg/kg, 0.3 mg/kg, 0.4 mg/kg, 0.5 mg/kg, 0.75 mg/kg, 1 mg/kg, 1.5 mg/kg, 2 mg/kg, 2.5 mg/kg, 3 mg/kg, 3.5 mg/kg, 4 mg/kg, 5 mg/kg, 6 mg/kg, 7 mg/kg, 8 mg/kg, 9 mg/kg, or 10 mg/kg.

62. The method of claim 54, wherein the at least one nucleic acid sequence is a sequence having at least 85% identity to any sequence set forth in **Table 1A** (SEQ ID NOs: 71-183, 556 and 626-633).

63. The method of any of claims 54-62, wherein the ceDNA vector is administered to a photoreceptor cell, or an RPE cell, or both.

64. The method of any of claims 54-63, wherein the ceDNA vector expresses the FVIII protein in a photoreceptor cell, or an RPE cell, or both.

65. The method of any of claims 54-64, wherein the ceDNA vector is administered by any one or more of: subretinal injection, suprachoroidal injection or intravitreal injection.

66. A pharmaceutical composition comprising the ceDNA vector of any one of claims 1-47.

67. A cell containing a ceDNA vector of any of claims 1-47 or 72-79.

68. The cell of claim 67, wherein the cell a photoreceptor cell, or an RPE cell, or both.

69. A composition comprising a ceDNA vector of any of claims 1-47 and a lipid.

70. The composition of claim 69, wherein the lipid is a lipid nanoparticle (LNP).

71. A kit comprising the ceDNA vector of any one of claims 1-47 or 72-79, the pharmaceutical composition of claim 66, the cell of claim 67 or claim 68, or the composition of claim 69 or claim 70.

72. A capsid-free close-ended DNA (ceDNA) vector comprising:

at least one nucleic acid sequence between flanking inverted terminal repeats (ITRs), wherein at least one nucleic acid sequence encodes at least one protein,

wherein the ceDNA vector comprises a promoter or promoter set operatively linked to the least one nucleic acid sequence that encodes the at least one protein, and wherein the promoter is selected from the group consisting of: human a1 antitrypsin (hAAT) promoter,

minimal transthyretin promoter (TTRm), hAAT_core_C06, hAAT_core_C07, hAAT_core_08, hAAT_core_C09, hAAT_core_C10, and hAAT_core_truncated.

73. The ceDNA vector of claim 72, wherein the promoter is selected from a nucleic acid sequence having at least 85% identity to any one of SEQ ID NOs: 210-217.

74. The ceDNA vector of claim 72, wherein the promoter set comprises a synthetic liver specific promoter set including enhancers and core promoter, without 5pUTR.

75. The ceDNA vector of claim 72, wherein the promoter set is selected from a nucleic acid sequence having at least 85 % identity to any one of SEQ ID NOs: 184-197, 400, 401, and 484.

76. The ceDNA vector of any of claims 72-75, wherein the ceDNA vector comprises an enhancer.

77. The ceDNA vector of claim 76, wherein the enhancer is selected from the group consisting of: a Serpin enhancer (SerpEnh), the transthyretin (TTRe) gene enhancer (TTRe), the Hepatic Nuclear Factor 1 binding site (HNF1), Hepatic Nuclear Factor 4 binding site (HNF4), Human apolipoprotein E/C-I liver specific enhancer (ApoE_Enh), the enhancer region from Pro-albumin gene (ProEnh), a CpG minimized version of the ApoE_Enh (Human apolipoprotein E/C-I liver specific enhancer) (ApoE_Enh_C03, ApoE_Enh_C04, ApoE_Enh_C09, and ApoE_Enh_C10), and Hepatic nuclear factor enhancer array embedded in GE-856 (Embedded_enhancer_HNF_array).

78. The ceDNA vector of claim 77, wherein the Serpin enhancer comprises a nucleic acid sequence at least 85% identical to SEQ ID NO: 198.

79. The ceDNA vector of claim 76, wherein the enhancer is selected from a nucleic acid sequence having at least 85% identity to any one of SEQ ID NOs: 198-209, 485 and 557-616.

80. A method of expressing a protein in a cell comprising contacting the cell with the ceDNA vector of any one of claims 72-79.

81. The method of claim 80, wherein the cell is a photoreceptor or a RPE cell.

82. The method of claim 80 or 81, wherein the cell in *in vitro* or *in vivo*.

83. The method of any one of claims 80-82, wherein the at least one nucleic acid sequence is codon optimized for expression in the eukaryotic cell.

84. The ceDNA vector of any one of claims 1-46, wherein the at least one nucleic acid sequence that encodes at least one FVIII protein is selected from a nucleic acid sequence having at least 85% identity to any one of SEQ ID NOs: 556 and 626-633, and wherein the ceDNA vector comprises an enhancer, wherein the enhancer is selected from a nucleic acid sequence having at least 85 % identity to any one of SEQ ID NOs: 557-616.

85. A DNA vector comprising a nucleic acid sequence at least 85% identical to SEQ ID NOs: 71-183, 556 and 626-633.

86. The DNA vector of claim 85, wherein the DNA vector comprises an enhancer sequence

having at least 95% identity to any one of SEQ ID NOs: 198-209, 485, 557-616.

87. The DNA vector of claim 86, wherein the DNA vector comprises a SerpEnh sequence having at least 95% identity to any one of SEQ ID NOs: 198 and 557-616.

88. The DNA vector of claim 87, wherein the DNA vector comprises a SerpEnh sequence having at least 95% identity to any one of SEQ ID NOs: 557-616.

89. The DNA vector of claim 88, wherein the DNA vector comprises a SerpEnh sequence having at least 95% identity to any one of SEQ ID NOs: 557-568.

90. The DNA vector of claim 88, wherein the DNA vector comprises a SerpEnh sequence having at least 95% identity to any one of SEQ ID NOs: 569 and 570.

91. The DNA vector of claim 88, wherein the DNA vector comprises a SerpEnh sequence having at least 95% identity to any one of SEQ ID NO: 571.

92. The DNA vector of claim 88, wherein the DNA vector comprises a SerpEnh sequence having at least 95% identity to any one of SEQ ID NO: 572.

93. The DNA vector of claim 88, wherein the DNA vector comprises a SerpEnh sequence having at least 95% identity to any one of SEQ ID NO: 611.

94. The DNA vector of claim 88, wherein the DNA vector comprises a SerpEnh sequence having at least 95% identity to any one of SEQ ID NO: 603.

95. The DNA vector of any one of claims 85-94, wherein the DNA vector comprises a TTRe sequence.

96. The DNA vector of claim 95, wherein the TTRe sequence is set forth in SEQ ID NO: 199 or a sequence having at least 95% identity thereof.

97. The DNA vector of claim 95, wherein the DNA vector comprises a TTR promoter.

98. The DNA vector of claim 95, wherein the TTR promoter is set forth in SEQ ID NO: 211 or a sequence having 95% identity thereof.

99. The DNA vector of claim 97, wherein the DNA vector comprises a 5' untranslated region (5' UTR) sequence selected from the group consisting of SEQ ID NO: 411, SEQ ID NO: 412, SEQ ID NO: 413, SEQ ID NO: 414, SEQ ID NO: 415 , SEQ ID NO: 416 , SEQ ID NO: 417, SEQ ID NO: 418, SEQ ID NO: 419, SEQ ID NO: 420, SEQ ID NO: 421, SEQ ID NO: 422, SEQ ID NO: 423, SEQ ID NO: 424, SEQ ID NO: 425, SEQ ID NO: 426, SEQ ID NO: 427, SEQ ID NO: 428, SEQ ID NO: 429, SEQ ID NO: 430, SEQ ID NO: 431, SEQ ID NO: 432, SEQ ID NO: 433, SEQ ID NO: 434, SEQ ID NO: 435, and SEQ ID NO: 436.

100. The DNA vector of claim 97, wherein the DNA vector comprises an intron sequence selected from the group consisting of SEQ ID NO: 235, SEQ ID NO: 236, SEQ ID NO: 237, SEQ ID NO: 238, SEQ ID NO: 239 , SEQ ID NO: 240 , SEQ ID NO: 241, SEQ ID NO: 242, SEQ ID NO: 243, SEQ ID NO: 245, SEQ ID NO: 246, SEQ ID NO: 247, and SEQ ID NO: 248.

101. The DNA vector of claim 97, wherein the DNA vector further comprises an intron

sequence having at least 95% identity to SEQ ID NO: 235.

102. The DNA vector of claim 97, wherein the DNA vector comprises a 3'UTR sequence.

103. The DNA vector of claim 102, wherein the 3'UTR sequence comprises a WPRE element and/or bGH poly A signal sequence or a sequence having at least 95% identity to any one of SEQ ID NOs: 283-291 and 634.

104. The DNA vector of claim 102, wherein the DNA vector comprises a mircroRNA (mir) sequence set forth in SEQ ID NO: 543 or a sequence having at least 95% identity thereof.

105. The DNA vector of claim 97, wherein the DNA vector comprises a spacer sequence selected from a sequence having at least 85% identity to any sequence set forth in **Table 15** (SEQ ID NOs:318-332 and 635-641).

106. The DNA vector of claim 85, wherein the DNA vector comprises at least one ITR flanking 5' and/or 3' end of the nucleic acid sequence at least 95% identical to SEQ ID NO:556.

107. The DNA vector of claim 106, wherein the at least one ITR flanking 5' and/or 3'is a wild-type AAV ITR(s).

108. The DNA vector of claim 85, wherein the DNA vector is a closed-ended DNA (ceDNA).

109. The DNA vector of claim 85, wherein the DNA vector is a plasmid.

110. The DNA vector of claim 85, wherein the DNA vector comprises a nucleic acid sequence encoding a single chain (SC) FVIII.

111. The DNA vector of claim 110, wherein the nucleic acid sequence is set forth in SEQ ID NO: 556 or a sequence having at least 99% identity thereto.

112. A ceDNA vector comprising a nucleic acid sequence of SEQ ID NO: 42 or a nucleic acid sequence at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 42.

113. A ceDNA vector comprising a nucleic acid sequence of SEQ ID NO: 642 or a nucleic acid sequence at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 642.

114. A ceDNA vector comprising a nucleic acid sequence of SEQ ID NO: 643 or a nucleic acid sequence at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 643.

115. A ceDNA vector comprising a nucleic acid sequence of SEQ ID NO: 644 or a nucleic acid sequence at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 644.

116. A ceDNA vector comprising a nucleic acid sequence of SEQ ID NO: 645 or a nucleic acid sequence at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 645.

117. A ceDNA vector comprising a nucleic acid sequence of SEQ ID NO: 646 or a nucleic

acid sequence at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 646.

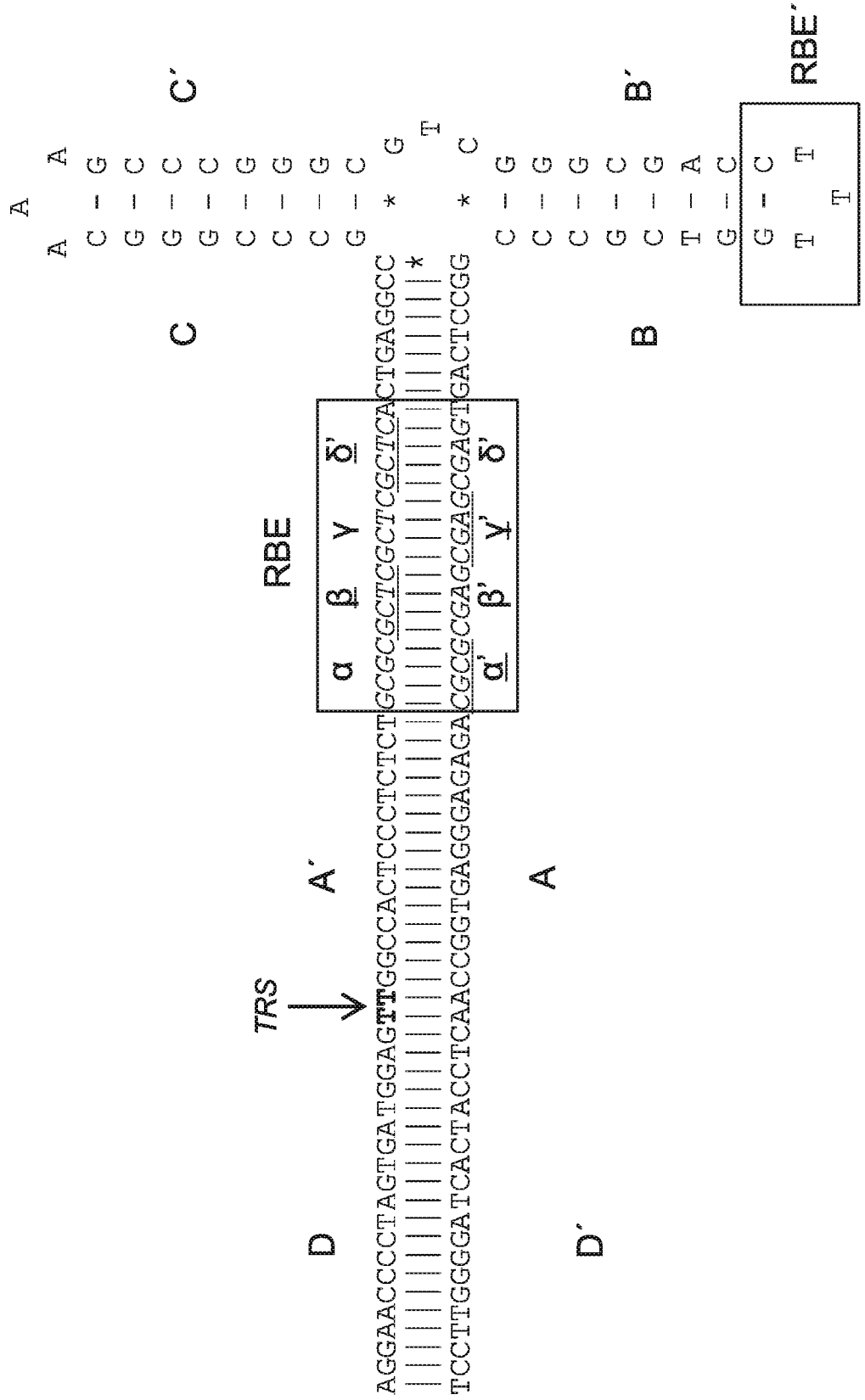

A
A
C – G
G – C
G – C
G – C
C – G
C – G
G – C
C
C'
A
A
G
T
*
*
C
C – G
C – G
C – G
G – C
C – G
T – A
G – C
B
B'
RBE'
G – C
T
T
T
RBE
α β γ δ'
α' β' γ' δ'
TRS
D
A'
A
D'
AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCC
TCCTTGGGGATCACTACCTCAACCGGTGAGGGAGACGCGCGAGCGAGCGAGTGACTCCGG

FIG. 1A

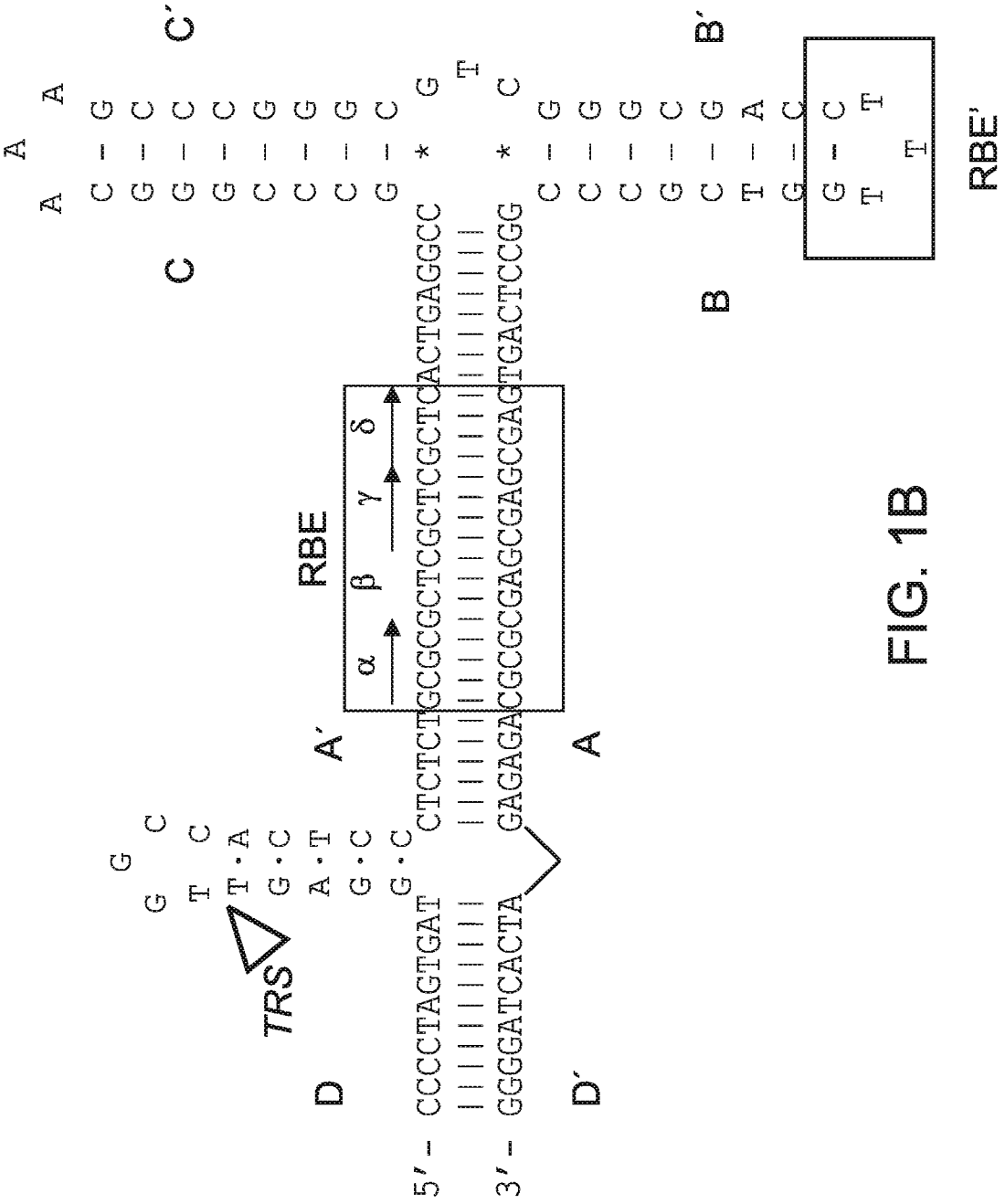


FIG. 1B

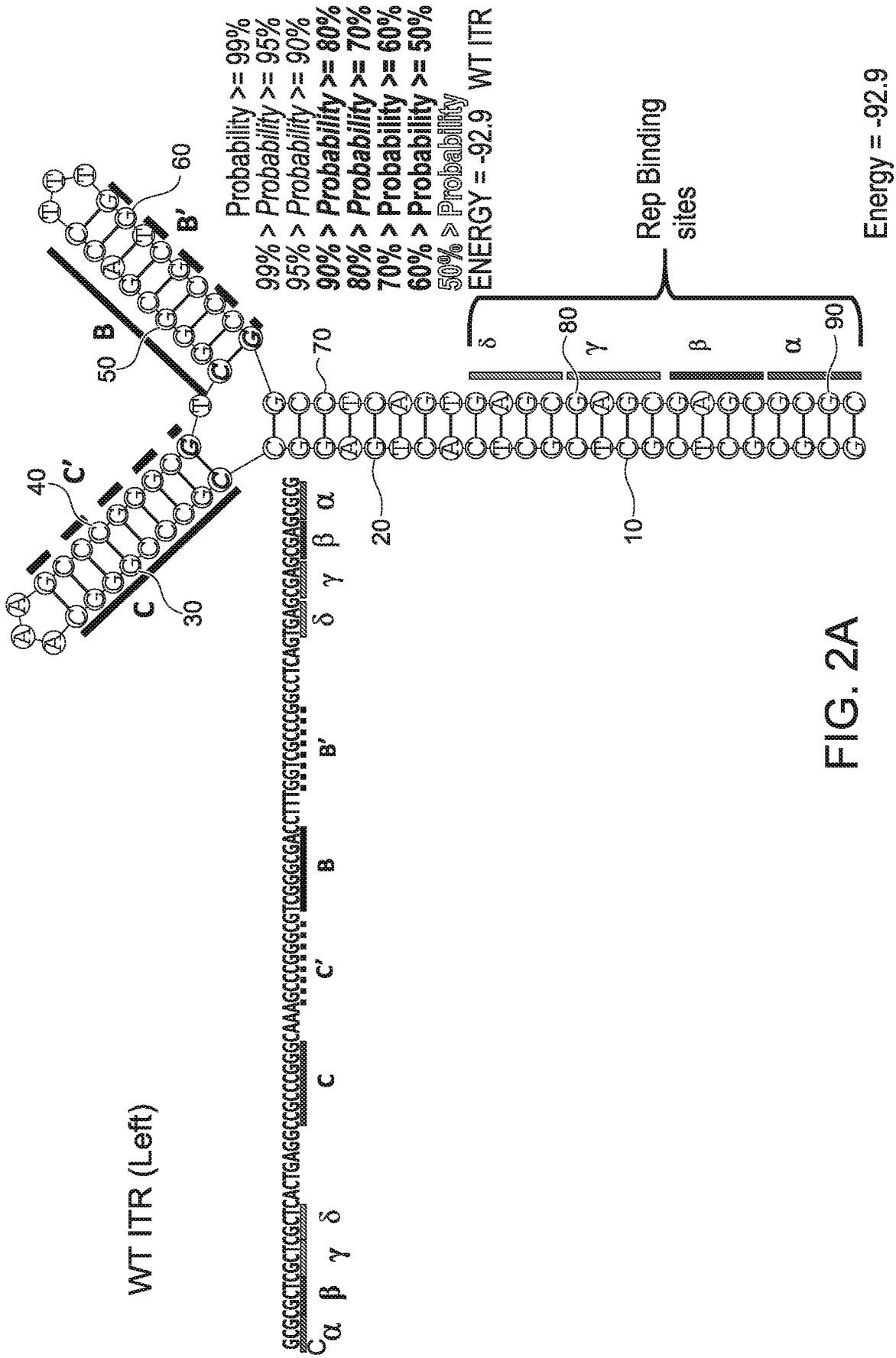

WT ITR (Left)
GCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCG
Cα β γ δ C C' B B' δ γ β α
Probability >= 99%
99% > Probability >= 95%
95% > Probability >= 90%
90% > Probability >= 80%
80% > Probability >= 70%
70% > Probability >= 60%
60% > Probability >= 50%
50% > Probability
ENERGY = -92.9 WT ITR
Rep Binding sites
Energy = -92.9
10
20
30
40
50
60
70
80
90

FIG. 2A

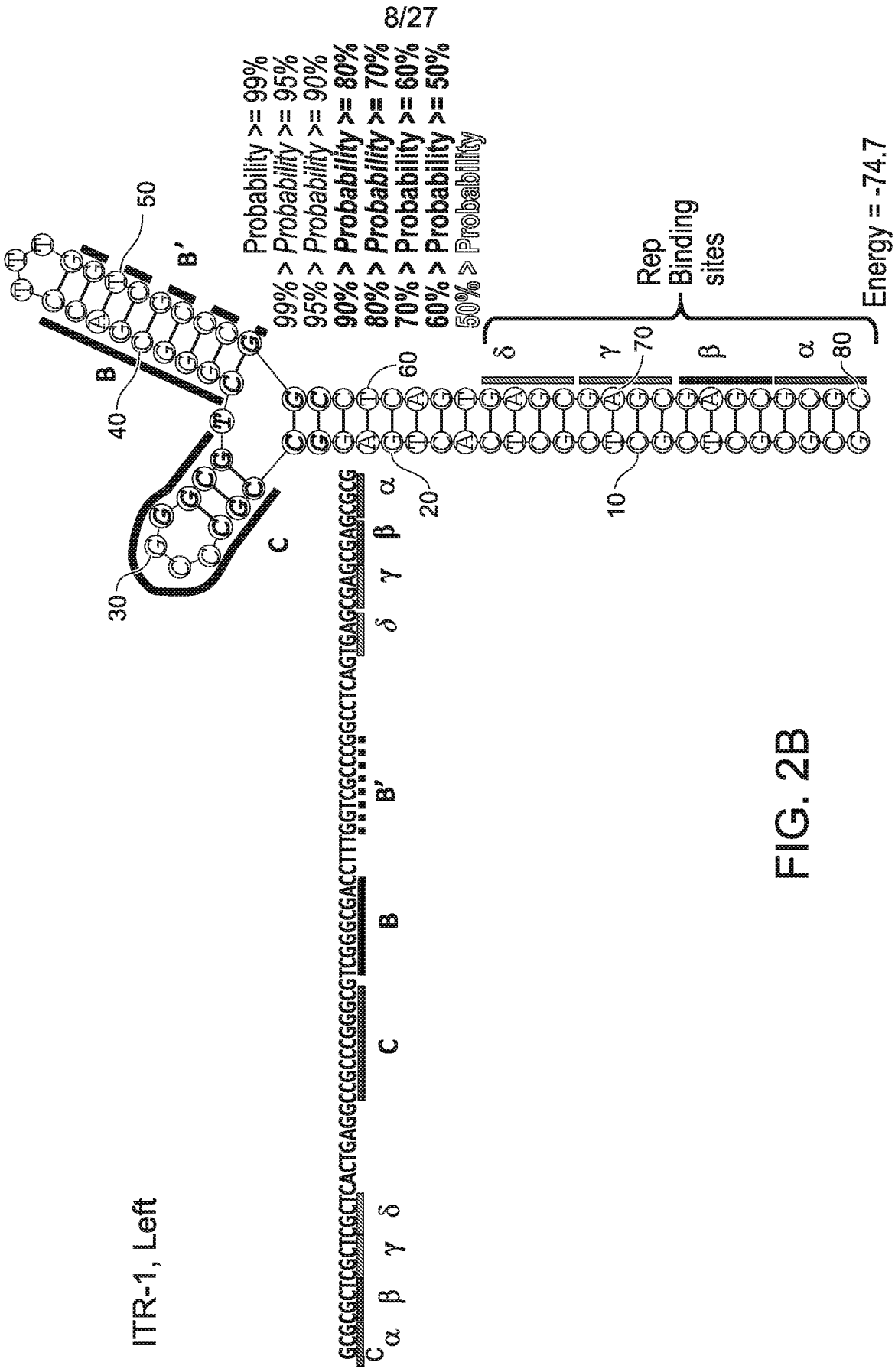

8/27
Probability >= 99%
99% > Probability >= 95%
95% > Probability >= 90%
90% > Probability >= 80%
80% > Probability >= 70%
70% > Probability >= 60%
60% > Probability >= 50%
50% > Probability
Rep Binding sites
Energy = -74.7
ITR-1, Left
GCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCG
B
B'
C
α β γ δ
δ γ β α
10
20
30
40
50
60
70
80

FIG. 2B

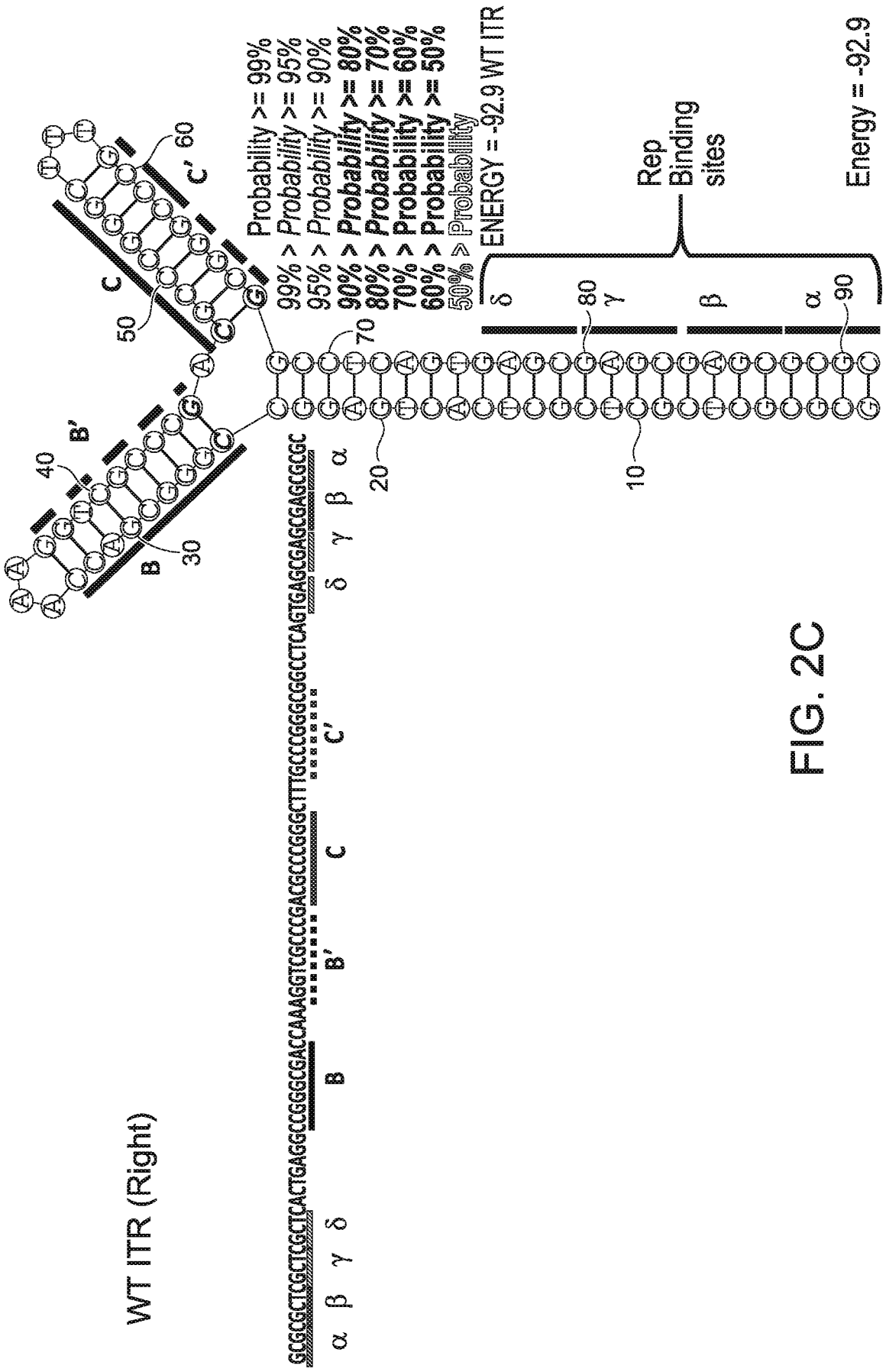

WT ITR (Right)
Probability >= 99%
99% > Probability >= 95%
95% > Probability >= 90%
90% > Probability >= 80%
80% > Probability >= 70%
70% > Probability >= 60%
60% > Probability >= 50%
50% > Probability
ENERGY = -92.9 WT ITR
Rep Binding sites
Energy = -92.9
α β γ δ
B
B'
C
C'
δ γ β α
10
20
30
40
50
60
70
80
90

FIG. 2C

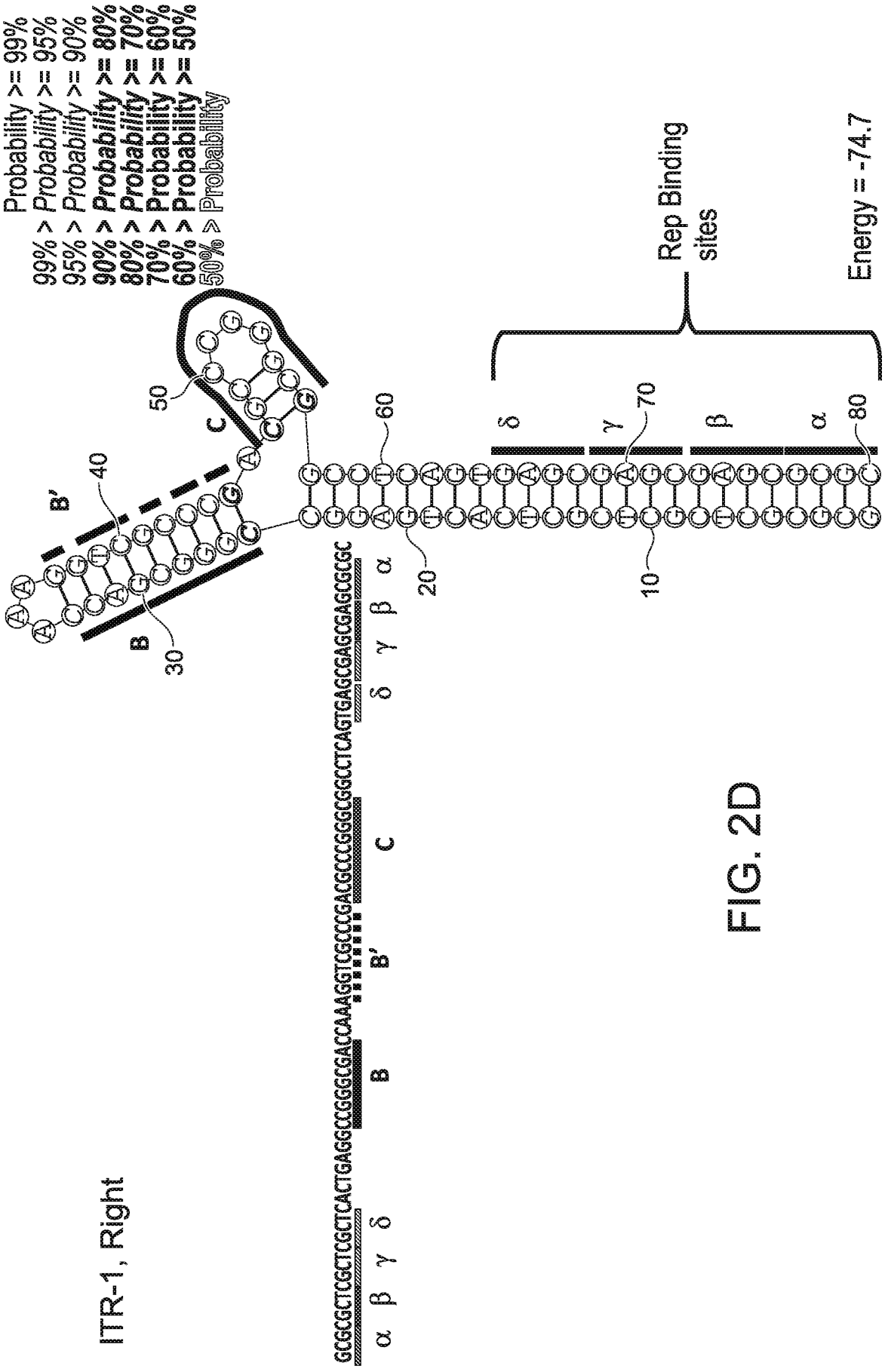

ITR-1, Right
Probability >= 99%
99% > Probability >= 95%
95% > Probability >= 90%
90% > Probability >= 80%
80% > Probability >= 70%
70% > Probability >= 60%
60% > Probability >= 50%
50% > Probability
Rep Binding sites
Energy = -74.7
α β γ δ
B
B'
C
δ γ β α
10
20
30
40
50
60
70
80

FIG. 2D

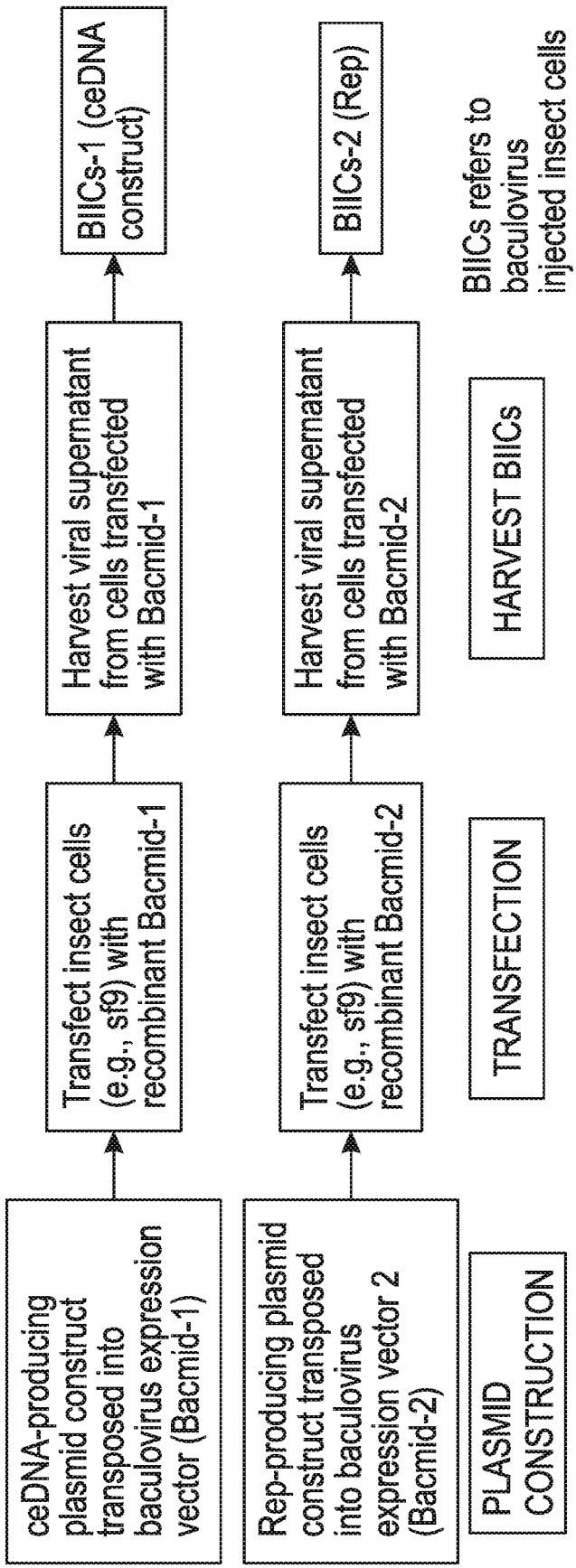

ceDNA Production
Upstream Process
ceDNA-producing plasmid construct transposed into baculovirus expression vector (Bacmid-1)
Rep-producing plasmid construct transposed into baculovirus expression vector 2 (Bacmid-2)
PLASMID CONSTRUCTION
Transfect insect cells (e.g., sf9) with recombinant Bacmid-1
Transfect insect cells (e.g., sf9) with recombinant Bacmid-2
TRANSFECTION
Harvest viral supernatant from cells transfected with Bacmid-1
Harvest viral supernatant from cells transfected with Bacmid-2
HARVEST BIICs
BIICs-1 (ceDNA construct)
BIICs-2 (Rep)
BIICs refers to baculovirus injected insect cells

FIG. 3A

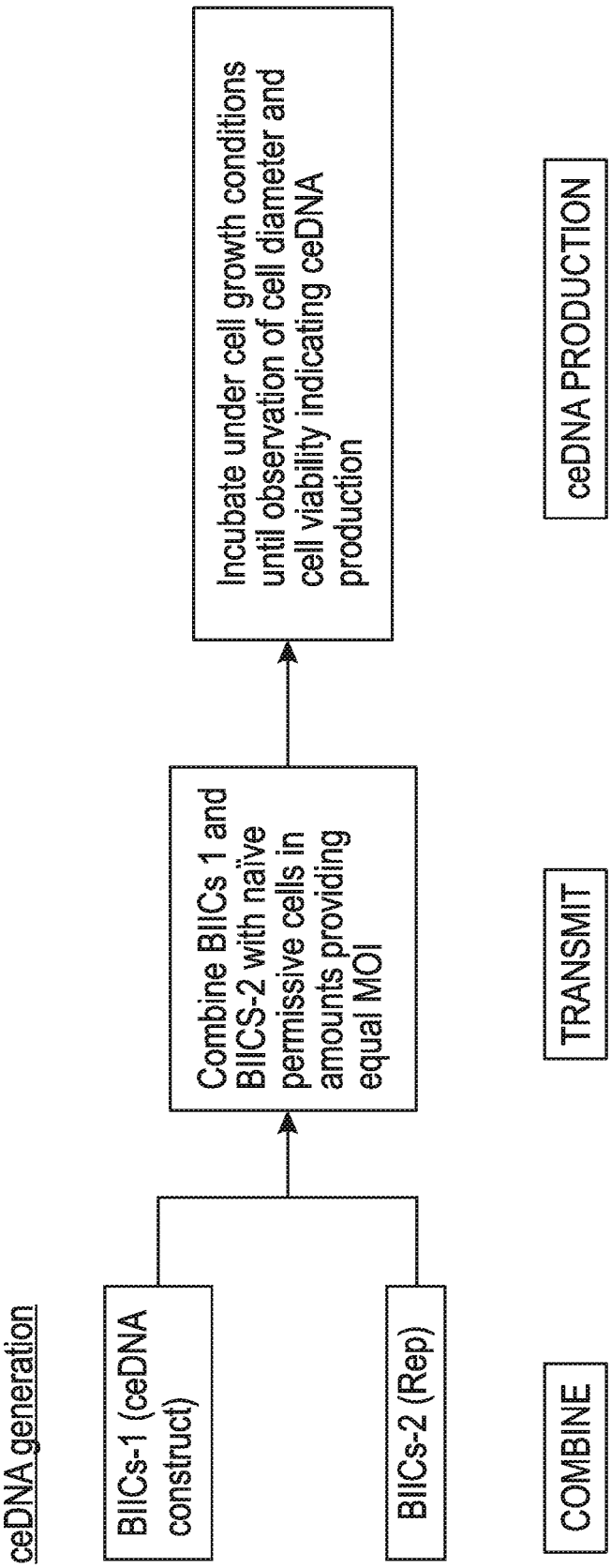

ceDNA Production
ceDNA generation
BIICs-1 (ceDNA construct)
BIICs-2 (Rep)
COMBINE
Combine BIICs 1 and BIICS-2 with naïve permissive cells in amounts providing equal MOI
TRANSMIT
Incubate under cell growth conditions until observation of cell diameter and cell viability indicating ceDNA production
ceDNA PRODUCTION

FIG. 3B

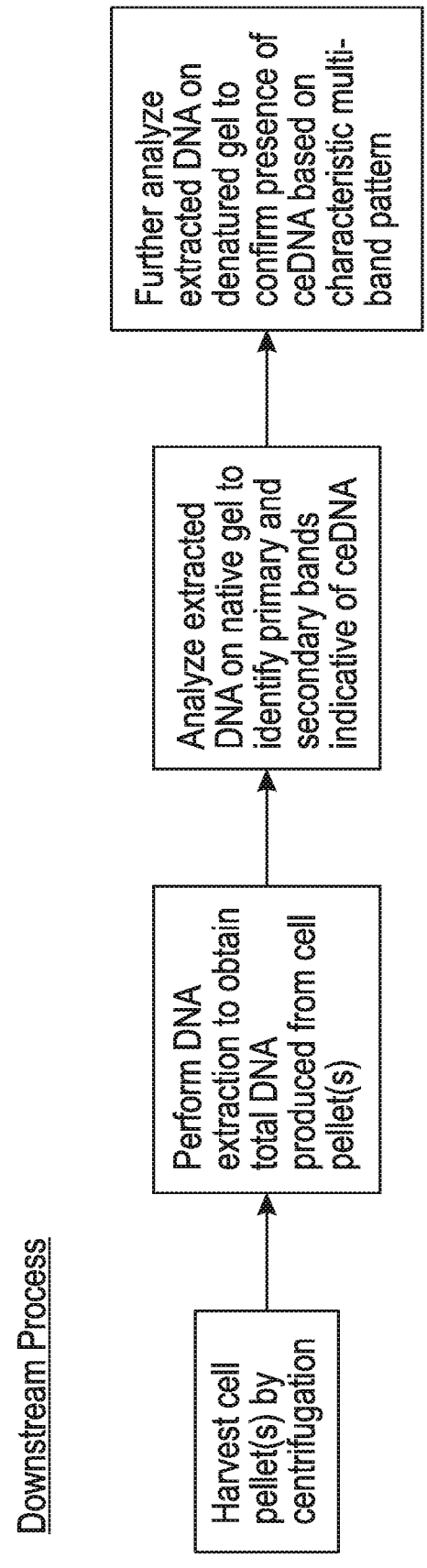

Downstream Process
ceDNA Production
Harvest cell pellet(s) by centrifugation
Perform DNA extraction to obtain total DNA produced from cell pellet(s)
Analyze extracted DNA on native gel to identify primary and secondary bands indicative of ceDNA
Further analyze extracted DNA on denatured gel to confirm presence of ceDNA based on characteristic multi-band pattern
HARVEST
EXTRACT
Confirm ceDNA Production

FIG. 3C

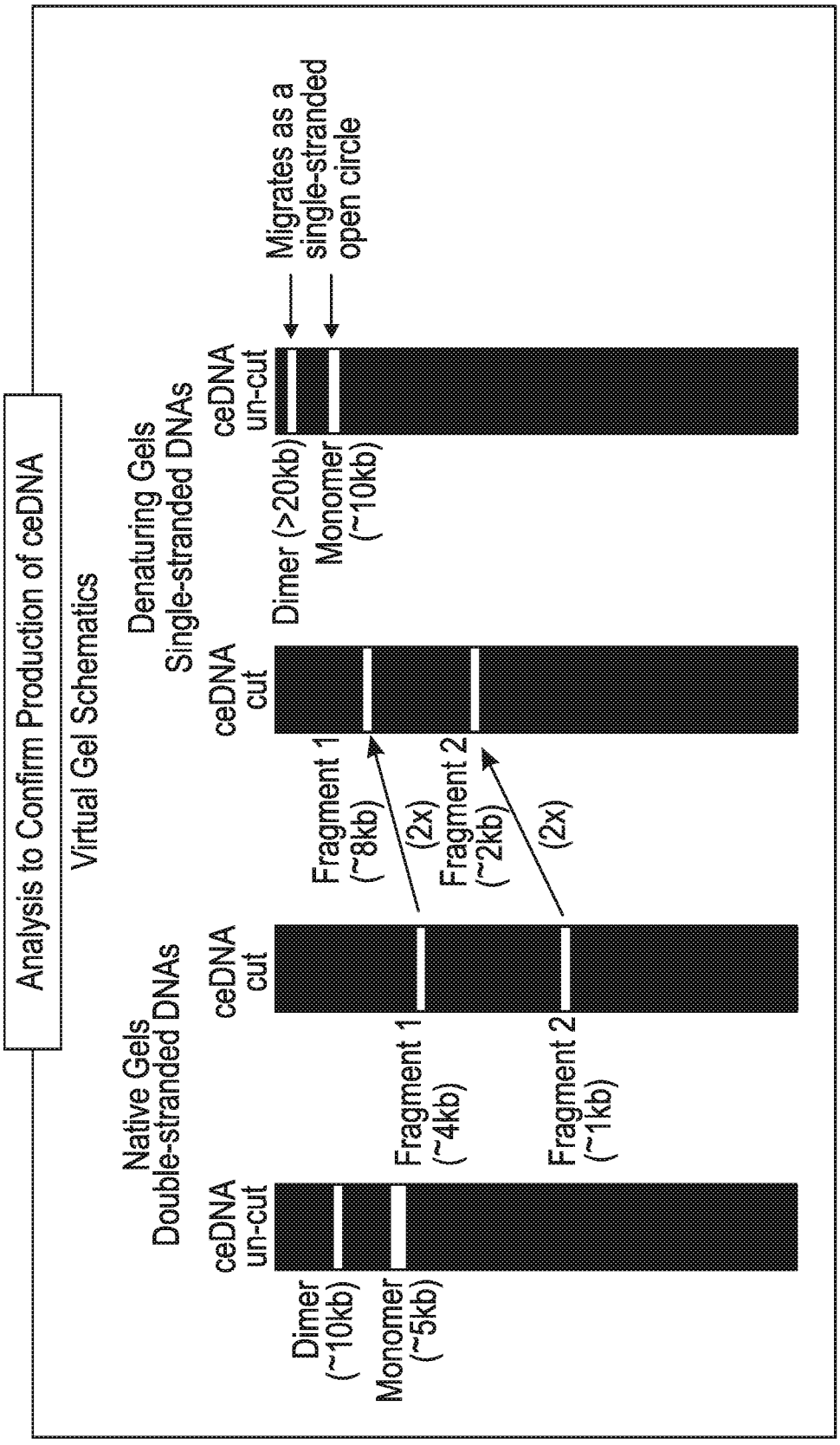

Analysis to Confirm Production of ceDNA
Virtual Gel Schematics
Native Gels
Double-stranded DNAs
ceDNA un-cut
Dimer (~10kb)
Monomer (~5kb)
ceDNA cut
Fragment 1 (~4kb)
Fragment 2 (~1kb)
(2x)
(2x)
Denaturing Gels
Single-stranded DNAs
ceDNA cut
Fragment 1 (~8kb)
Fragment 2 (~2kb)
ceDNA un-cut
Dimer (>20kb)
Monomer (~10kb)
Migrates as a single-stranded open circle

FIG. 3D

FIG. 3E

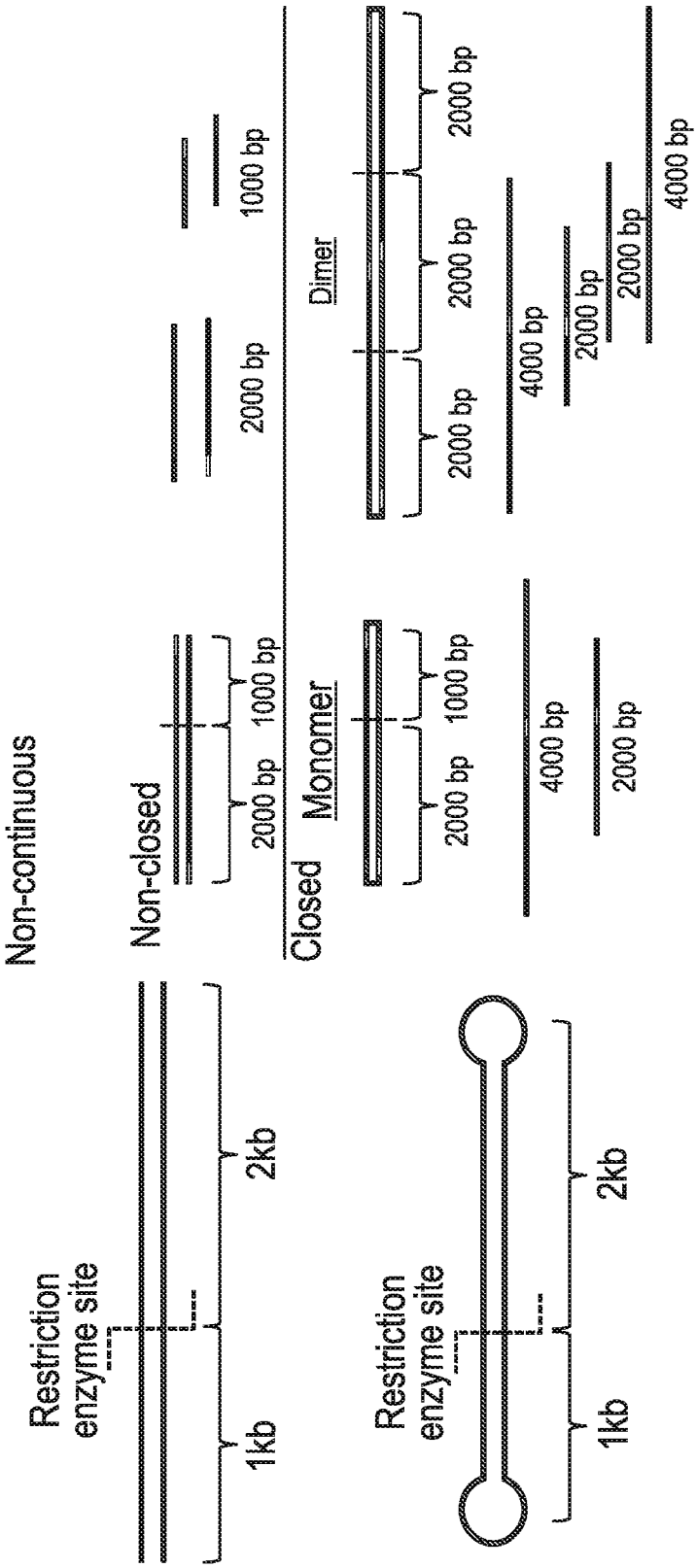

Non-continuous
Restriction enzyme site
1kb
2kb
Non-closed
2000 bp
1000 bp
Closed
Monomer
2000 bp
1000 bp
4000 bp
2000 bp
Dimer
2000 bp
2000 bp
2000 bp
4000 bp
2000 bp
2000 bp
2000 bp
4000 bp
Linear and continuous
Restriction enzyme site
1kb
2kb

FIG. 4

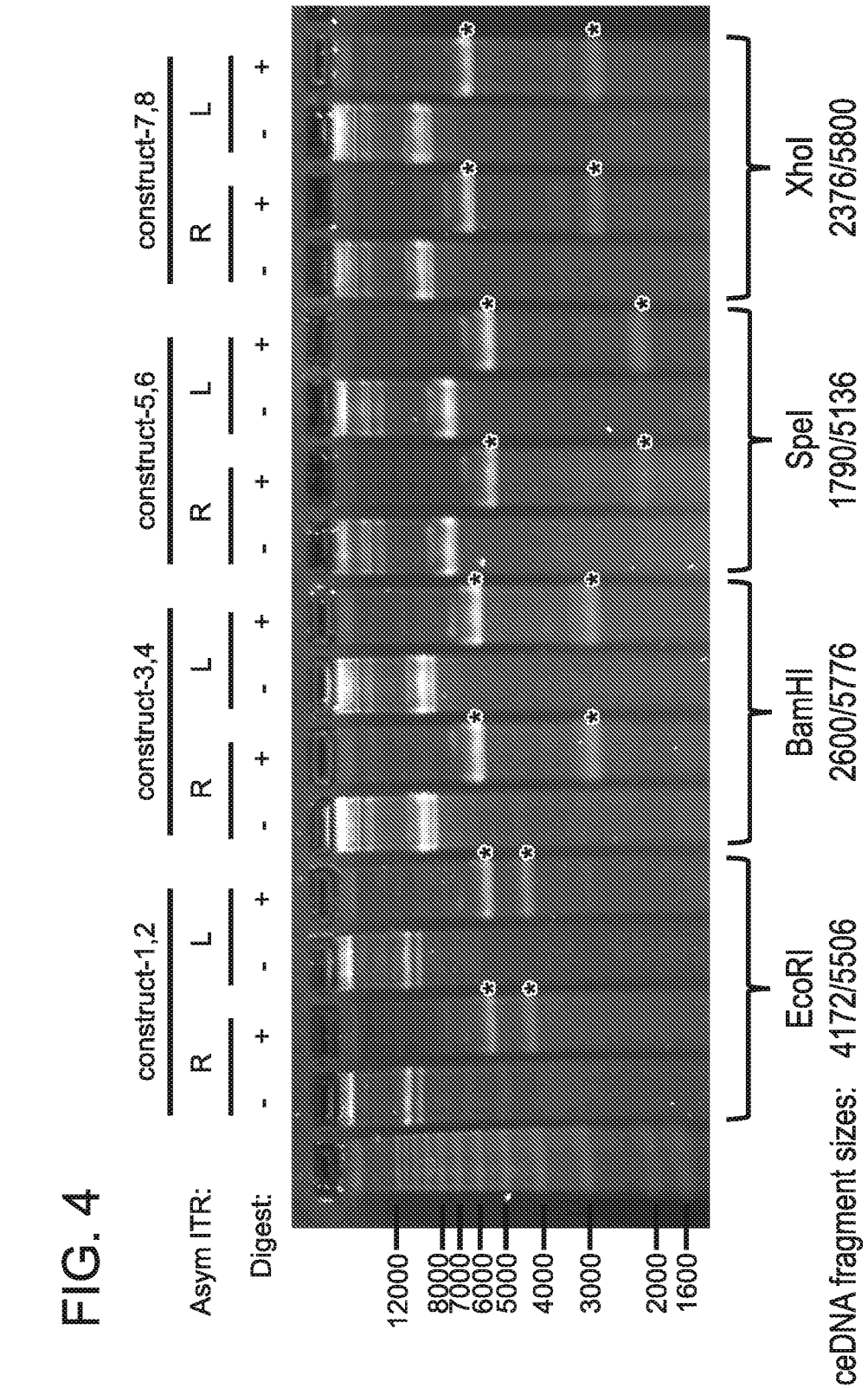
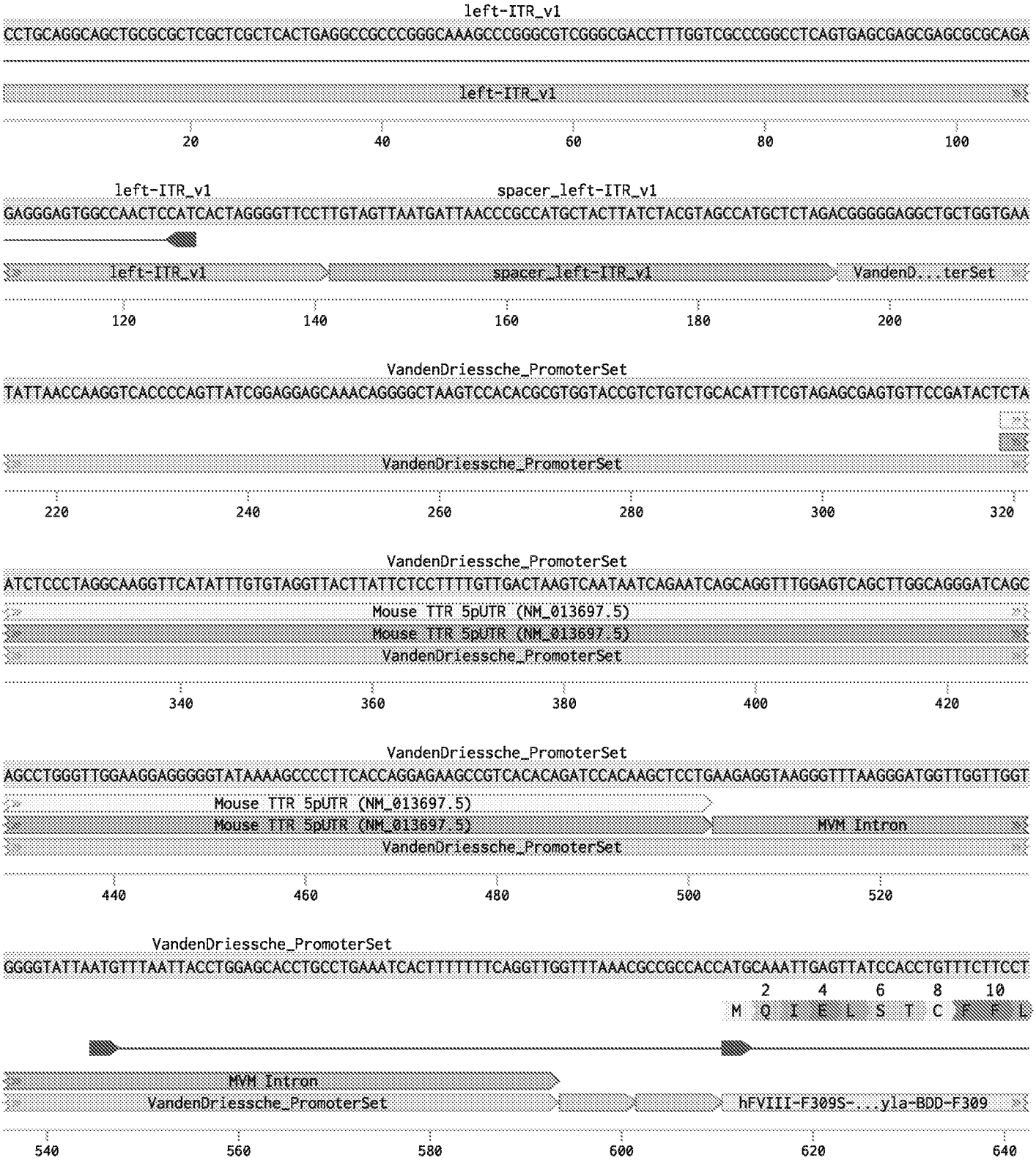
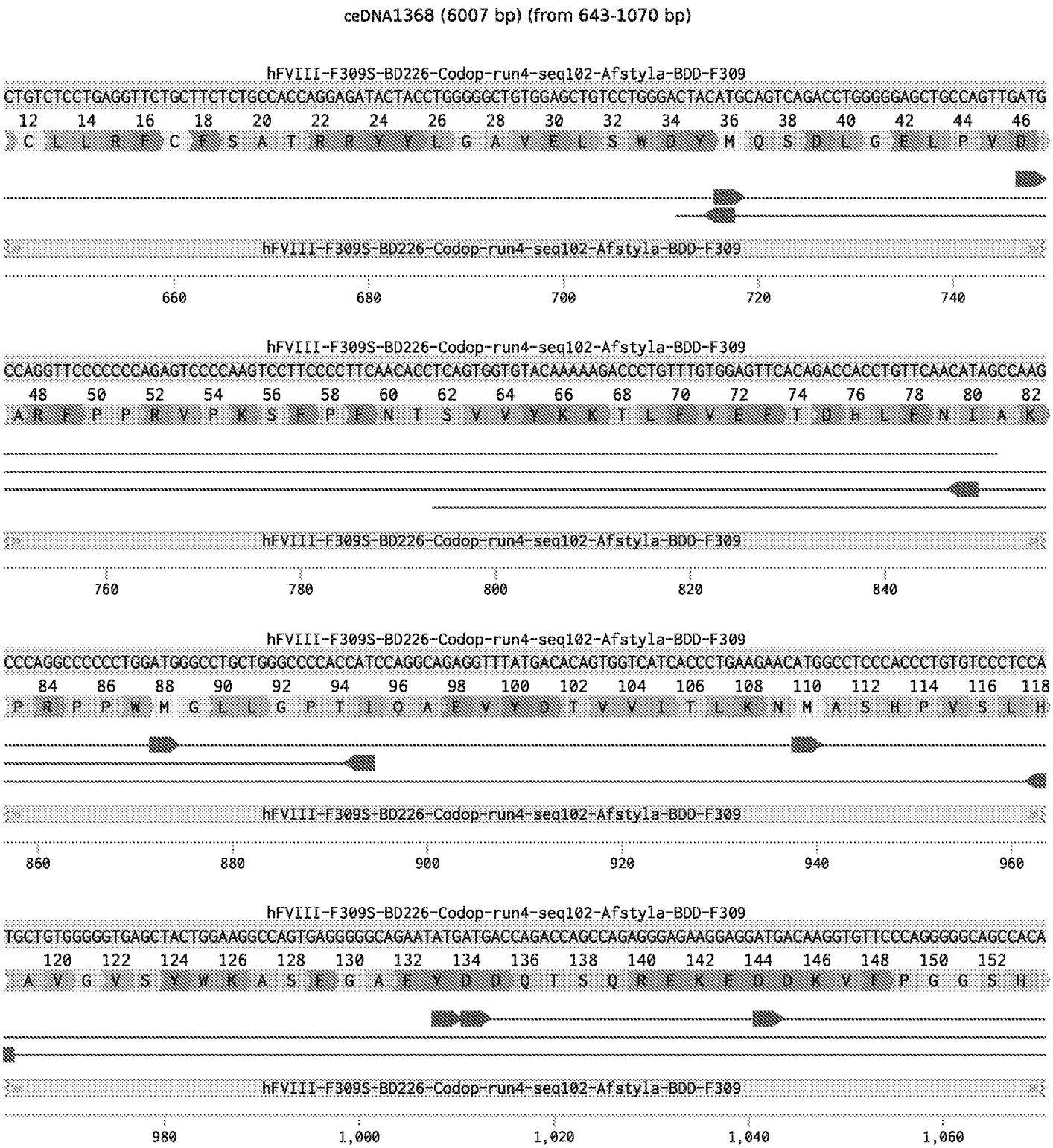

construct-1,2
construct-3,4
construct-5,6
construct-7,8
Asym ITR:
R
L
Digest:
-
+
12000
8000
7000
6000
5000
4000
3000
2000
1600
ceDNA fragment sizes:
EcoRI 4172/5506
BamHI 2600/5776
SpeI 1790/5136
XhoI 2376/5800

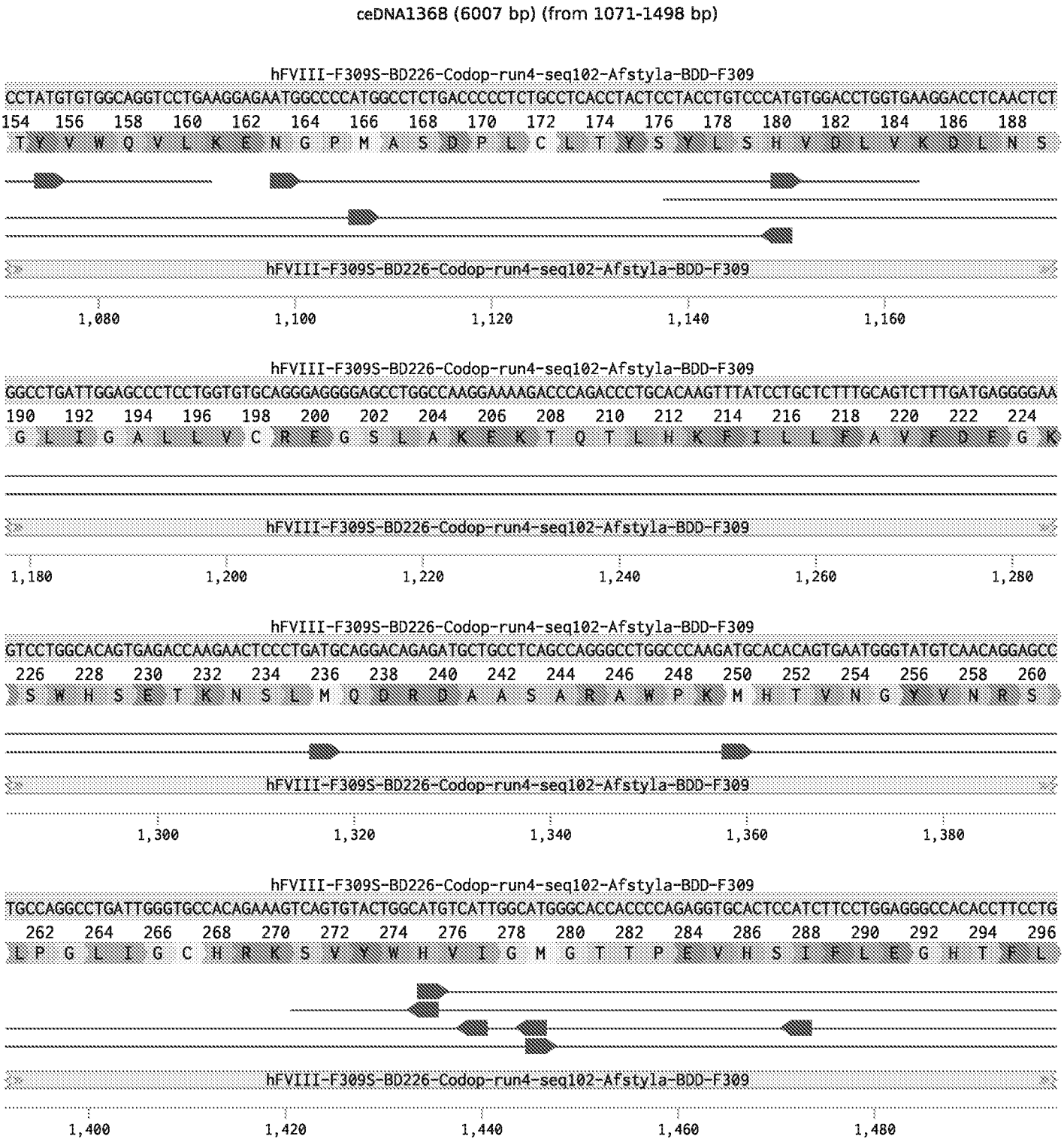
FIG. 5

ceDNA1368 (6007 bp)

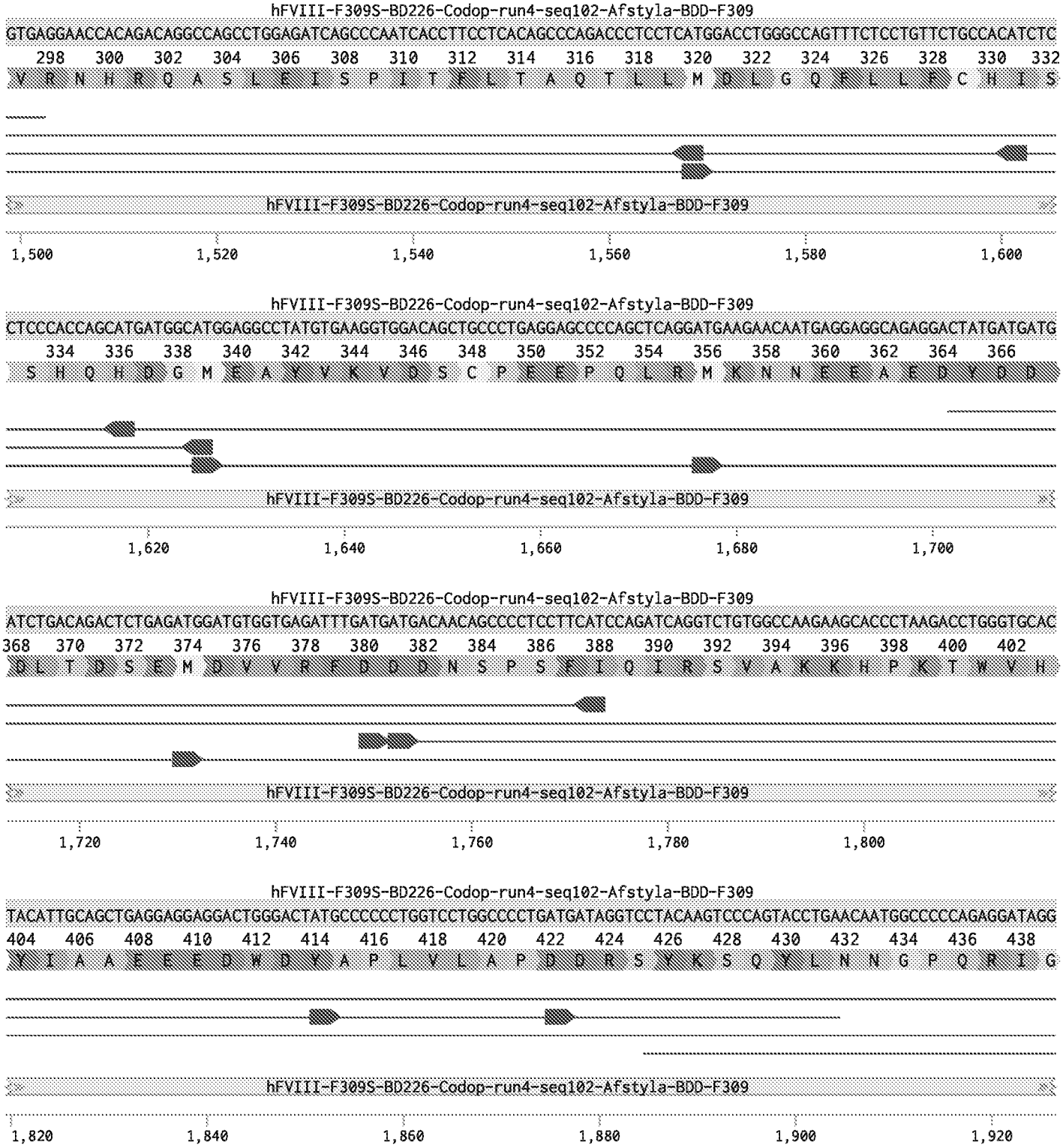
(from 1-642 bp)

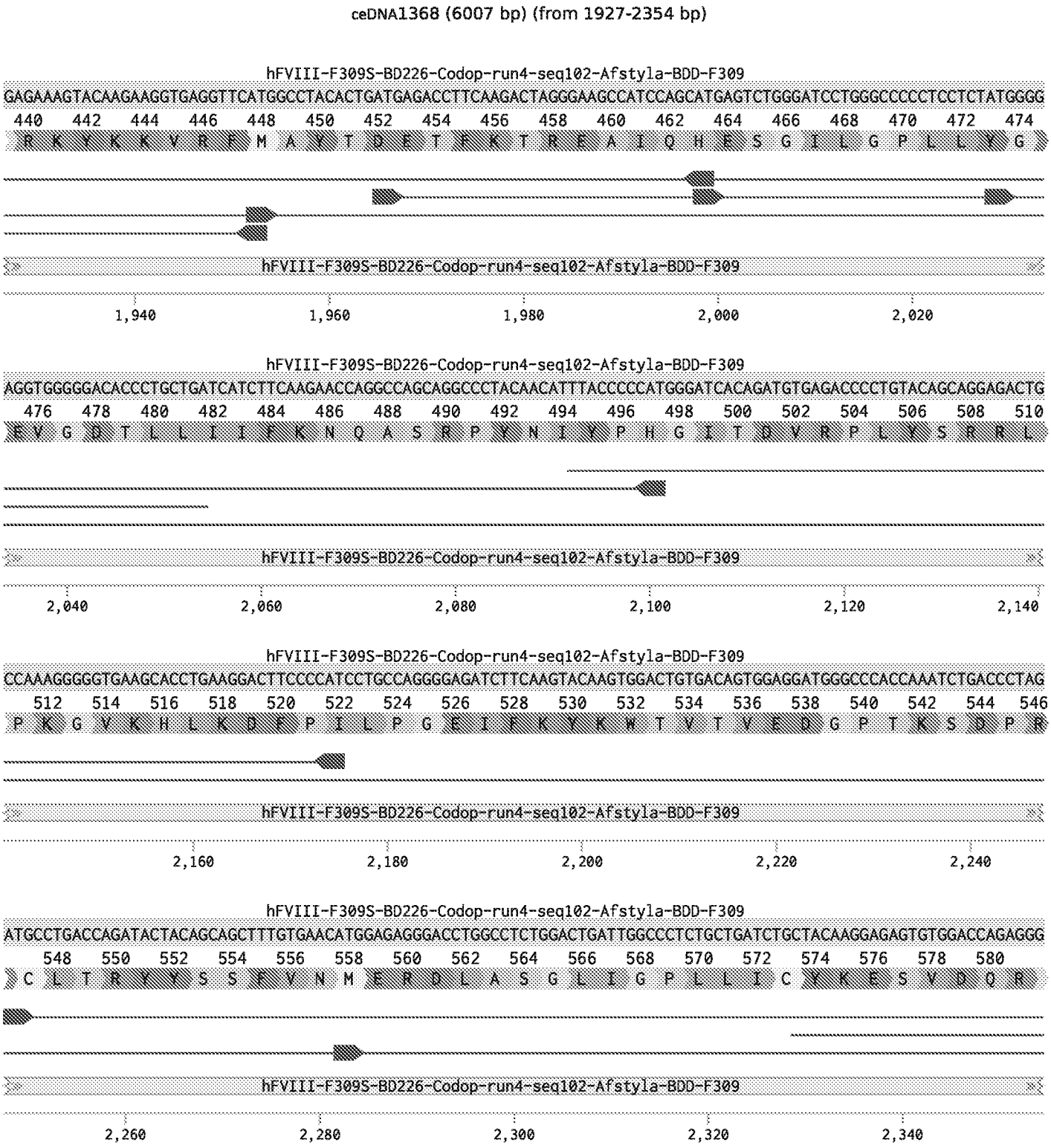


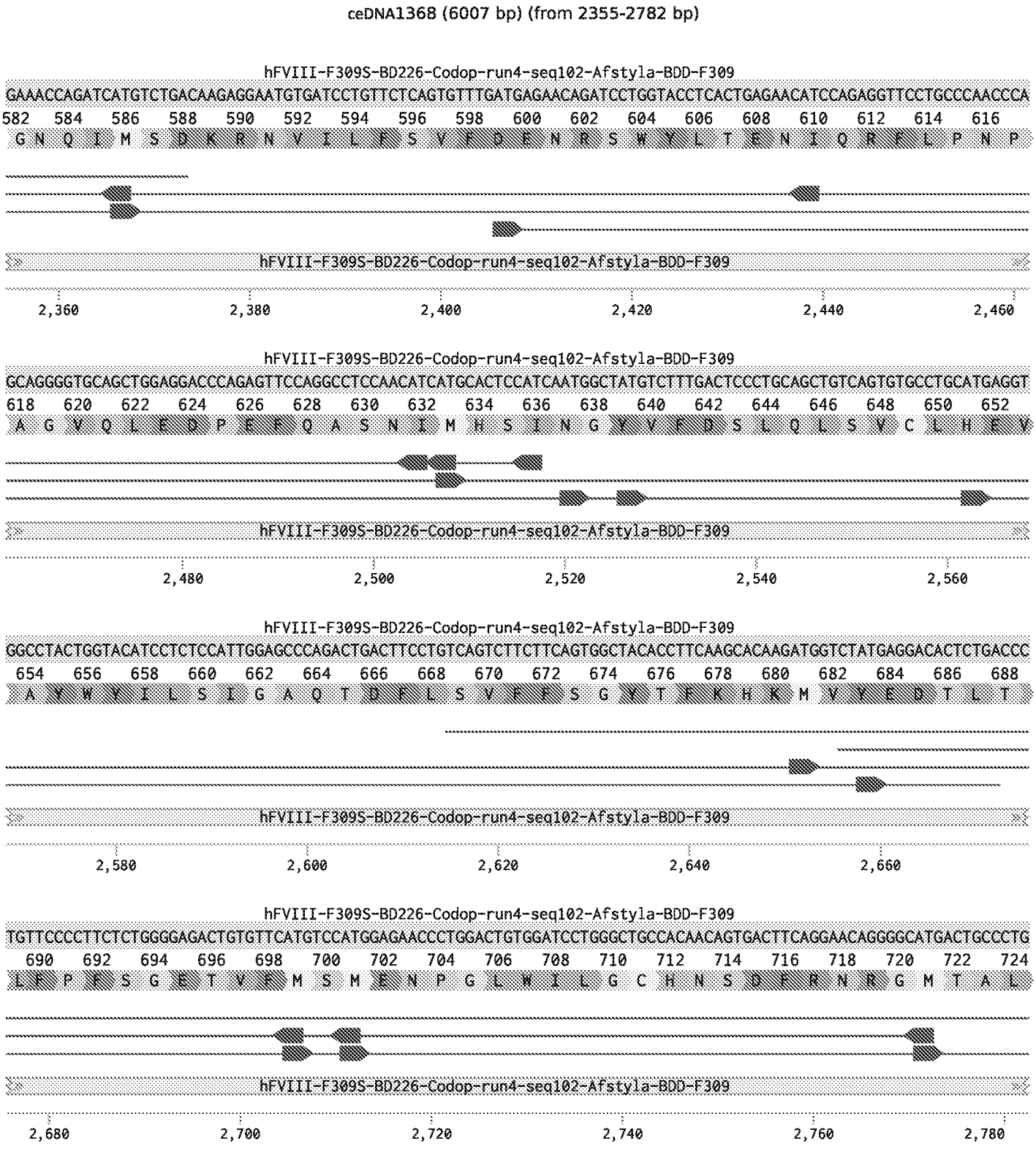

ceDNA1368 (6007 bp) (from 643-1070 bp)
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
CTGTCTCCTGAGGTTCTGCTTCTCTGCCACCAGGAGATACTACCTGGGGGCTGTGGAGCTGTCCTGGGACTACATGCAGTCAGACCTGGGGGAGCTGCCAGTTGATG
12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
C L L R F C F S A T R R Y Y L G A V E L S W D Y M Q S D L G E L P V D
660 680 700 720 740
CCAGGTTCCCCCCCAGAGTCCCCAAGTCCTTCCCCTTCAACACCTCAGTGGTGTACAAAAAGACCCTGTTTGTGGAGTTCACAGACCACCTGTTCAACATAGCCAAG
48 50 52 54 56 58 60 62 64 66 68 70 72 74 76 78 80 82
A R F P P R V P K S F P F N T S V V Y K K T L F V E F T D H L F N I A K
760 780 800 820 840
CCCAGGCCCCCCTGGATGGGCCTGCTGGGCCCCACCATCCAGGCAGAGGTTTATGACACAGTGGTCATCACCCTGAAGAACATGGCCTCCCACCCTGTGTCCCTCCA
84 86 88 90 92 94 96 98 100 102 104 106 108 110 112 114 116 118
P R P P W M G L L G P T I Q A E V Y D T V V I T L K N M A S H P V S L H
860 880 900 920 940 960
TGCTGTGGGGGTGAGCTACTGGAAGGCCAGTGAGGGGGCAGAATATGATGACCAGACCAGCCAGAGGGAGAAGGAGGATGACAAGGTGTTCCCAGGGGGCAGCCACA
120 122 124 126 128 130 132 134 136 138 140 142 144 146 148 150 152
A V G V S Y W K A S E G A E Y D D Q T S Q R E K E D D K V F P G G S H
980 1,000 1,020 1,040 1,060

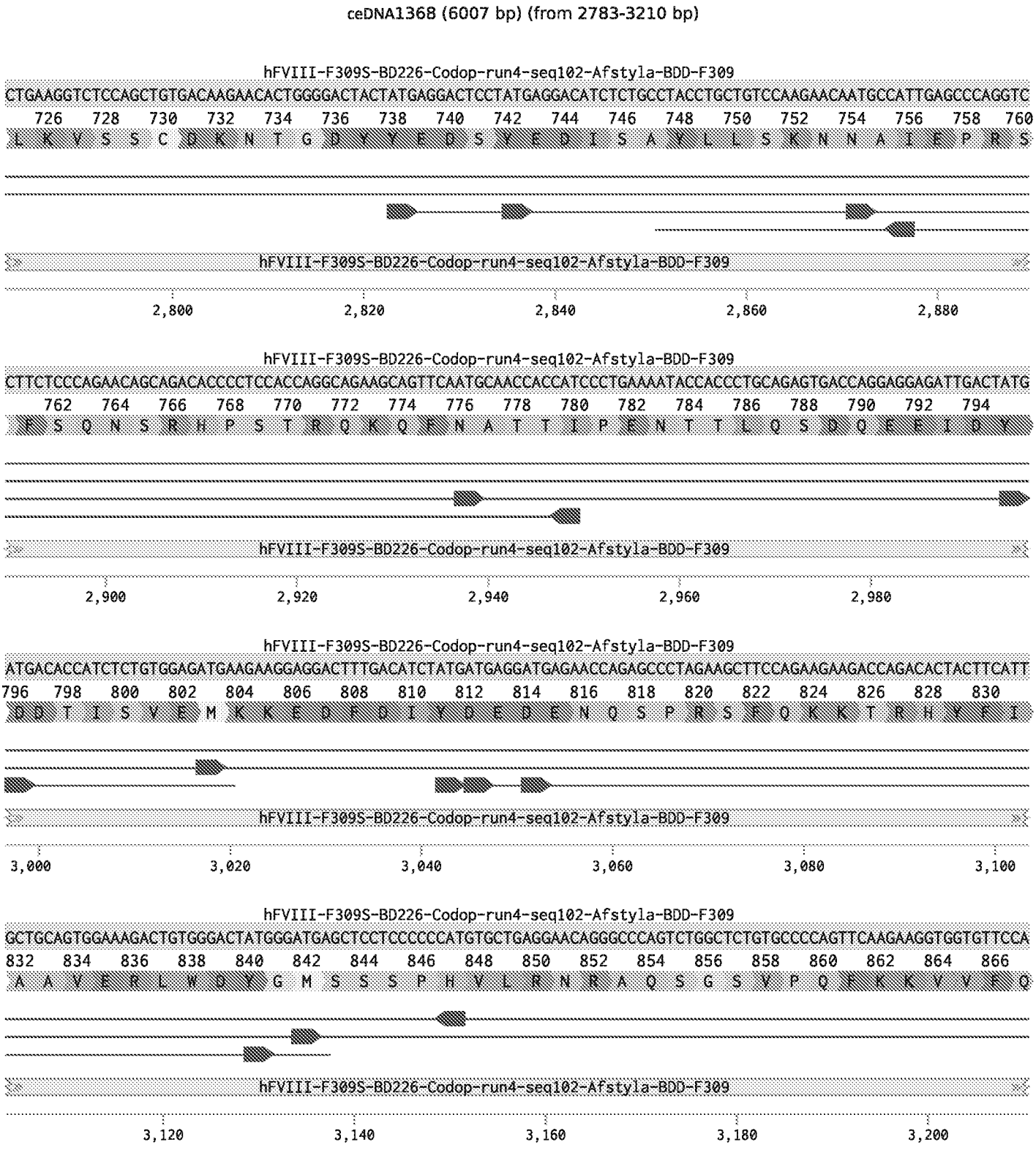

ceDNA1368 (6007 bp) (from 1071-1498 bp)
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
CCTATGTGTGGCAGGTCCTGAAGGAGAATGGCCCCATGGCCTCTGACCCCCTCTGCCTCACCTACTCCTACCTGTCCCATGTGGACCTGGTGAAGGACCTCAACTCT
154 156 158 160 162 164 166 168 170 172 174 176 178 180 182 184 186 188
T Y V W Q V L K E N G P M A S D P L C L T Y S Y L S H V D L V K D L N S
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
1,080 1,100 1,120 1,140 1,160
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
GGCCTGATTGGAGCCCTCCTGGTGTGCAGGGAGGGGAGCCTGGCCAAGGAAAAGACCCAGACCCTGCACAAGTTTATCCTGCTCTTTGCAGTCTTTGATGAGGGGAA
190 192 194 196 198 200 202 204 206 208 210 212 214 216 218 220 222 224
G L I G A L L V C R E G S L A K E K T Q T L H K F I L L F A V F D E G K
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
1,180 1,200 1,220 1,240 1,260 1,280
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
GTCCTGGCACAGTGAGACCAAGAACTCCCTGATGCAGGACAGAGATGCTGCCTCAGCCAGGGCCTGGCCCAAGATGCACACAGTGAATGGGTATGTCAACAGGAGCC
226 228 230 232 234 236 238 240 242 244 246 248 250 252 254 256 258 260
S W H S E T K N S L M Q D R D A A S A R A W P K M H T V N G Y V N R S
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
1,300 1,320 1,340 1,360 1,380
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
TGCCAGGCCTGATTGGGTGCCACAGAAAGTCAGTGTACTGGCATGTCATTGGCATGGGCACCACCCCAGAGGTGCACTCCATCTTCCTGGAGGGCCACACCTTCCTG
262 264 266 268 270 272 274 276 278 280 282 284 286 288 290 292 294 296
L P G L I G C H R K S V Y W H V I G M G T T P E V H S I F L E G H T F L
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
1,400 1,420 1,440 1,460 1,480

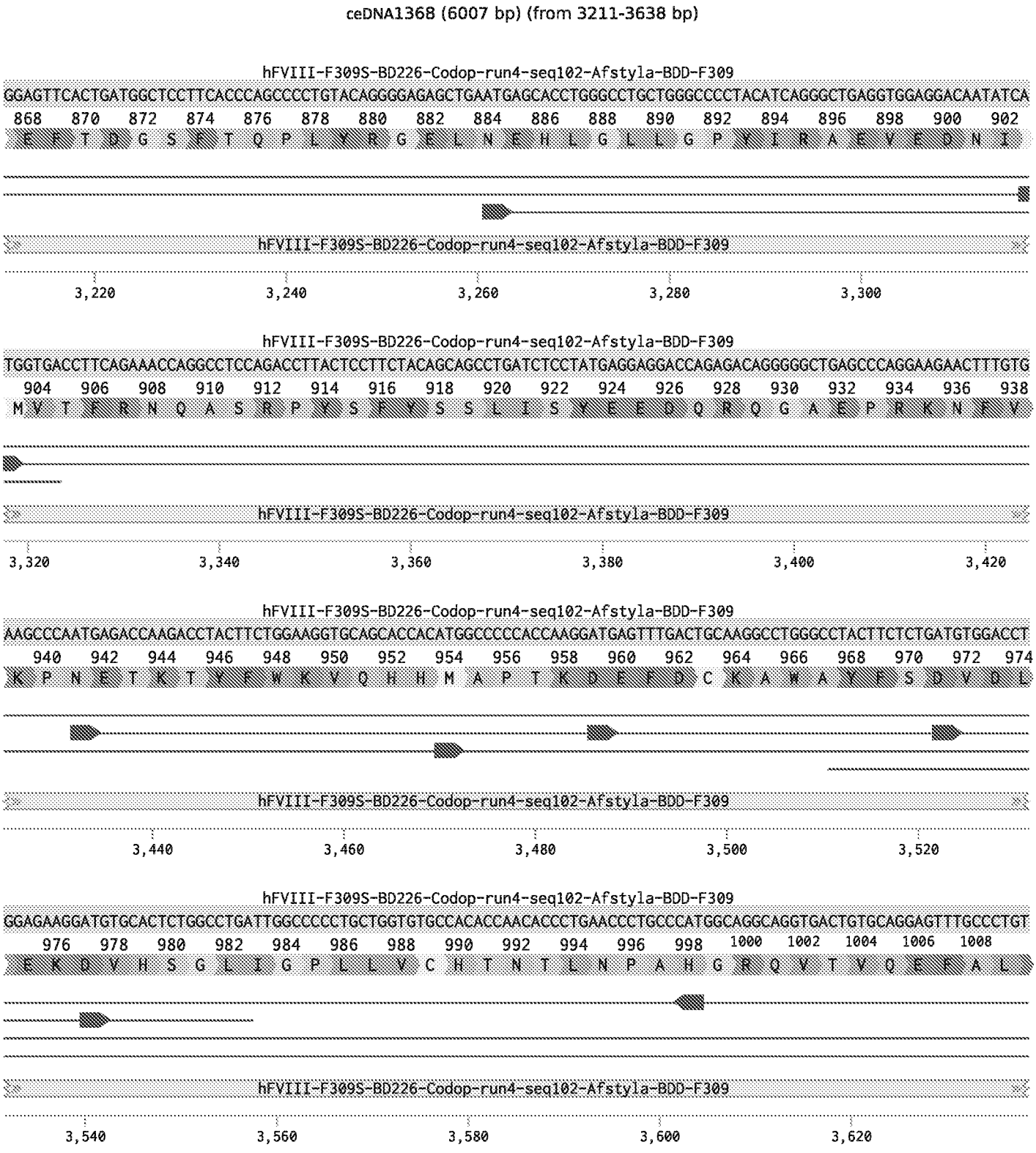
ceDNA1368 (6007 bp) (from 1499-1926 bp)

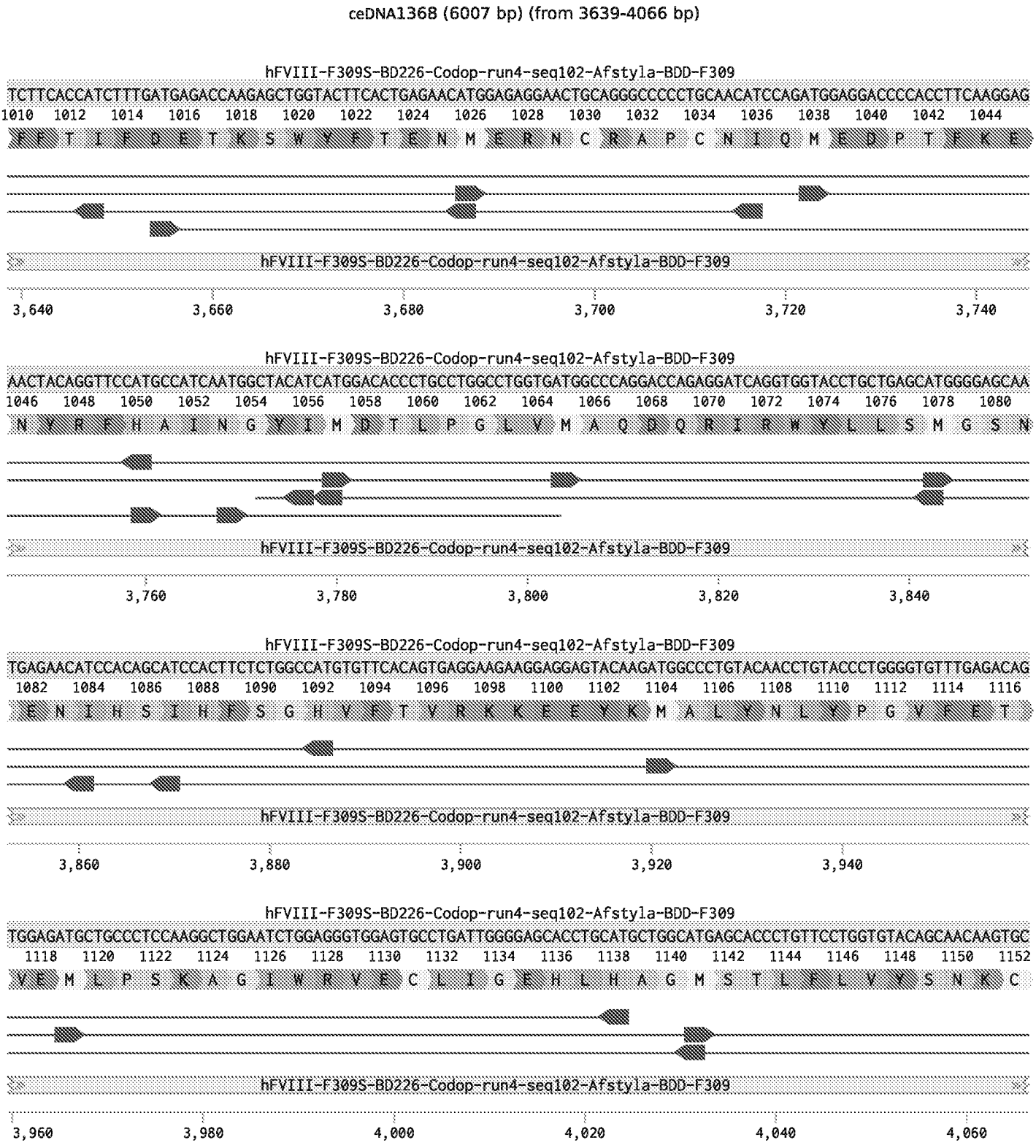


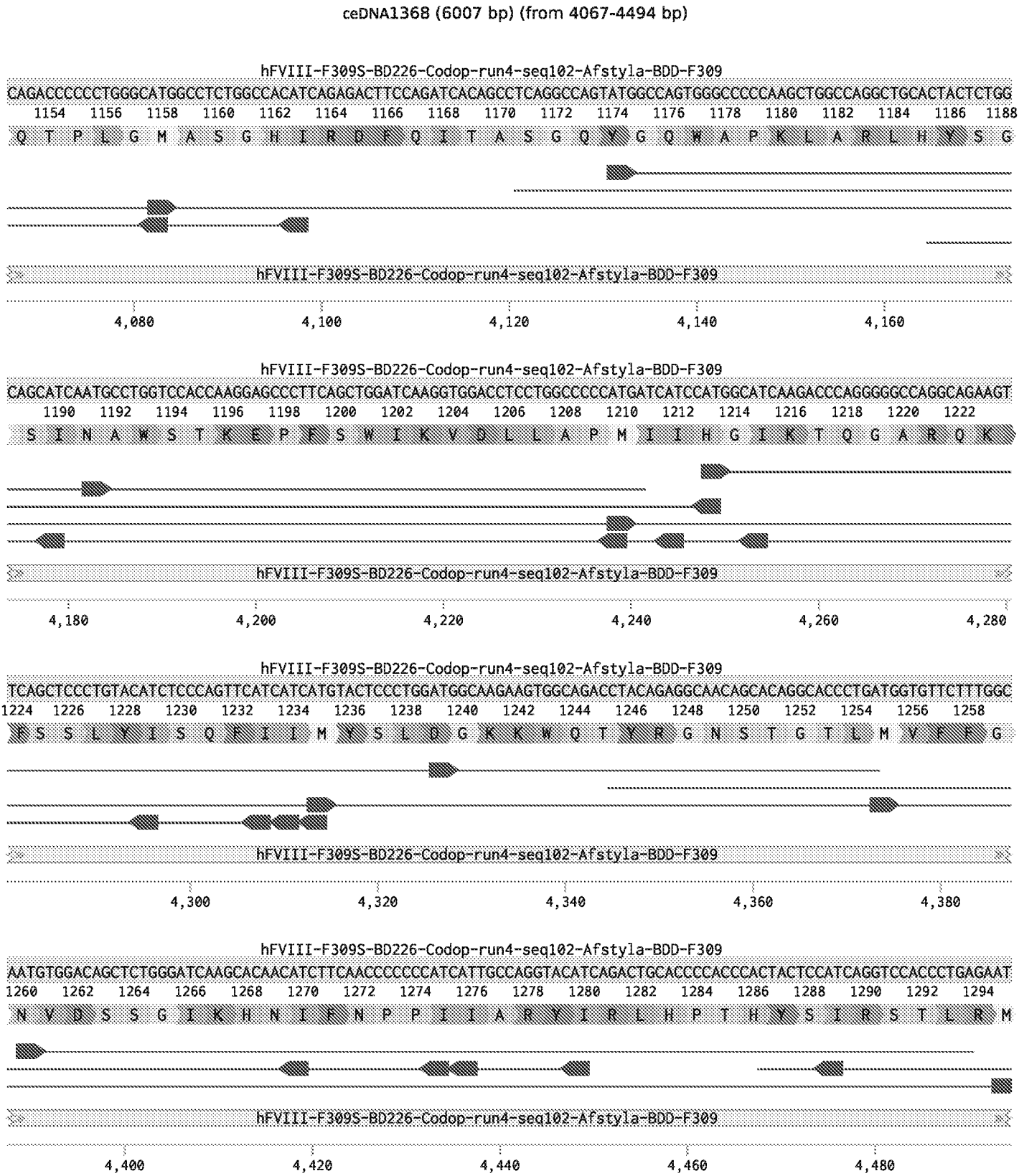

ceDNA1368 (6007 bp) (from 1927-2354 bp)
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
GAGAAAGTACAAGAAGGTGAGGTTCATGGCCTACACTGATGAGACCTTCAAGACTAGGGAAGCCATCCAGCATGAGTCTGGGATCCTGGGCCCCCTCCTCTATGGGG
440 442 444 446 448 450 452 454 456 458 460 462 464 466 468 470 472 474
R K Y K K V R F M A Y T D E T F K T R E A I Q H E S G I L G P L L Y G
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
1,940 1,960 1,980 2,000 2,020
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
AGGTGGGGGACACCCTGCTGATCATCTTCAAGAACCAGGCCAGCAGGCCCTACAACATTTACCCCCATGGGATCACAGATGTGAGACCCCTGTACAGCAGGAGACTG
476 478 480 482 484 486 488 490 492 494 496 498 500 502 504 506 508 510
E V G D T L L I I F K N Q A S R P Y N I Y P H G I T D V R P L Y S R R L
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
2,040 2,060 2,080 2,100 2,120 2,140
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
CCAAAGGGGGTGAAGCACCTGAAGGACTTCCCCATCCTGCCAGGGGAGATCTTCAAGTACAAGTGGACTGTGACAGTGGAGGATGGGCCCACCAAATCTGACCCTAG
512 514 516 518 520 522 524 526 528 530 532 534 536 538 540 542 544 546
P K G V K H L K D F P I L P G E I F K Y K W T V T V E D G P T K S D P R
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
2,160 2,180 2,200 2,220 2,240
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
ATGCCTGACCAGATACTACAGCAGCTTTGTGAACATGGAGAGGGACCTGGCCTCTGGACTGATTGGCCCTCTGCTGATCTGCTACAAGGAGAGTGTGGACCAGAGGG
548 550 552 554 556 558 560 562 564 566 568 570 572 574 576 578 580
C L T R Y Y S S F V N M E R D L A S G L I G P L L I C Y K E S V D Q R
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
2,260 2,280 2,300 2,320 2,340

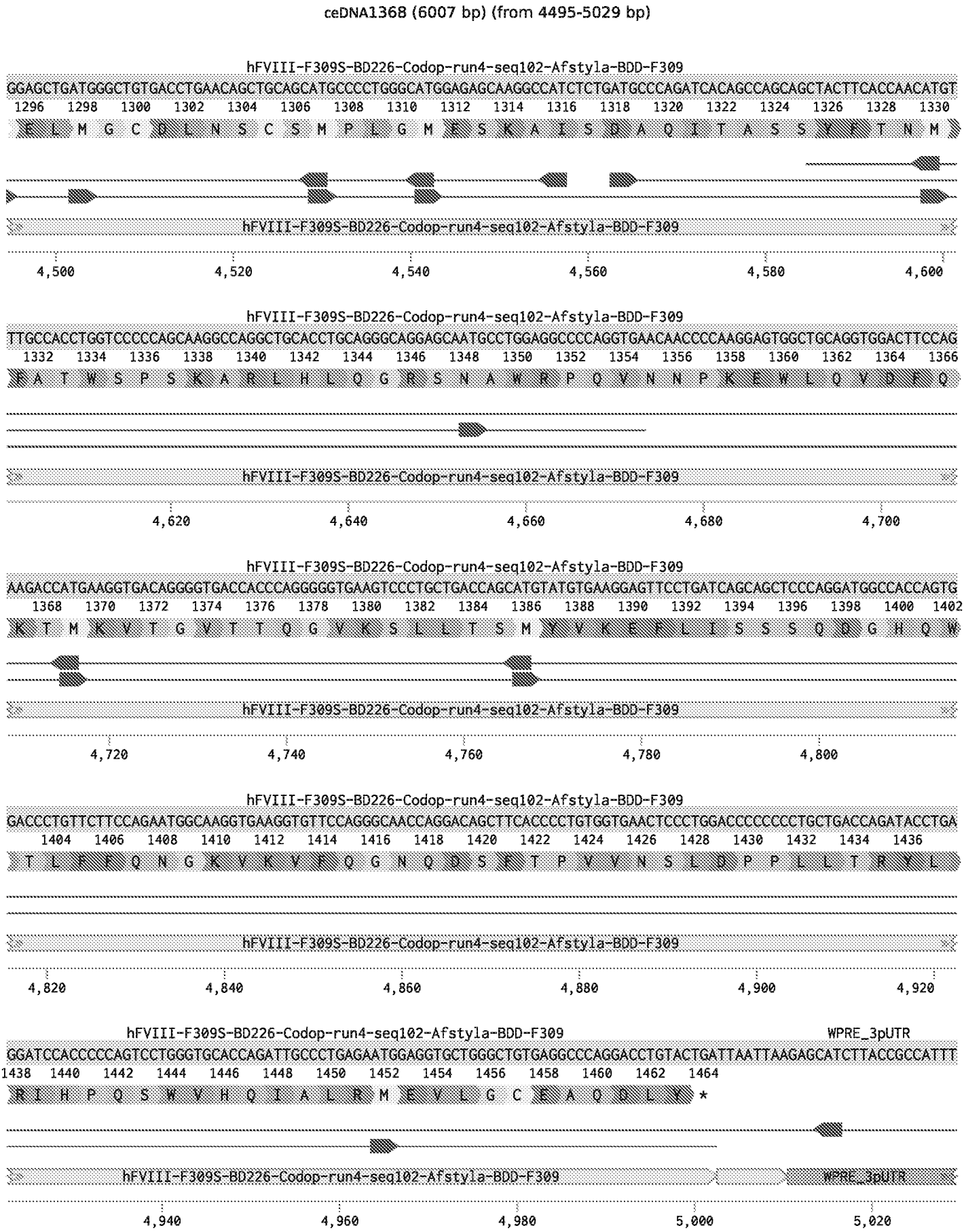

ceDNA1368 (6007 bp) (from 2355-2782 bp)
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
GAAACCAGATCATGTCTGACAAGAGGAATGTGATCCTGTTCTCAGTGTTTGATGAGAACAGATCCTGGTACCTCACTGAGAACATCCAGAGGTTCCTGCCCAACCCA
582 584 586 588 590 592 594 596 598 600 602 604 606 608 610 612 614 616
G N Q I M S D K R N V I L F S V F D E N R S W Y L T E N I Q R F L P N P
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
2,360 2,380 2,400 2,420 2,440 2,460
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
GCAGGGGTGCAGCTGGAGGACCCAGAGTTCCAGGCCTCCAACATCATGCACTCCATCAATGGCTATGTCTTTGACTCCCTGCAGCTGTCAGTGTGCCTGCATGAGGT
618 620 622 624 626 628 630 632 634 636 638 640 642 644 646 648 650 652
A G V Q L E D P E F Q A S N I M H S I N G Y V F D S L Q L S V C L H E V
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
2,480 2,500 2,520 2,540 2,560
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
GGCCTACTGGTACATCCTCTCCATTGGAGCCCAGACTGACTTCCTGTCAGTCTTCTTCAGTGGCTACACCTTCAAGCACAAGATGGTCTATGAGGACACTCTGACCC
654 656 658 660 662 664 666 668 670 672 674 676 678 680 682 684 686 688
A Y W Y I L S I G A Q T D F L S V F F S G Y T F K H K M V Y E D T L T
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
2,580 2,600 2,620 2,640 2,660
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
TGTTCCCCTTCTCTGGGGAGACTGTGTTCATGTCCATGGAGAACCCTGGACTGTGGATCCTGGGCTGCCACAACAGTGACTTCAGGAACAGGGGCATGACTGCCCTG
690 692 694 696 698 700 702 704 706 708 710 712 714 716 718 720 722 724
L T P F S G E T V F M S M E N P G L W I L G C H N S D F R N R G M T A L
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
2,680 2,700 2,720 2,740 2,760 2,780

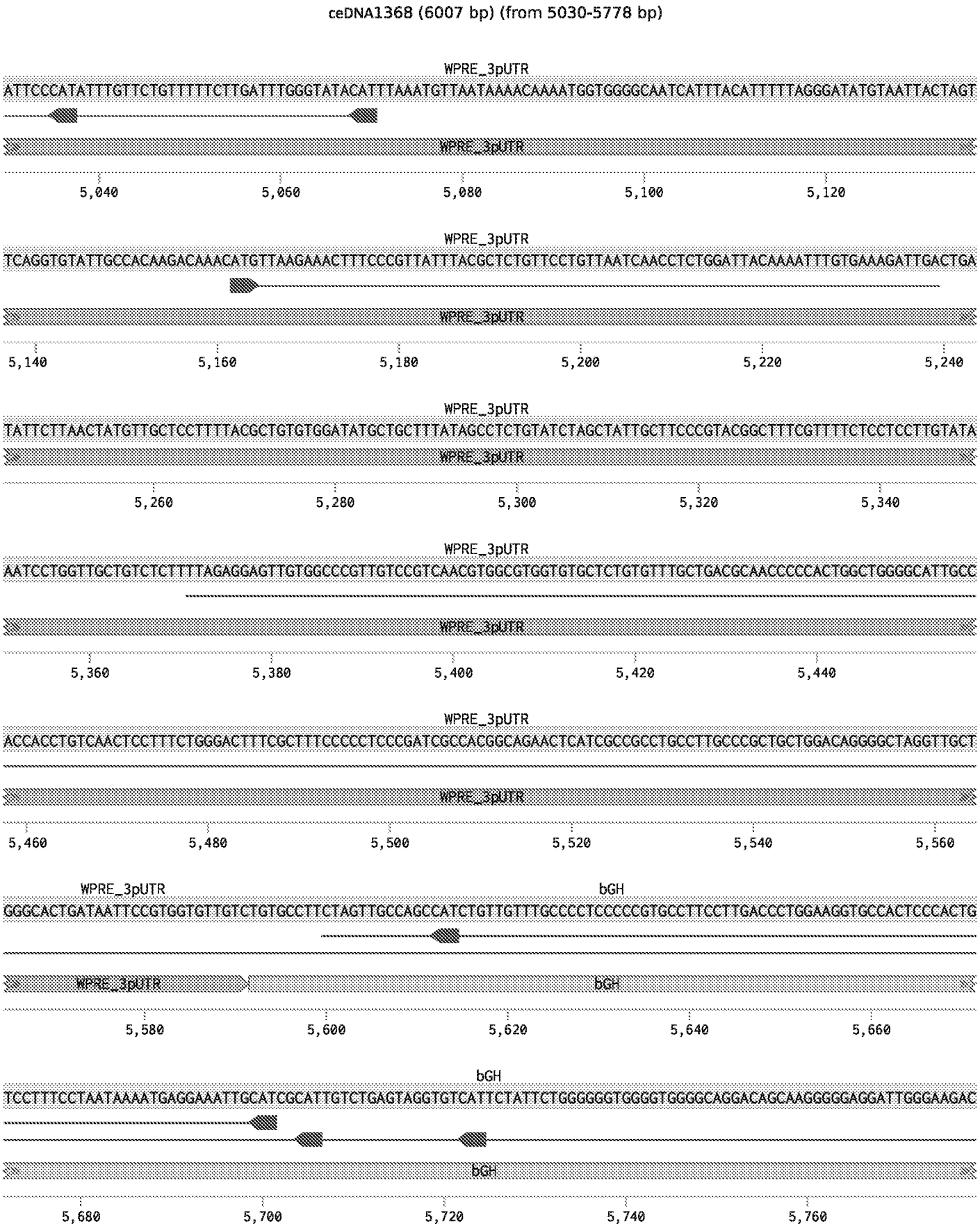

ceDNA1368 (6007 bp) (from 2783-3210 bp)
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
CTGAAGGTCTCCAGCTGTGACAAGAACACTGGGGACTACTATGAGGACTCCTATGAGGACATCTCTGCCTACCTGCTGTCCAAGAACAATGCCATTGAGCCCAGGTC
726 728 730 732 734 736 738 740 742 744 746 748 750 752 754 756 758 760
L K V S S C D K N T G D Y Y E D S Y E D I S A Y L L S K N N A I E P R S
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
2,800 2,820 2,840 2,860 2,880
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
CTTCTCCCAGAACAGCAGACACCCCTCCACCAGGCAGAAGCAGTTCAATGCAACCACCATCCCTGAAAATACCACCCTGCAGAGTGACCAGGAGGAGATTGACTATG
762 764 766 768 770 772 774 776 778 780 782 784 786 788 790 792 794
F S Q N S R H P S T R Q K Q F N A T T I P E N T T L Q S D Q E F I D Y
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
2,900 2,920 2,940 2,960 2,980
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
ATGACACCATCTCTGTGGAGATGAAGAAGGAGGACTTTGACATCTATGATGAGGATGAGAACCAGAGCCCTAGAAGCTTCCAGAAGAAGACCAGACACTACTTCATT
796 798 800 802 804 806 808 810 812 814 816 818 820 822 824 826 828 830
D D T I S V E M K K E D F D I Y D E D E N Q S P R S F Q K K T R H Y F I
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
3,000 3,020 3,040 3,060 3,080 3,100
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
GCTGCAGTGGAAAGACTGTGGGACTATGGGATGAGCTCCTCCCCCCATGTGCTGAGGAACAGGGCCCAGTCTGGCTCTGTGCCCCAGTTCAAGAAGGTGGTGTTCCA
832 834 836 838 840 842 844 846 848 850 852 854 856 858 860 862 864 866
A A V E R L W D Y G M S S S P H V L R N R A Q S G S V P Q F K K V V F Q
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
3,120 3,140 3,160 3,180 3,200


ceDNA1368 (6007 bp) (from 3211-3638 bp)
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
GGAGTTCACTGATGGCTCCTTCACCCAGCCCCTGTACAGGGGAGAGCTGAATGAGCACCTGGGCCTGCTGGGCCCCTACATCAGGGCTGAGGTGGAGGACAATATCA
868 870 872 874 876 878 880 882 884 886 888 890 892 894 896 898 900 902
E F T D G S F T Q P L Y R G E L N E H L G L L G P Y I R A E V E D N I
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
3,220 3,240 3,260 3,280 3,300
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
TGGTGACCTTCAGAAACCAGGCCTCCAGACCTTACTCCTTCTACAGCAGCCTGATCTCCTATGAGGAGGACCAGAGACAGGGGGCTGAGCCCAGGAAGAACTTTGTG
904 906 908 910 912 914 916 918 920 922 924 926 928 930 932 934 936 938
M V T F R N Q A S R P Y S F Y S S L I S Y E E D Q R Q G A E P R K N F V
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
3,320 3,340 3,360 3,380 3,400 3,420
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
AAGCCCAATGAGACCAAGACCTACTTCTGGAAGGTGCAGCACCACATGGCCCCCACCAAGGATGAGTTTGACTGCAAGGCCTGGGCCTACTTCTCTGATGTGGACCT
940 942 944 946 948 950 952 954 956 958 960 962 964 966 968 970 972 974
K P N E T K T Y F W K V Q H H M A P T K D E F D C K A W A Y F S D V D L
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
3,440 3,460 3,480 3,500 3,520
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
GGAGAAGGATGTGCACTCTGGCCTGATTGGCCCCCTGCTGGTGTGCCACACCAACACCCTGAACCCTGCCCATGGCAGGCAGGTGACTGTGCAGGAGTTTGCCCTGT
976 978 980 982 984 986 988 990 992 994 996 998 1000 1002 1004 1006 1008
E K D V H S G L I G P L L V C H T N T L N P A H G R Q V T V Q E F A L
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
3,540 3,560 3,580 3,600 3,620


ceDNA1368 (6007 bp) (from 3639-4066 bp)
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
TCTTCACCATCTTTGATGAGACCAAGAGCTGGTACTTCACTGAGAACATGGAGAGGAACTGCAGGGCCCCCTGCAACATCCAGATGGAGGACCCCACCTTCAAGGAG
1010 1012 1014 1016 1018 1020 1022 1024 1026 1028 1030 1032 1034 1036 1038 1040 1042 1044
F F T I F D E T K S W Y F T E N M E R N C R A P C N I Q M E D P T F K E
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
3,640 3,660 3,680 3,700 3,720 3,740
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
AACTACAGGTTCCATGCCATCAATGGCTACATCATGGACACCCTGCCTGGCCTGGTGATGGCCCAGGACCAGAGGATCAGGTGGTACCTGCTGAGCATGGGGAGCAA
1046 1048 1050 1052 1054 1056 1058 1060 1062 1064 1066 1068 1070 1072 1074 1076 1078 1080
N Y R F H A I N G Y I M D T L P G L V M A Q D Q R I R W Y L L S M G S N
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
3,760 3,780 3,800 3,820 3,840
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
TGAGAACATCCACAGCATCCACTTCTCTGGCCATGTGTTCACAGTGAGGAAGAAGGAGGAGTACAAGATGGCCCTGTACAACCTGTACCCTGGGGTGTTTGAGACAG
1082 1084 1086 1088 1090 1092 1094 1096 1098 1100 1102 1104 1106 1108 1110 1112 1114 1116
E N I H S I H F S G H V F T V R K K E E Y K M A L Y N L Y P G V F E T
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
3,860 3,880 3,900 3,920 3,940
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
TGGAGATGCTGCCCTCCAAGGCTGGAATCTGGAGGGTGGAGTGCCTGATTGGGGAGCACCTGCATGCTGGCATGAGCACCCTGTTCCTGGTGTACAGCAACAAGTGC
1118 1120 1122 1124 1126 1128 1130 1132 1134 1136 1138 1140 1142 1144 1146 1148 1150 1152
V F M L P S K A G I W R V E C L I G E H L H A G M S T L F L V Y S N K C
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
3,960 3,980 4,000 4,020 4,040 4,060


ceDNA1368 (6007 bp) (from 4067-4494 bp)
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
CAGACCCCCCTGGGCATGGCCTCTGGCCACATCAGAGACTTCCAGATCACAGCCTCAGGCCAGTATGGCCAGTGGGCCCCCAAGCTGGCCAGGCTGCACTACTCTGG
1154 1156 1158 1160 1162 1164 1166 1168 1170 1172 1174 1176 1178 1180 1182 1184 1186 1188
Q T P L G M A S G H I R D F Q I T A S G Q Y G Q W A P K L A R L H Y S G
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
4,080 4,100 4,120 4,140 4,160
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
CAGCATCAATGCCTGGTCCACCAAGGAGCCCTTCAGCTGGATCAAGGTGGACCTCCTGGCCCCCATGATCATCCATGGCATCAAGACCCAGGGGGCCAGGCAGAAGT
1190 1192 1194 1196 1198 1200 1202 1204 1206 1208 1210 1212 1214 1216 1218 1220 1222
S I N A W S T K E P F S W I K V D L L A P M I I H G I K T Q G A R Q K
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
4,180 4,200 4,220 4,240 4,260 4,280
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
TCAGCTCCCTGTACATCTCCCAGTTCATCATCATGTACTCCCTGGATGGCAAGAAGTGGCAGACCTACAGAGGCAACAGCACAGGCACCCTGATGGTGTTCTTTGGC
1224 1226 1228 1230 1232 1234 1236 1238 1240 1242 1244 1246 1248 1250 1252 1254 1256 1258
F S S L Y I S Q F I I M Y S L D G K K W Q T Y R G N S T G T L M V F F G
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
4,300 4,320 4,340 4,360 4,380
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
AATGTGGACAGCTCTGGGATCAAGCACAACATCTTCAACCCCCCCATCATTGCCAGGTACATCAGACTGCACCCCACCCACTACTCCATCAGGTCCACCCTGAGAAT
1260 1262 1264 1266 1268 1270 1272 1274 1276 1278 1280 1282 1284 1286 1288 1290 1292 1294
N V D S S G I K H N I F N P P I I A R Y I R L H P T H Y S I R S T L R M
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
4,400 4,420 4,440 4,460 4,480


ceDNA1368 (6007 bp) (from 4495-5029 bp)
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
GGAGCTGATGGGCTGTGACCTGAACAGCTGCAGCATGCCCCTGGGCATGGAGAGCAAGGCCATCTCTGATGCCCAGATCACAGCCAGCAGCTACTTCACCAACATGT
1296 1298 1300 1302 1304 1306 1308 1310 1312 1314 1316 1318 1320 1322 1324 1326 1328 1330
E L M G C D L N S C S M P L G M E S K A I S D A Q I T A S S Y F T N M
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
4,500 4,520 4,540 4,560 4,580 4,600
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
TTGCCACCTGGTCCCCCAGCAAGGCCAGGCTGCACCTGCAGGGCAGGAGCAATGCCTGGAGGCCCCAGGTGAACAACCCCAAGGAGTGGCTGCAGGTGGACTTCCAG
1332 1334 1336 1338 1340 1342 1344 1346 1348 1350 1352 1354 1356 1358 1360 1362 1364 1366
F A T W S P S K A R L H L Q G R S N A W R P Q V N N P K E W L Q V D F Q
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
4,620 4,640 4,660 4,680 4,700
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
AAGACCATGAAGGTGACAGGGGTGACCACCCAGGGGGTGAAGTCCCTGCTGACCAGCATGTATGTGAAGGAGTTCCTGATCAGCAGCTCCCAGGATGGCCACCAGTG
1368 1370 1372 1374 1376 1378 1380 1382 1384 1386 1388 1390 1392 1394 1396 1398 1400 1402
K T M K V T G V T T Q G V K S L L T S M Y V K E F L I S S S Q D G H Q W
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
4,720 4,740 4,760 4,780 4,800
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
GACCCTGTTCTTCCAGAATGGCAAGGTGAAGGTGTTCCAGGGCAACCAGGACAGCTTCACCCCTGTGGTGAACTCCCTGGACCCCCCCCCTGCTGACCAGATACCTGA
1404 1406 1408 1410 1412 1414 1416 1418 1420 1422 1424 1426 1428 1430 1432 1434 1436
T L F F Q N G K V K V F Q G N Q D S F T P V V N S L D P P L L T R Y L
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
4,820 4,840 4,860 4,880 4,900 4,920
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
WPRE_3pUTR
GGATCCACCCCCAGTCCTGGGTGCACCAGATTGCCCTGAGAATGGAGGTGCTGGGCTGTGAGGCCCAGGACCTGTACTGATTAATTAAGAGCATCTTACCGCCATTT
1438 1440 1442 1444 1446 1448 1450 1452 1454 1456 1458 1460 1462 1464
R I H P Q S W V H Q I A L R M E V L G C E A Q D L Y *
hFVIII-F309S-BD226-Codop-run4-seq102-Afstyla-BDD-F309
WPRE_3pUTR
4,940 4,960 4,980 5,000 5,020


ceDNA1368 (6007 bp) (from 5030-5778 bp)
WPRE_3pUTR
ATTCCCATATTTGTTCTGTTTTTCTTGATTTGGGTATACATTTAAATGTTAATAAAACAAAATGGTGGGGCAATCATTTACATTTTTAGGGATATGTAATTACTAGT
WPRE_3pUTR
5,040
5,060
5,080
5,100
5,120
WPRE_3pUTR
TCAGGTGTATTGCCACAAGACAAACATGTTAAGAAACTTTCCCGTTATTTACGCTCTGTTCCTGTTAATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGA
WPRE_3pUTR
5,140
5,160
5,180
5,200
5,220
5,240
WPRE_3pUTR
TATTCTTAACTATGTTGCTCCTTTTACGCTGTGTGGATATGCTGCTTTATAGCCTCTGTATCTAGCTATTGCTTCCCGTACGGCTTTCGTTTTCTCCTCCTTGTATA
WPRE_3pUTR
5,260
5,280
5,300
5,320
5,340
WPRE_3pUTR
AATCCTGGTTGCTGTCTCTTTTAGAGGAGTTGTGGCCCGTTGTCCGTCAACGTGGCGTGGTGTGCTCTGTGTTTGCTGACGCAACCCCCACTGGCTGGGGCATTGCC
WPRE_3pUTR
5,360
5,380
5,400
5,420
5,440
WPRE_3pUTR
ACCACCTGTCAACTCCTTTCTGGGACTTTCGCTTTCCCCCTCCCGATCGCCACGGCAGAACTCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCTAGGTTGCT
WPRE_3pUTR
5,460
5,480
5,500
5,520
5,540
5,560
WPRE_3pUTR
bGH
GGGCACTGATAATTCCGTGGTGTTGTCTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTG
WPRE_3pUTR
bGH
5,580
5,600
5,620
5,640
5,660
bGH
TCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGAC
bGH
5,680
5,700
5,720
5,740
5,760

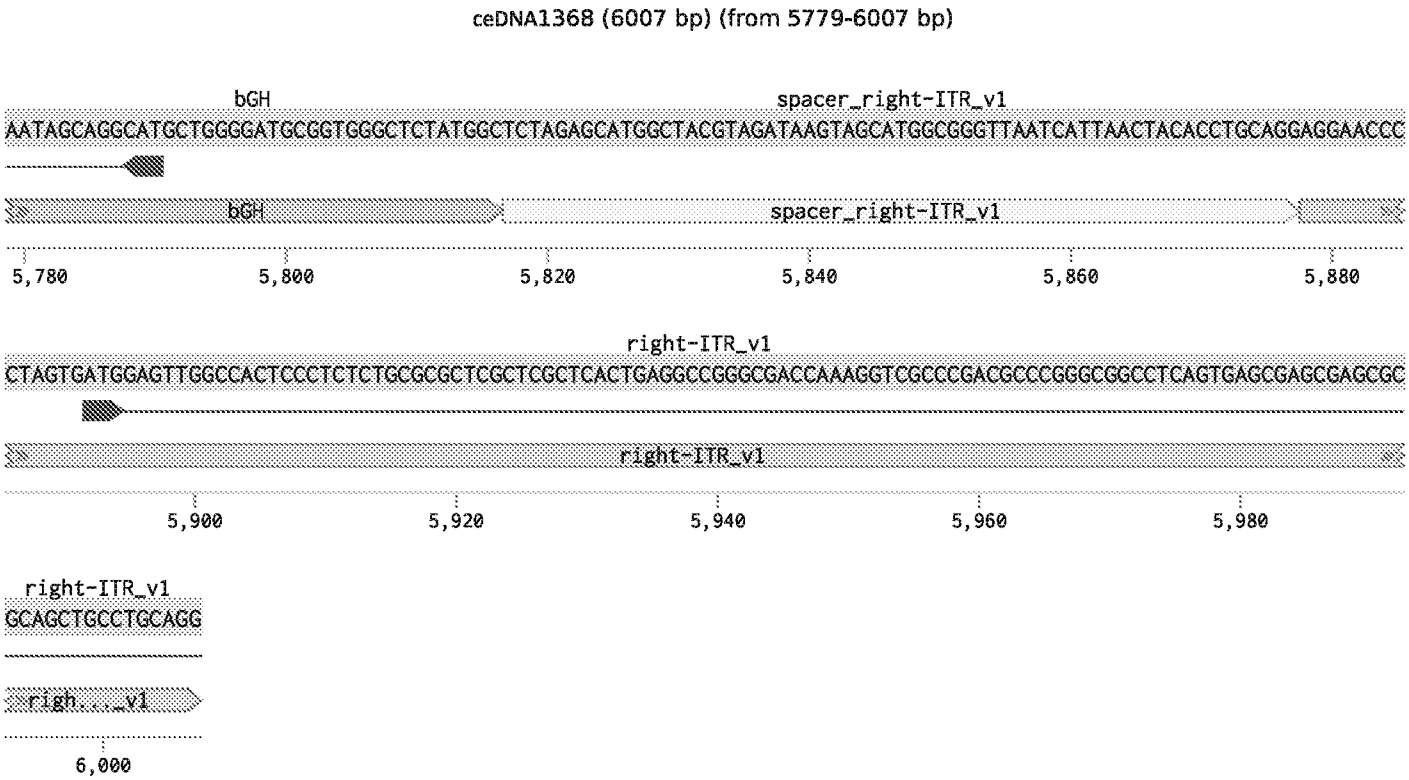
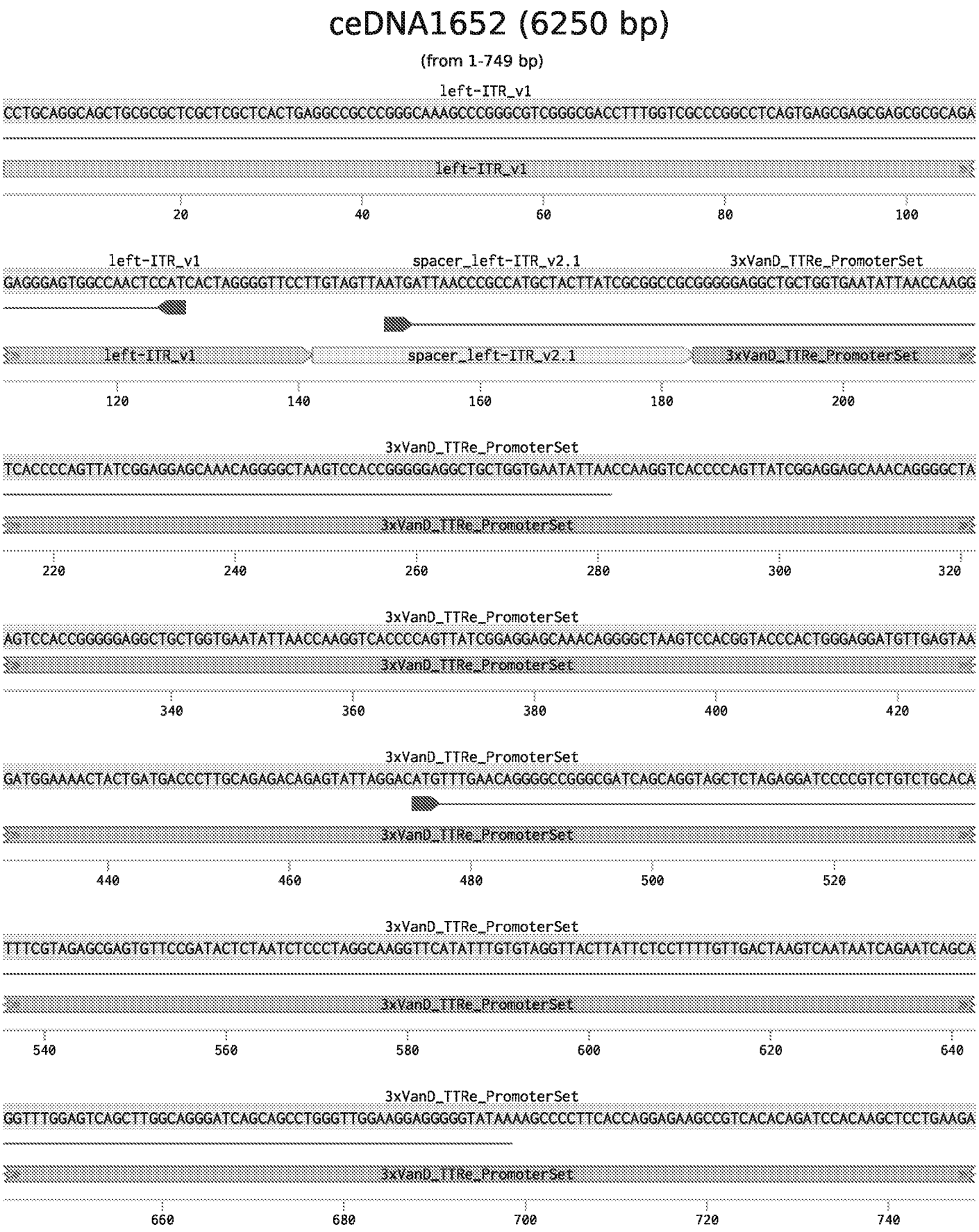
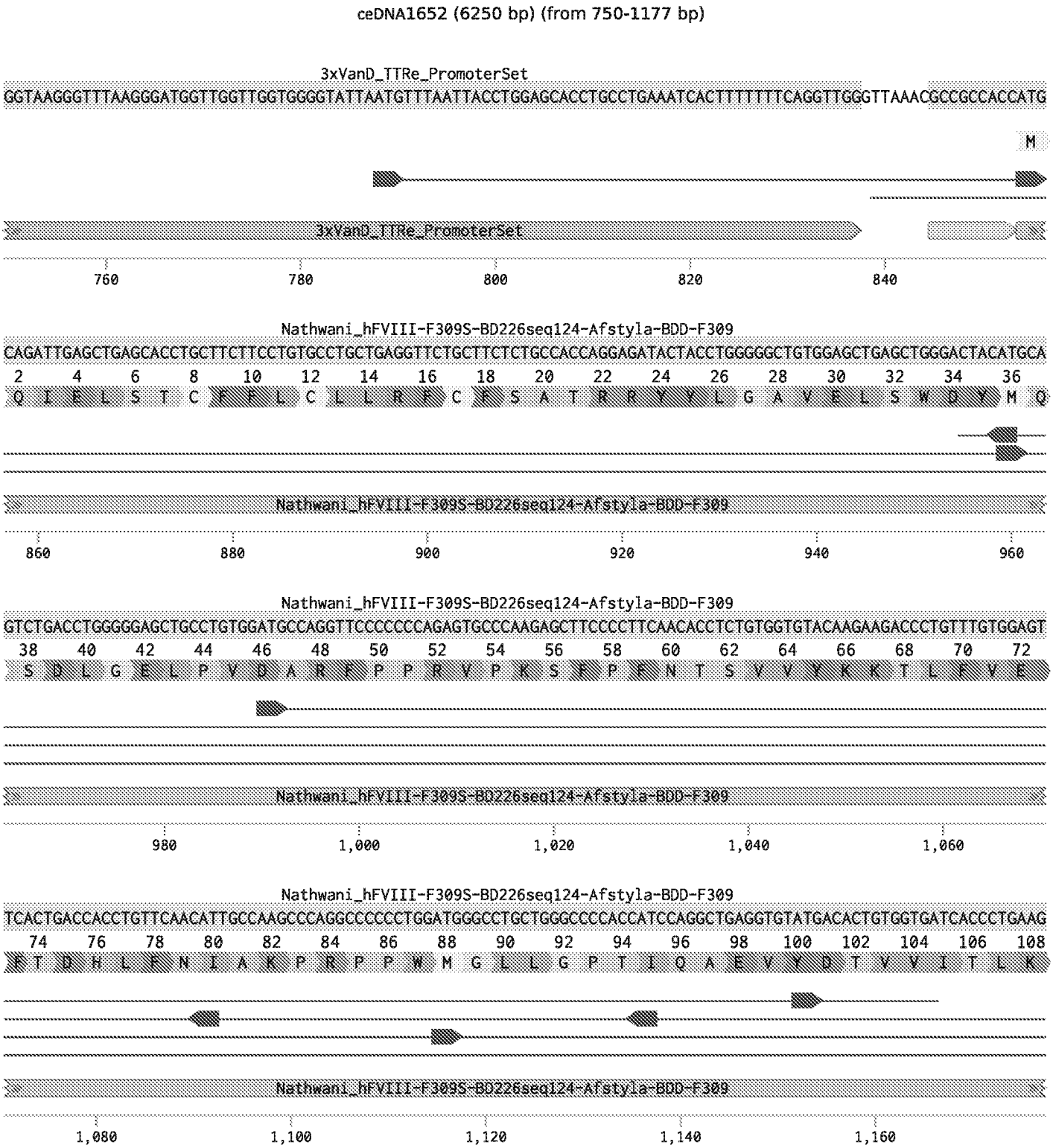
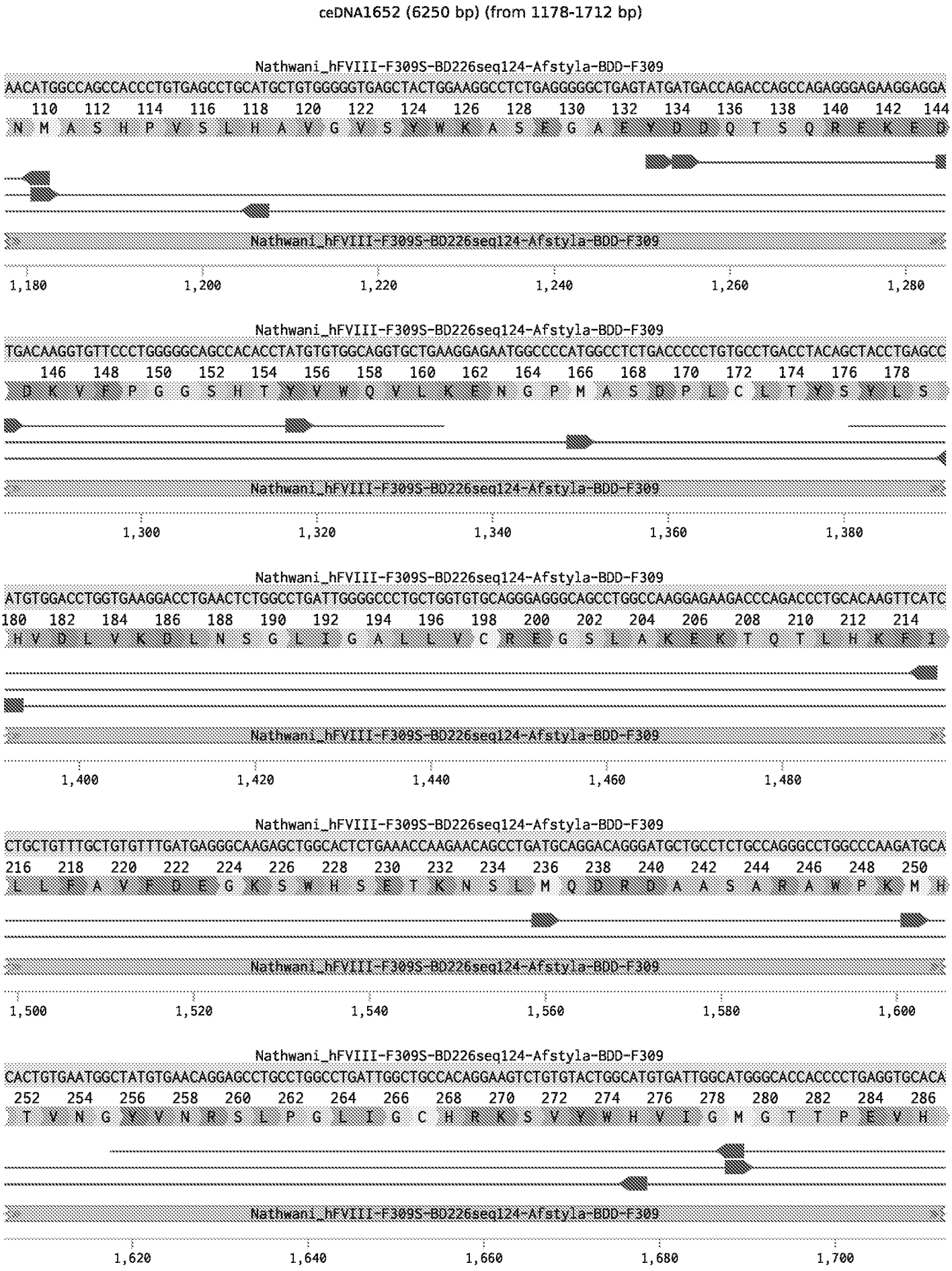
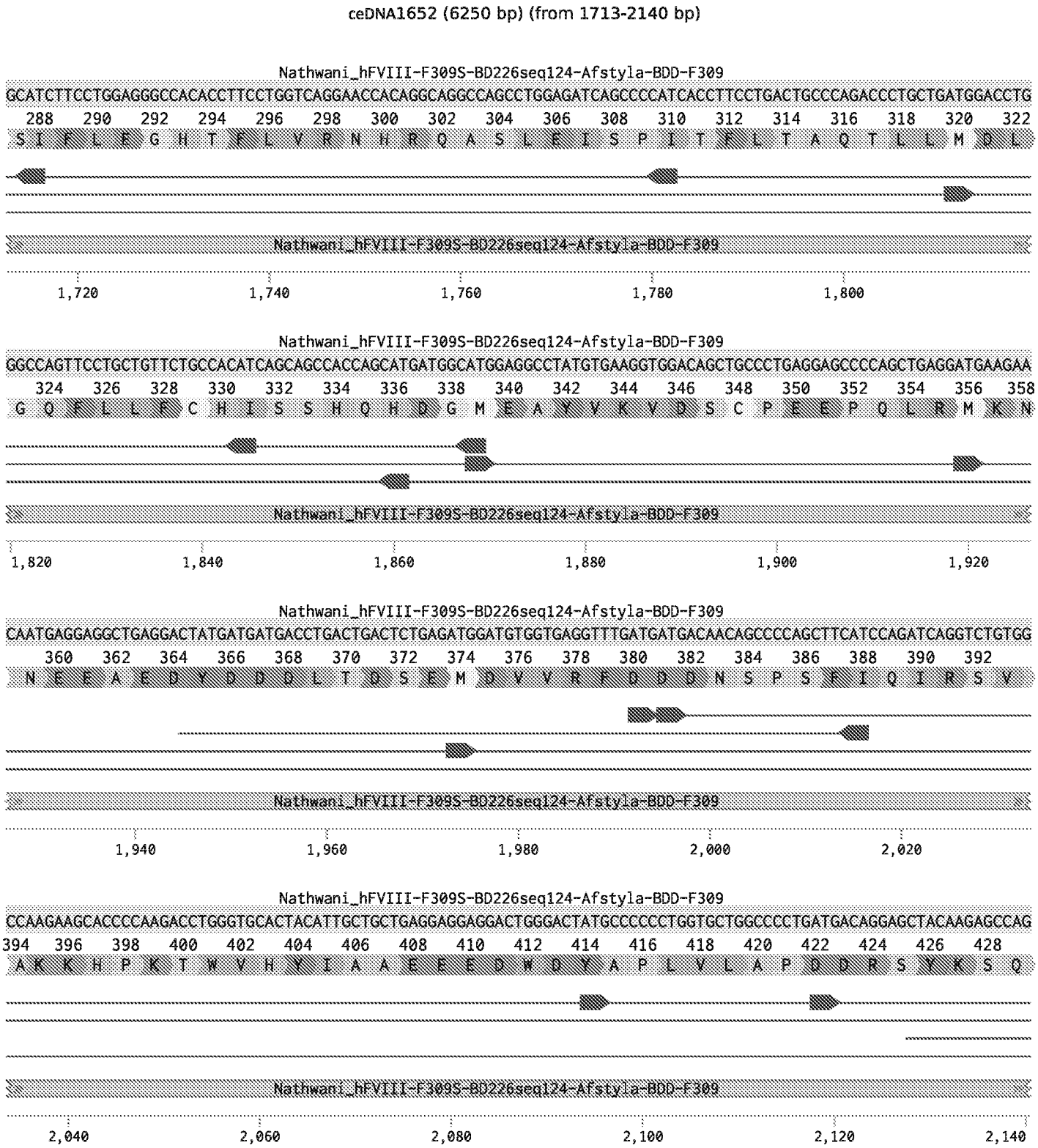

ceDNA1368 (6007 bp) (from 5779-6007 bp)
bGH
spacer_right-ITR_v1
AATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGCTCTAGAGCATGGCTACGTAGATAAGTAGCATGGCGGGTTAATCATTAACTACACCTGCAGGAGGAACCC
bGH
spacer_right-ITR_v1
5,780
5,800
5,820
5,840
5,860
5,880
right-ITR_v1
CTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGC
right-ITR_v1
5,900
5,920
5,940
5,960
5,980
right-ITR_v1
GCAGCTGCCTGCAGG
righ..._v1
6,000

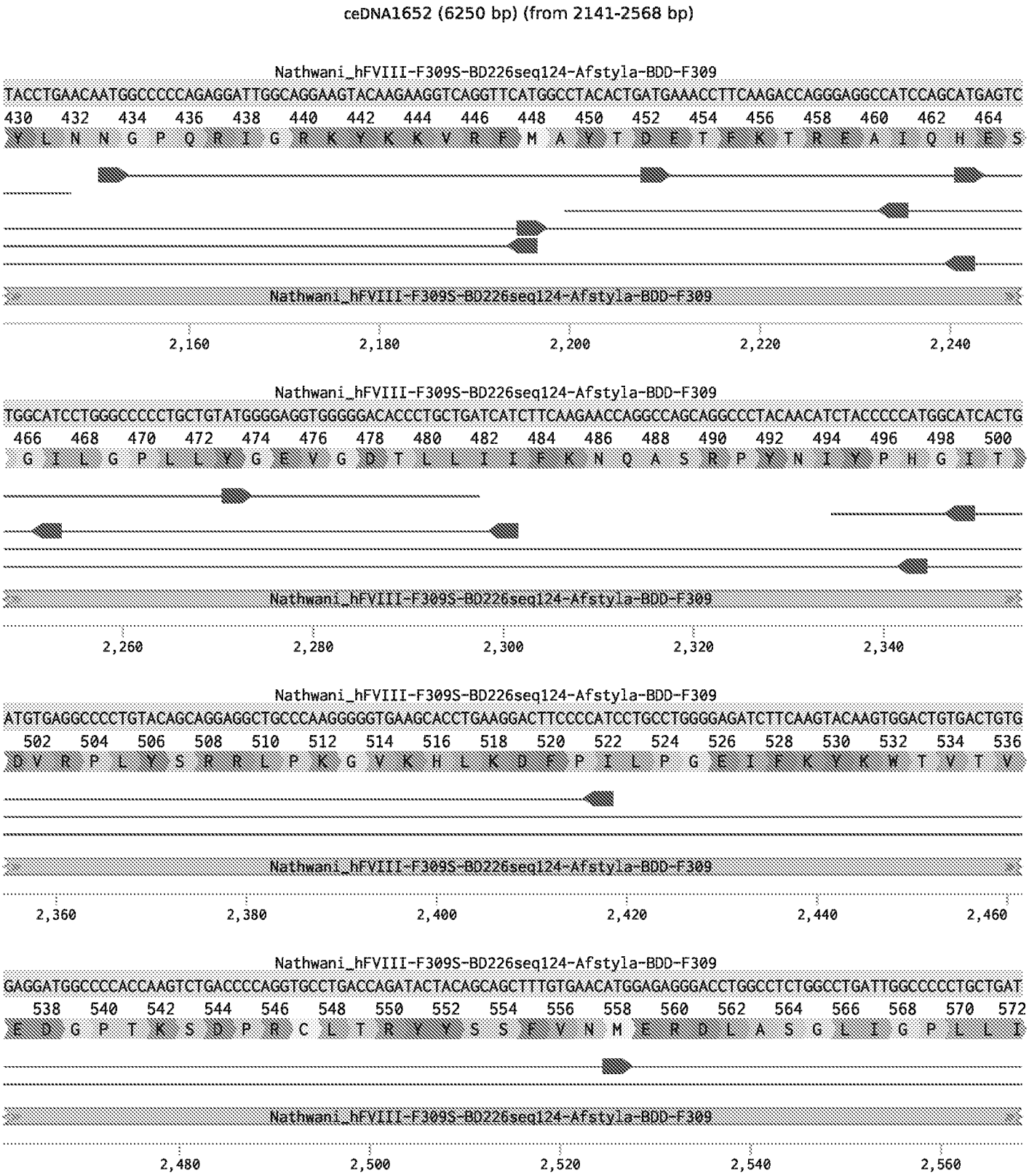
FIG. 6

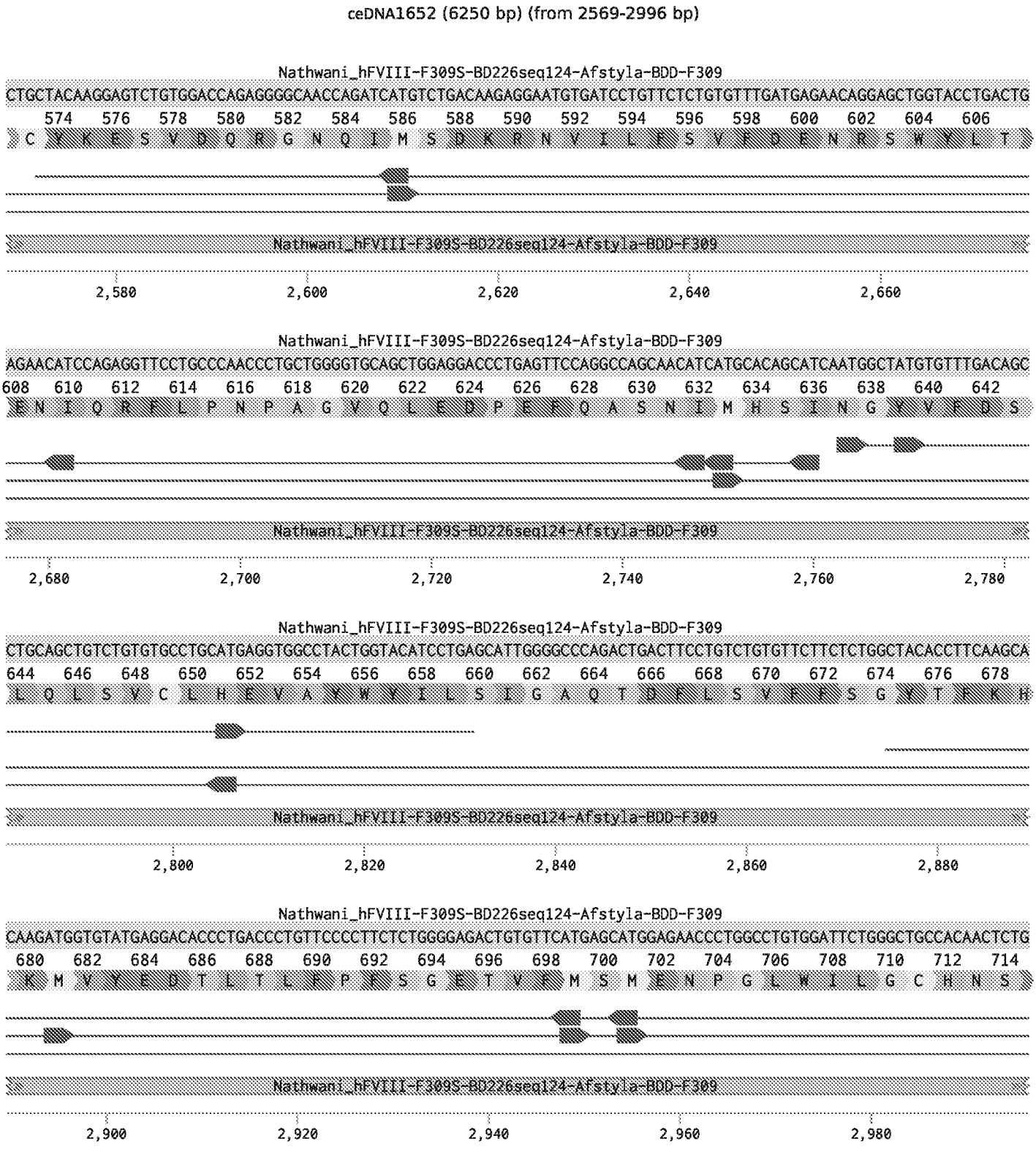

ceDNA1652 (6250 bp)
(from 1-749 bp)
left-ITR_v1
CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGA
left-ITR_v1
20
40
60
80
100
left-ITR_v1
spacer_left-ITR_v2.1
3xVanD_TTRe_PromoterSet
GAGGGAGTGGCCAACTCCATCACTAGGGGTTCCTTGTAGTTAATGATTAACCCGCCATGCTACTTATCGCGGCCGCGGGGGAGGCTGCTGGTGAATATTAACCAAGG
left-ITR_v1
spacer_left-ITR_v2.1
3xVanD_TTRe_PromoterSet
120
140
160
180
200
3xVanD_TTRe_PromoterSet
TCACCCCAGTTATCGGAGGAGCAAACAGGGGCTAAGTCCACCGGGGGAGGCTGCTGGTGAATATTAACCAAGGTCACCCCAGTTATCGGAGGAGCAAACAGGGGCTA
3xVanD_TTRe_PromoterSet
220
240
260
280
300
320
3xVanD_TTRe_PromoterSet
AGTCCACCGGGGGAGGCTGCTGGTGAATATTAACCAAGGTCACCCCAGTTATCGGAGGAGCAAACAGGGGCTAAGTCCACGGTACCCACTGGGAGGATGTTGAGTAA
3xVanD_TTRe_PromoterSet
340
360
380
400
420
3xVanD_TTRe_PromoterSet
GATGGAAAACTACTGATGACCCTTGCAGAGACAGAGTATTAGGACATGTTTGAACAGGGGCCGGGCGATCAGCAGGTAGCTCTAGAGGATCCCCGTCTGTCTGCACA
3xVanD_TTRe_PromoterSet
440
460
480
500
520
3xVanD_TTRe_PromoterSet
TTTCGTAGAGCGAGTGTTCCGATACTCTAATCTCCCTAGGCAAGGTTCATATTTGTGTAGGTTACTTATTCTCCTTTTGTTGACTAAGTCAATAATCAGAATCAGCA
3xVanD_TTRe_PromoterSet
540
560
580
600
620
640
3xVanD_TTRe_PromoterSet
GGTTTGGAGTCAGCTTGGCAGGGATCAGCAGCCTGGGTTGGAAGGAGGGGGTATAAAAGCCCCTTCACCAGGAGAAGCCGTCACACAGATCCACAAGCTCCTGAAGA
3xVanD_TTRe_PromoterSet
660
680
700
720
740

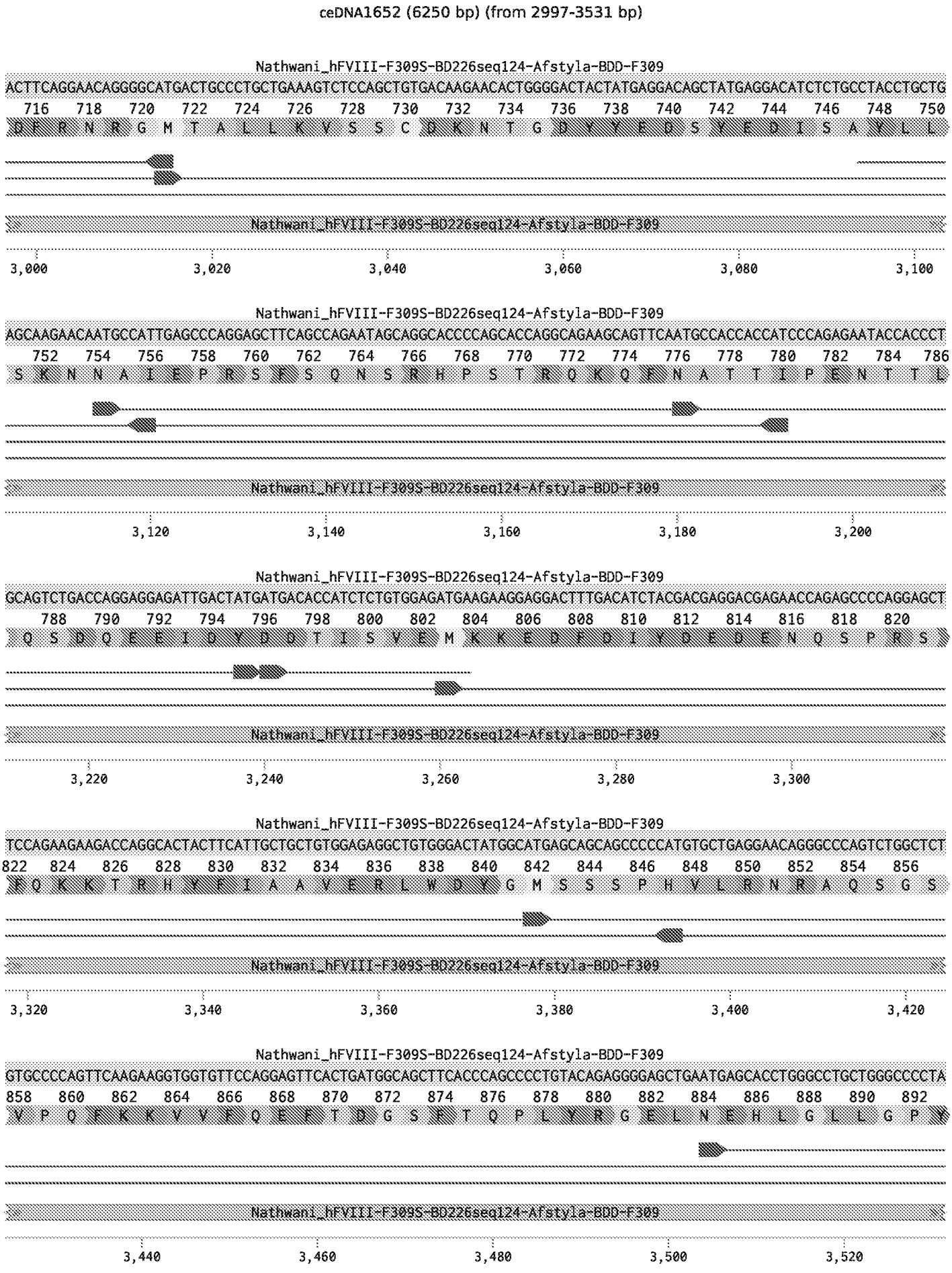

ceDNA1652 (6250 bp) (from 750-1177 bp)
3xVanD_TTRe_PromoterSet
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M
3xVanD_TTRe_PromoterSet
760 780 800 820 840
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
CAGATTGAGCTGAGCACCTGCTTCTTCCTGTGCCTGCTGAGGTTCTGCTTCTCTGCCACCAGGAGATACTACCTGGGGGCTGTGGAGCTGAGCTGGGACTACATGCA
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36
Q I E L S T C F F L C L L R F C F S A T R R Y Y L G A V E L S W D Y M Q
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
860 880 900 920 940 960
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
GTCTGACCTGGGGGAGCTGCCTGTGGATGCCAGGTTCCCCCCCAGAGTGCCCAAGAGCTTCCCCTTCAACACCTCTGTGGTGTACAAGAAGACCCTGTTTGTGGAGT
38 40 42 44 46 48 50 52 54 56 58 60 62 64 66 68 70 72
S D L G E L P V D A R F P P R V P K S F P F N T S V V Y K K T L F V E
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
980 1,000 1,020 1,040 1,060
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
TCACTGACCACCTGTTCAACATTGCCAAGCCCAGGCCCCCCTGGATGGGCCTGCTGGGCCCCACCATCCAGGCTGAGGTGTATGACACTGTGGTGATCACCCTGAAG
74 76 78 80 82 84 86 88 90 92 94 96 98 100 102 104 106 108
F T D H L F N I A K P R P P W M G L L G P T I Q A E V Y D T V V I T L K
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
1,080 1,100 1,120 1,140 1,160

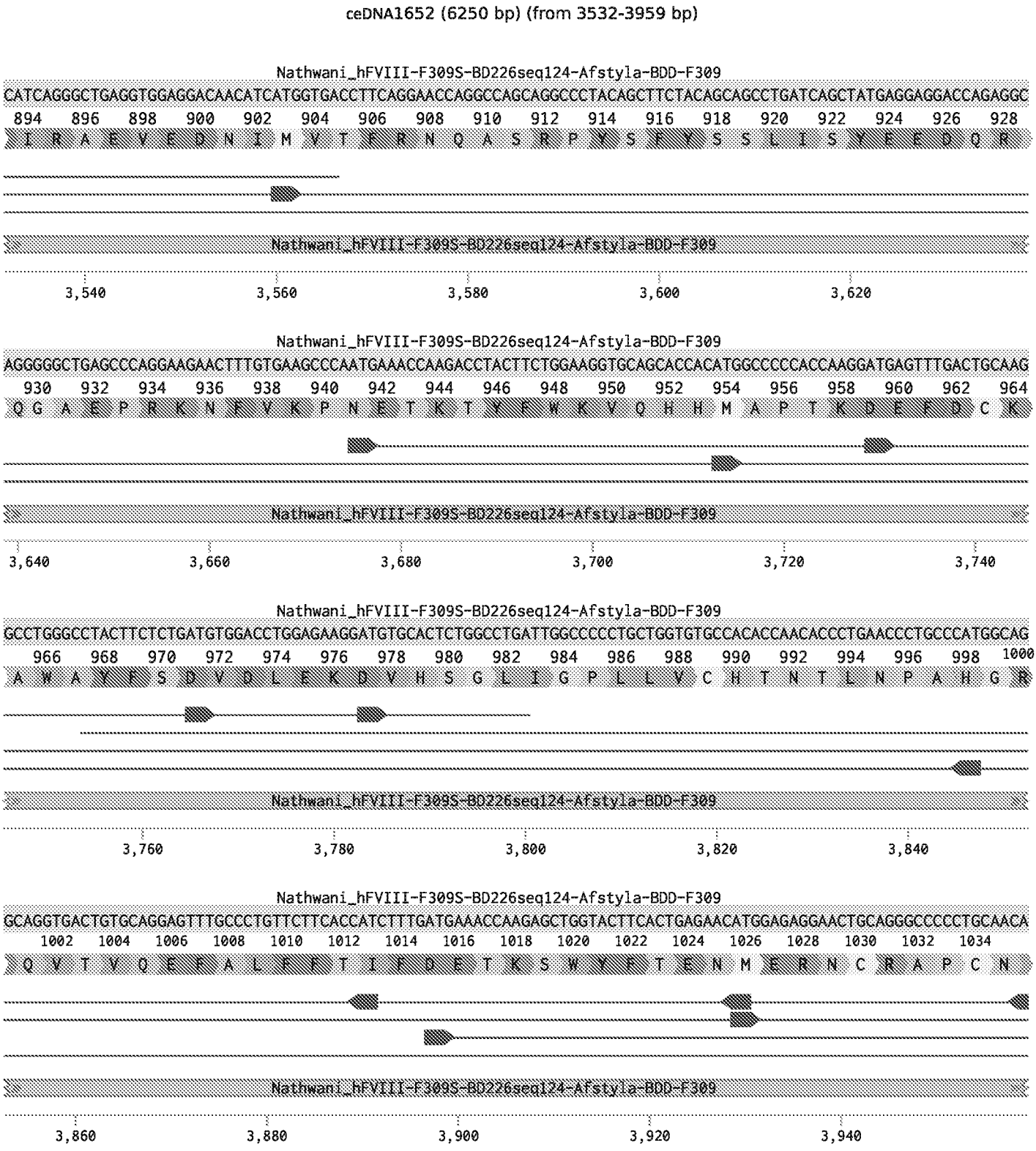

ceDNA1652 (6250 bp) (from 1178-1712 bp)
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110 112 114 116 118 120 122 124 126 128 130 132 134 136 138 140 142 144
N M A S H P V S L H A V G V S Y W K A S E G A E Y D D Q T S Q R E K E D
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
1,180 1,200 1,220 1,240 1,260 1,280
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
TGACAAGGTGTTCCCTGGGGGCAGCCACACCTATGTGTGGCAGGTGCTGAAGGAGAATGGCCCCATGGCCTCTGACCCCCTGTGCCTGACCTACAGCTACCTGAGCC
146 148 150 152 154 156 158 160 162 164 166 168 170 172 174 176 178
D K V F P G G S H T Y V W Q V L K E N G P M A S D P L C L T Y S Y L S
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
1,300 1,320 1,340 1,360 1,380
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
ATGTGGACCTGGTGAAGGACCTGAACTCTGGCCTGATTGGGGCCCTGCTGGTGTGCAGGGAGGGCAGCCTGGCCAAGGAGAAGACCCAGACCCTGCACAAGTTCATC
180 182 184 186 188 190 192 194 196 198 200 202 204 206 208 210 212 214
H V D L V K D L N S G L I G A L L V C R E G S L A K E K T Q T L H K F I
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
1,400 1,420 1,440 1,460 1,480
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
CTGCTGTTTGCTGTGTTTGATGAGGGCAAGAGCTGGCACTCTGAAACCAAGAACAGCCTGATGCAGGACAGGGATGCTGCCTCTGCCAGGGCCTGGCCCAAGATGCA
216 218 220 222 224 226 228 230 232 234 236 238 240 242 244 246 248 250
L L F A V F D E G K S W H S E T K N S L M Q D R D A A S A R A W P K M H
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
1,500 1,520 1,540 1,560 1,580 1,600
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
CACTGTGAATGGCTATGTGAACAGGAGCCTGCCTGGCCTGATTGGCTGCCACAGGAAGTCTGTGTACTGGCATGTGATTGGCATGGGCACCACCCCTGAGGTGCACA
252 254 256 258 260 262 264 266 268 270 272 274 276 278 280 282 284 286
T V N G Y V N R S L P G L I G C H R K S V Y W H V I G M G T T P E V H
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
1,620 1,640 1,660 1,680 1,700

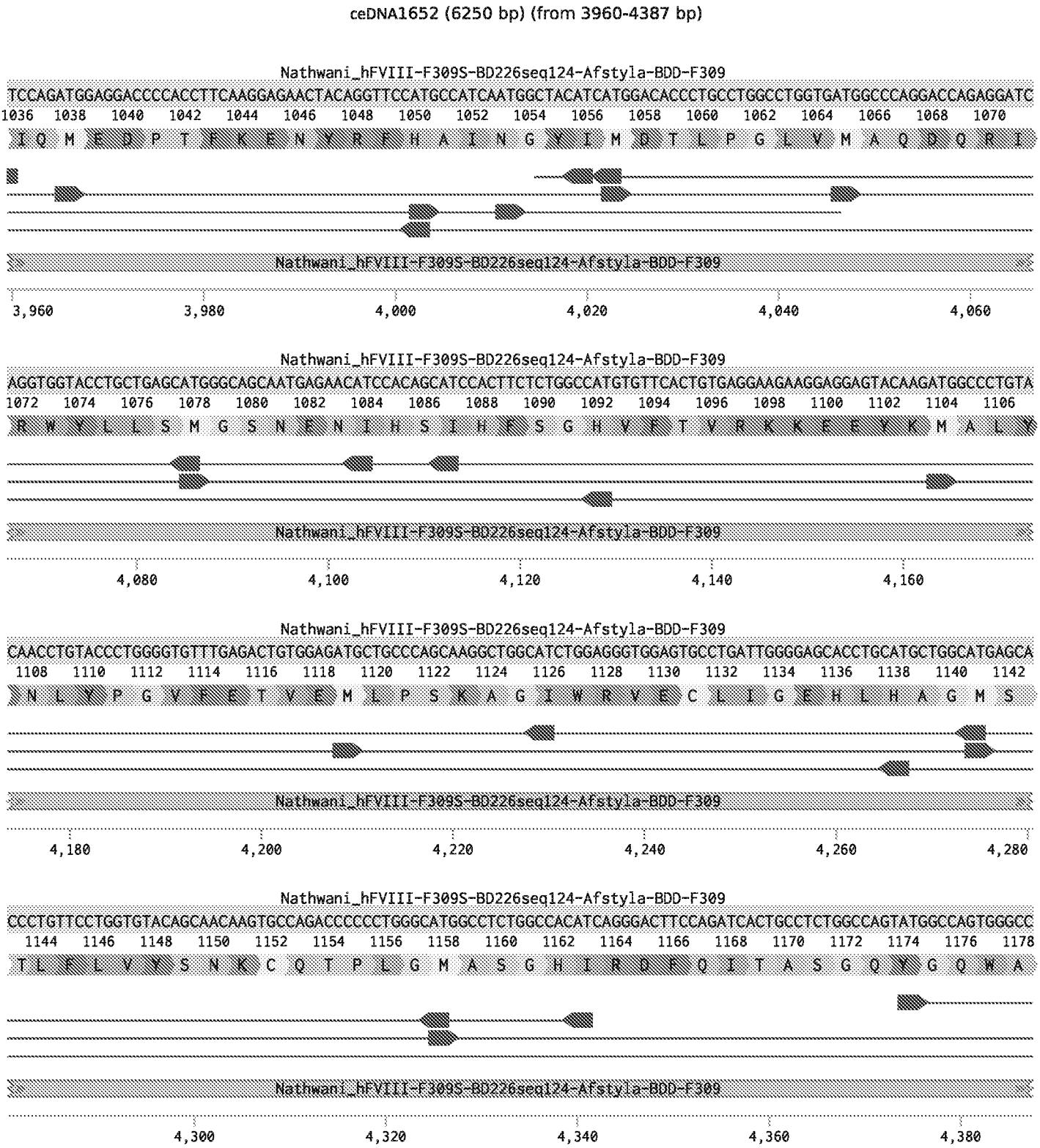

ceDNA1652 (6250 bp) (from 1713-2140 bp)
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288 290 292 294 296 298 300 302 304 306 308 310 312 314 316 318 320 322
S I F L E G H T F L V R N H R Q A S L E I S P I T F L T A Q T L L M D L
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
1,720 1,740 1,760 1,780 1,800
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
GGCCAGTTCCTGCTGTTCTGCCACATCAGCAGCCACCAGCATGATGGCATGGAGGCCTATGTGAAGGTGGACAGCTGCCCTGAGGAGCCCCAGCTGAGGATGAAGAA
324 326 328 330 332 334 336 338 340 342 344 346 348 350 352 354 356 358
G Q F L L F C H I S S H Q H D G M E A Y V K V D S C P E E P Q L R M K N
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
1,820 1,840 1,860 1,880 1,900 1,920
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
CAATGAGGAGGCTGAGGACTATGATGATGACCTGACTGACTCTGAGATGGATGTGGTGAGGTTTGATGATGACAACAGCCCCAGCTTCATCCAGATCAGGTCTGTGG
360 362 364 366 368 370 372 374 376 378 380 382 384 386 388 390 392
N E E A E D Y D D D L T D S E M D V V R F D D D N S P S F I Q I R S V
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
1,940 1,960 1,980 2,000 2,020
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
CCAAGAAGCACCCCAAGACCTGGGTGCACTACATTGCTGCTGAGGAGGAGGACTGGGACTATGCCCCCCTGGTGCTGGCCCCTGATGACAGGAGCTACAAGAGCCAG
394 396 398 400 402 404 406 408 410 412 414 416 418 420 422 424 426 428
A K K H P K T W V H Y I A A E E E D W D Y A P L V L A P D D R S Y K S Q
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
2,040 2,060 2,080 2,100 2,120 2,140


ceDNA1652 (6250 bp) (from 2141-2568 bp)
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
TACCTGAACAATGGCCCCCAGAGGATTGGCAGGAAGTACAAGAAGGTCAGGTTCATGGCCTACACTGATGAAACCTTCAAGACCAGGGAGGCCATCCAGCATGAGTC
430 432 434 436 438 440 442 444 446 448 450 452 454 456 458 460 462 464
Y L N N G P Q R I G R K Y K K V R F M A Y T D E T F K T R E A I Q H E S
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
2,160 2,180 2,200 2,220 2,240
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
TGGCATCCTGGGCCCCCTGCTGTATGGGGAGGTGGGGGACACCCTGCTGATCATCTTCAAGAACCAGGCCAGCAGGCCCTACAACATCTACCCCCATGGCATCACTG
466 468 470 472 474 476 478 480 482 484 486 488 490 492 494 496 498 500
G I L G P L L Y G E V G D T L L I I F K N Q A S R P Y N I Y P H G I T
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
2,260 2,280 2,300 2,320 2,340
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
ATGTGAGGCCCCTGTACAGCAGGAGGCTGCCCAAGGGGGTGAAGCACCTGAAGGACTTCCCCATCCTGCCTGGGGAGATCTTCAAGTACAAGTGGACTGTGACTGTG
502 504 506 508 510 512 514 516 518 520 522 524 526 528 530 532 534 536
D V R P L Y S R R L P K G V K H L K D F P I L P G E I F K Y K W T V T V
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
2,360 2,380 2,400 2,420 2,440 2,460
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
GAGGATGGCCCCACCAAGTCTGACCCCAGGTGCCTGACCAGATACTACAGCAGCTTTGTGAACATGGAGAGGGACCTGGCCTCTGGCCTGATTGGCCCCCTGCTGAT
538 540 542 544 546 548 550 552 554 556 558 560 562 564 566 568 570 572
E D G P T K S D P R C L T R Y Y S S F V N M E R D L A S G L I G P L L I
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
2,480 2,500 2,520 2,540 2,560

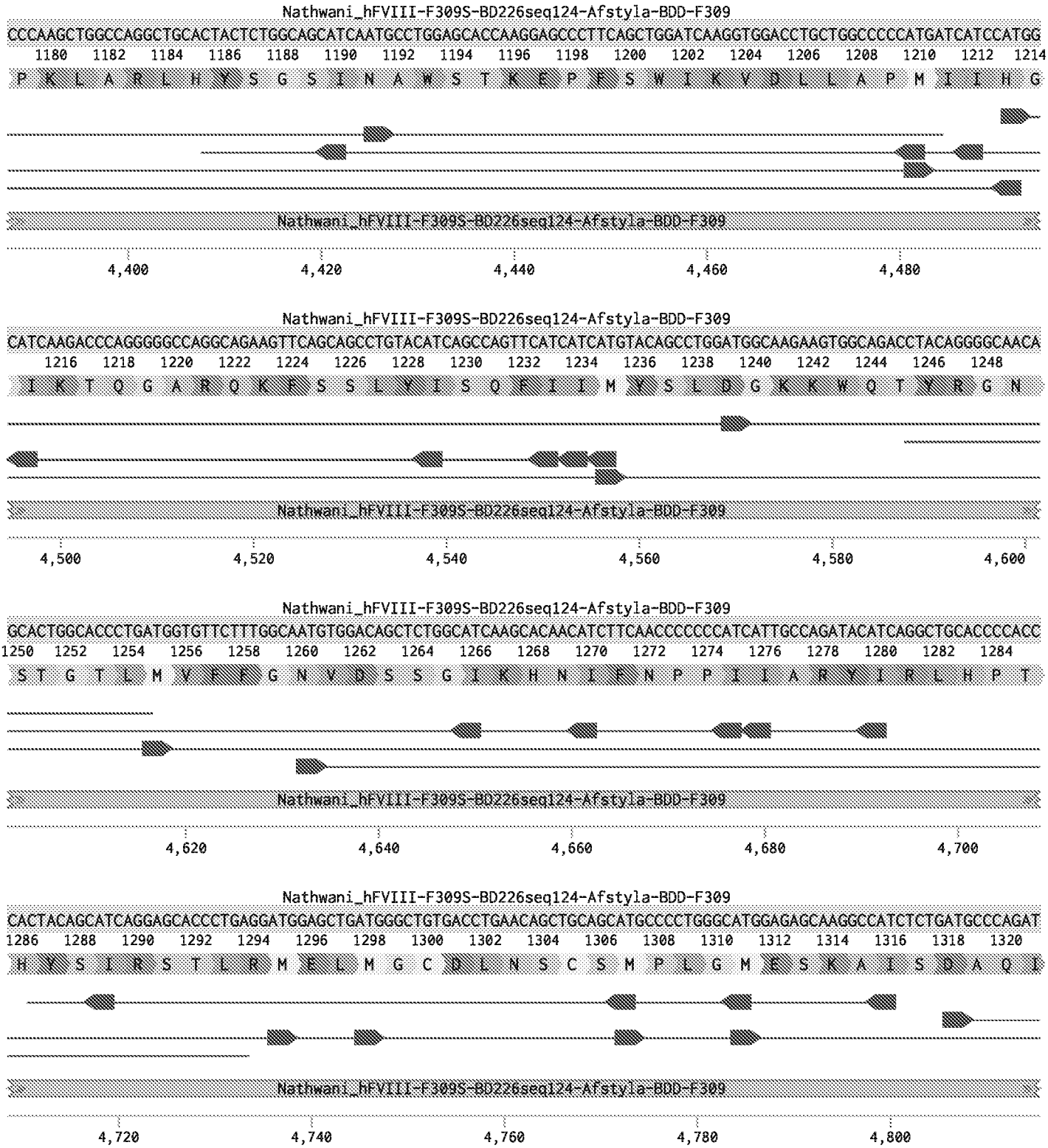

ceDNA1652 (6250 bp) (from 2569-2996 bp)
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574 576 578 580 582 584 586 588 590 592 594 596 598 600 602 604 606
C Y K E S V D Q R G N Q I M S D K R N V I L F S V F D E N R S W Y L T
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
2,580 2,600 2,620 2,640 2,660
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
AGAACATCCAGAGGTTCCTGCCCAACCCTGCTGGGGTGCAGCTGGAGGACCCTGAGTTCCAGGCCAGCAACATCATGCACAGCATCAATGGCTATGTGTTTGACAGC
608 610 612 614 616 618 620 622 624 626 628 630 632 634 636 638 640 642
E N I Q R F L P N P A G V Q L E D P E F Q A S N I M H S I N G Y V F D S
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
2,680 2,700 2,720 2,740 2,760 2,780
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
CTGCAGCTGTCTGTGTGCCTGCATGAGGTGGCCTACTGGTACATCCTGAGCATTGGGGCCCAGACTGACTTCCTGTCTGTGTTCTTCTCTGGCTACACCTTCAAGCA
644 646 648 650 652 654 656 658 660 662 664 666 668 670 672 674 676 678
L Q L S V C L H E V A Y W Y I L S I G A Q T D F L S V F F S G Y T F K H
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
2,800 2,820 2,840 2,860 2,880
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
CAAGATGGTGTATGAGGACACCCTGACCCTGTTCCCCTTCTCTGGGGAGACTGTGTTCATGAGCATGGAGAACCCTGGCCTGTGGATTCTGGGCTGCCACAACTCTG
680 682 684 686 688 690 692 694 696 698 700 702 704 706 708 710 712 714
K M V Y E D T L T L F P F S G E T V F M S M E N P G L W I L G C H N S
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
2,900 2,920 2,940 2,960 2,980


ceDNA1652 (6250 bp) (from 2997-3531 bp)
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
ACTTCAGGAACAGGGGCATGACTGCCCTGCTGAAAGTCTCCAGCTGTGACAAGAACACTGGGGACTACTATGAGGACAGCTATGAGGACATCTCTGCCTACCTGCTG
716 718 720 722 724 726 728 730 732 734 736 738 740 742 744 746 748 750
D F R N R G M T A L L K V S S C D K N T G D Y Y E D S Y E D I S A Y L L
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
3,000 3,020 3,040 3,060 3,080 3,100
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
AGCAAGAACAATGCCATTGAGCCCAGGAGCTTCAGCCAGAATAGCAGGCACCCCAGCACCAGGCAGAAGCAGTTCAATGCCACCACCATCCCAGAGAATACCACCCT
752 754 756 758 760 762 764 766 768 770 772 774 776 778 780 782 784 786
S K N N A I E P R S F S Q N S R H P S T R Q K Q F N A T T I P E N T T L
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
3,120 3,140 3,160 3,180 3,200
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
GCAGTCTGACCAGGAGGAGATTGACTATGATGACACCATCTCTGTGGAGATGAAGAAGGAGGACTTTGACATCTACGACGAGGACGAGAACCAGAGCCCCAGGAGCT
788 790 792 794 796 798 800 802 804 806 808 810 812 814 816 818 820
Q S D Q E E I D Y D D T I S V E M K K E D F D I Y D E D E N Q S P R S
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
3,220 3,240 3,260 3,280 3,300
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
TCCAGAAGAAGACCAGGCACTACTTCATTGCTGCTGTGGAGAGGCTGTGGGACTATGGCATGAGCAGCAGCCCCCATGTGCTGAGGAACAGGGCCCAGTCTGGCTCT
822 824 826 828 830 832 834 836 838 840 842 844 846 848 850 852 854 856
F Q K K T R H Y F I A A V E R L W D Y G M S S S P H V L R N R A Q S G S
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
3,320 3,340 3,360 3,380 3,400 3,420
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
GTGCCCCAGTTCAAGAAGGTGGTGTTCCAGGAGTTCACTGATGGCAGCTTCACCCAGCCCCTGTACAGAGGGGAGCTGAATGAGCACCTGGGCCTGCTGGGCCCCTA
858 860 862 864 866 868 870 872 874 876 878 880 882 884 886 888 890 892
V P Q F K K V V F Q E F T D G S F T Q P L Y R G E L N E H L G L L G P Y
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
3,440 3,460 3,480 3,500 3,520

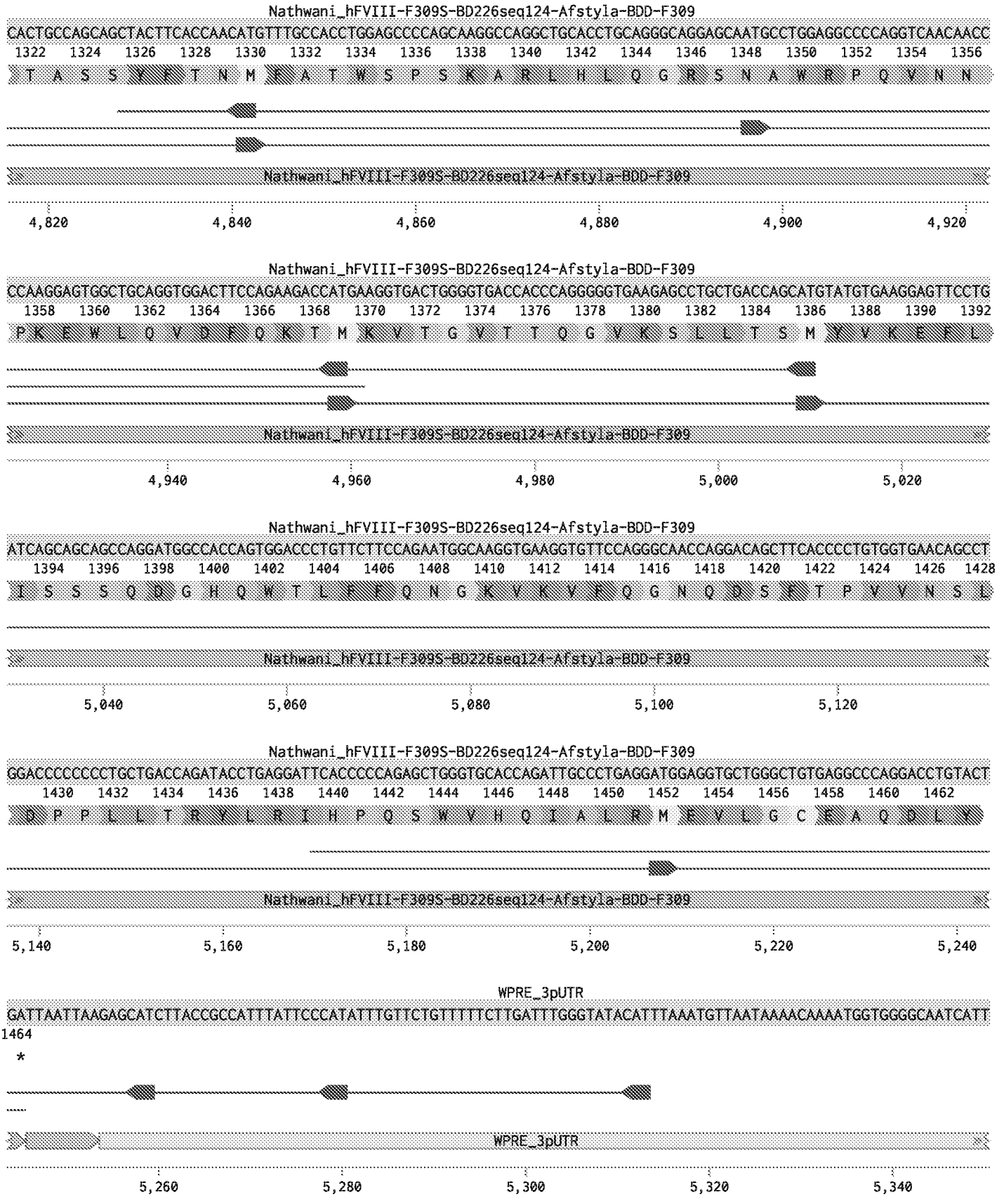

ceDNA1652 (6250 bp) (from 3532-3959 bp)
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894 896 898 900 902 904 906 908 910 912 914 916 918 920 922 924 926 928
I R A E V E D N I M V T F R N Q A S R P Y S F Y S S L I S Y E E D Q R
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
3,540 3,560 3,580 3,600 3,620
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
AGGGGGCTGAGCCCAGGAAGAACTTTGTGAAGCCCAATGAAACCAAGACCTACTTCTGGAAGGTGCAGCACCACATGGCCCCCACCAAGGATGAGTTTGACTGCAAG
930 932 934 936 938 940 942 944 946 948 950 952 954 956 958 960 962 964
Q G A E P R K N F V K P N E T K T Y F W K V Q H H M A P T K D E F D C K
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
3,640 3,660 3,680 3,700 3,720 3,740
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
GCCTGGGCCTACTTCTCTGATGTGGACCTGGAGAAGGATGTGCACTCTGGCCTGATTGGCCCCCTGCTGGTGTGCCACACCAACACCCTGAACCCTGCCCATGGCAG
966 968 970 972 974 976 978 980 982 984 986 988 990 992 994 996 998 1000
A W A Y F S D V D L E K D V H S G L I G P L L V C H T N T L N P A H G R
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
3,760 3,780 3,800 3,820 3,840
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
GCAGGTGACTGTGCAGGAGTTTGCCCTGTTCTTCACCATCTTTGATGAAACCAAGAGCTGGTACTTCACTGAGAACATGGAGAGGAACTGCAGGGCCCCCTGCAACA
1002 1004 1006 1008 1010 1012 1014 1016 1018 1020 1022 1024 1026 1028 1030 1032 1034
Q V T V Q E F A L F F T I F D E T K S W Y F T E N M E R N C R A P C N
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
3,860 3,880 3,900 3,920 3,940

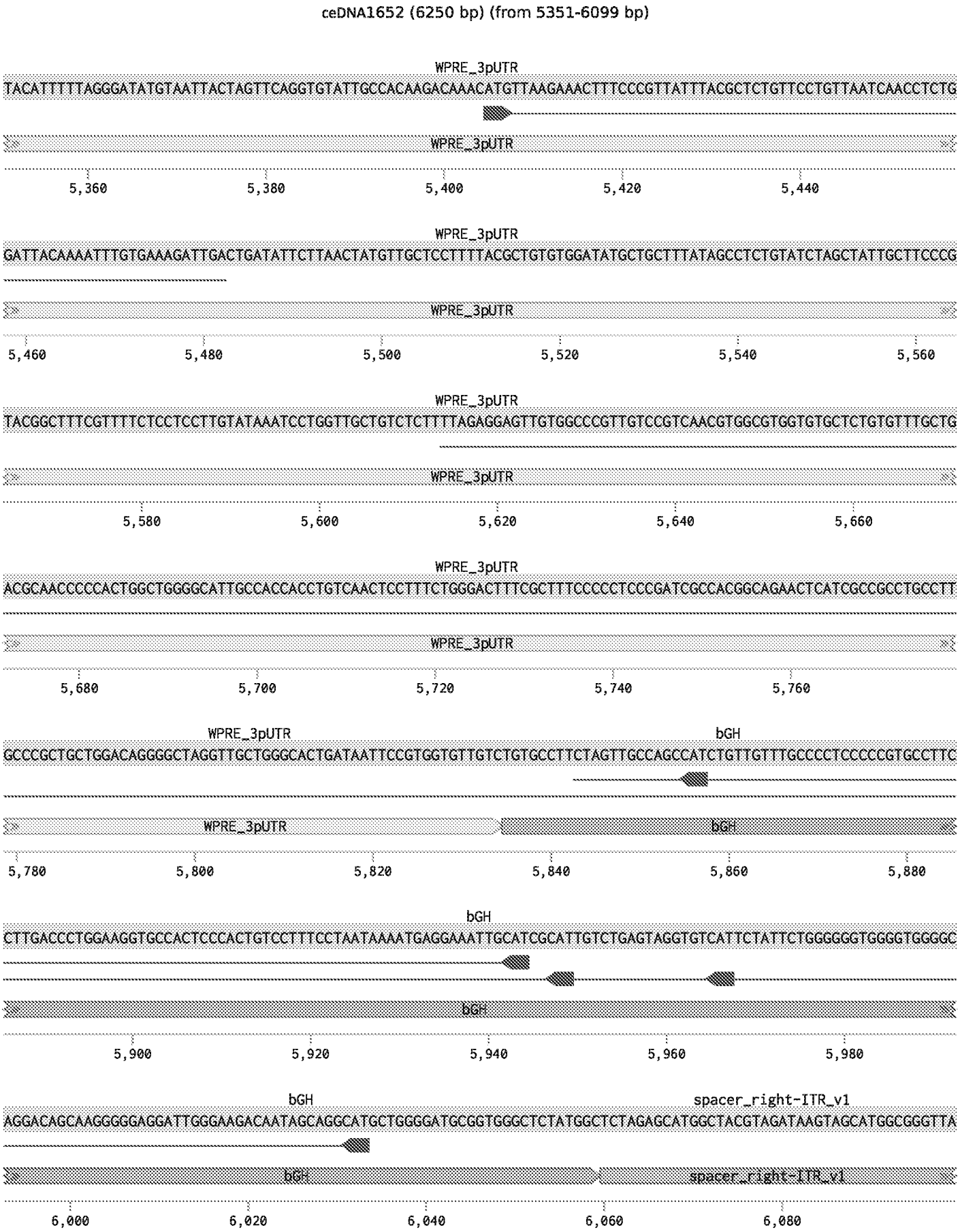

ceDNA1652 (6250 bp) (from 3960-4387 bp)
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
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1036 1038 1040 1042 1044 1046 1048 1050 1052 1054 1056 1058 1060 1062 1064 1066 1068 1070
I Q M E D P T F K E N Y R F H A I N G Y I M D T L P G L V M A Q D Q R I
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
3,960 3,980 4,000 4,020 4,040 4,060
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
AGGTGGTACCTGCTGAGCATGGGCAGCAATGAGAACATCCACAGCATCCACTTCTCTGGCCATGTGTTCACTGTGAGGAAGAAGGAGGAGTACAAGATGGCCCTGTA
1072 1074 1076 1078 1080 1082 1084 1086 1088 1090 1092 1094 1096 1098 1100 1102 1104 1106
R W Y L L S M G S N E N I H S I H F S G H V F T V R K K E E Y K M A L Y
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
4,080 4,100 4,120 4,140 4,160
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
CAACCTGTACCCTGGGGTGTTTGAGACTGTGGAGATGCTGCCCAGCAAGGCTGGCATCTGGAGGGTGGAGTGCCTGATTGGGGAGCACCTGCATGCTGGCATGAGCA
1108 1110 1112 1114 1116 1118 1120 1122 1124 1126 1128 1130 1132 1134 1136 1138 1140 1142
N L Y P G V F E T V E M L P S K A G I W R V E C L I G E H L H A G M S
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
4,180 4,200 4,220 4,240 4,260 4,280
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
CCCTGTTCCTGGTGTACAGCAACAAGTGCCAGACCCCCCTGGGCATGGCCTCTGGCCACATCAGGGACTTCCAGATCACTGCCTCTGGCCAGTATGGCCAGTGGGCC
1144 1146 1148 1150 1152 1154 1156 1158 1160 1162 1164 1166 1168 1170 1172 1174 1176 1178
T L F L V Y S N K C Q T P L G M A S G H I R D F Q I T A S G Q Y G Q W A
Nathwani_hFVIII-F309S-BD226seq124-Afstyla-BDD-F309
4,300 4,320 4,340 4,360 4,380

ceDNA1652 (6250 bp) (from 4388-4815 bp)



ceDNA1652 (6250 bp) (from 4816-5350 bp)




ceDNA1652 (6250 bp) (from 5351-6099 bp)
WPRE_3pUTR
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WPRE_3pUTR
5,360
5,380
5,400
5,420
5,440
WPRE_3pUTR
GATTACAAAATTTGTGAAAGATTGACTGATATTCTTAACTATGTTGCTCCTTTTACGCTGTGTGGATATGCTGCTTTATAGCCTCTGTATCTAGCTATTGCTTCCCG
WPRE_3pUTR
5,460
5,480
5,500
5,520
5,540
5,560
WPRE_3pUTR
TACGGCTTTCGTTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTCTTTTAGAGGAGTTGTGGCCCGTTGTCCGTCAACGTGGCGTGGTGTGCTCTGTGTTTGCTG
WPRE_3pUTR
5,580
5,600
5,620
5,640
5,660
WPRE_3pUTR
ACGCAACCCCCACTGGCTGGGGCATTGCCACCACCTGTCAACTCCTTTCTGGGACTTTCGCTTTCCCCCTCCCGATCGCCACGGCAGAACTCATCGCCGCCTGCCTT
WPRE_3pUTR
5,680
5,700
5,720
5,740
5,760
WPRE_3pUTR
bGH
GCCCGCTGCTGGACAGGGGCTAGGTTGCTGGGCACTGATAATTCCGTGGTGTTGTCTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTC
WPRE_3pUTR
bGH
5,780
5,800
5,820
5,840
5,860
5,880
bGH
CTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGC
bGH
5,900
5,920
5,940
5,960
5,980
bGH
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bGH
spacer_right-ITR_v1
6,000
6,020
6,040
6,060
6,080

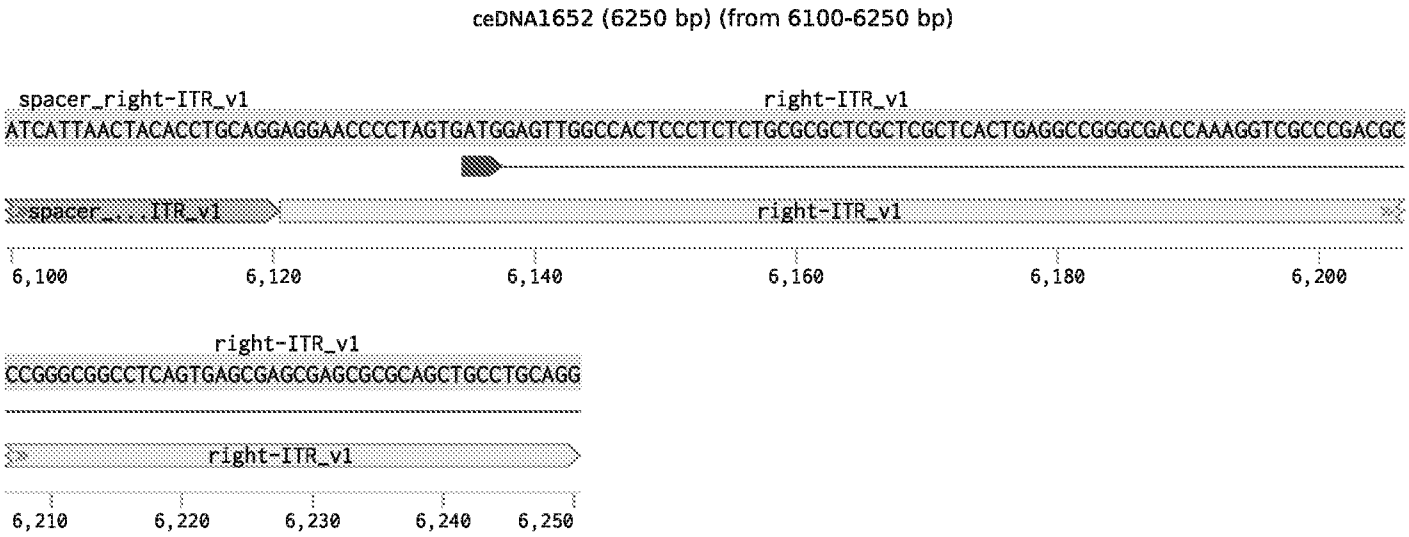
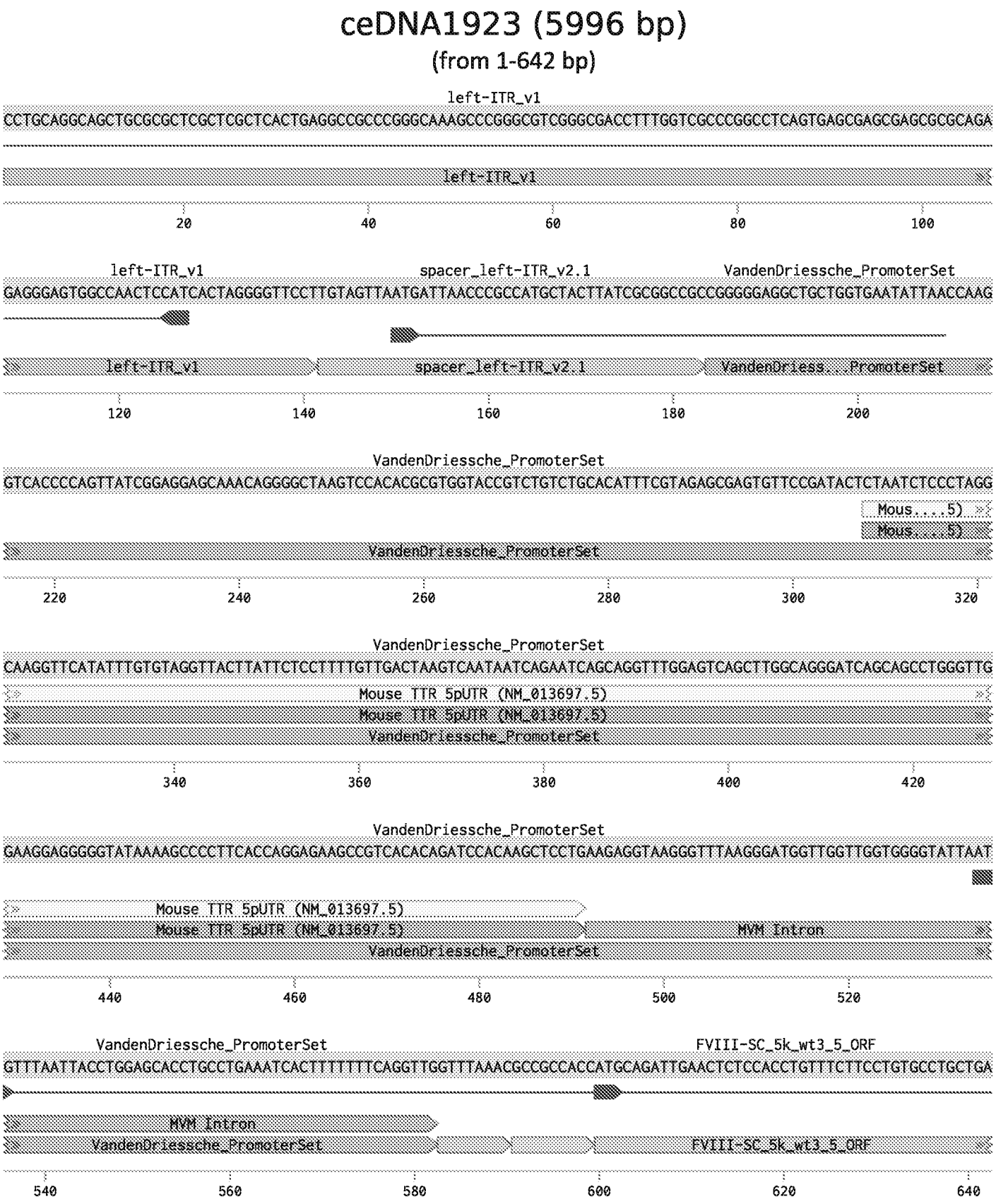
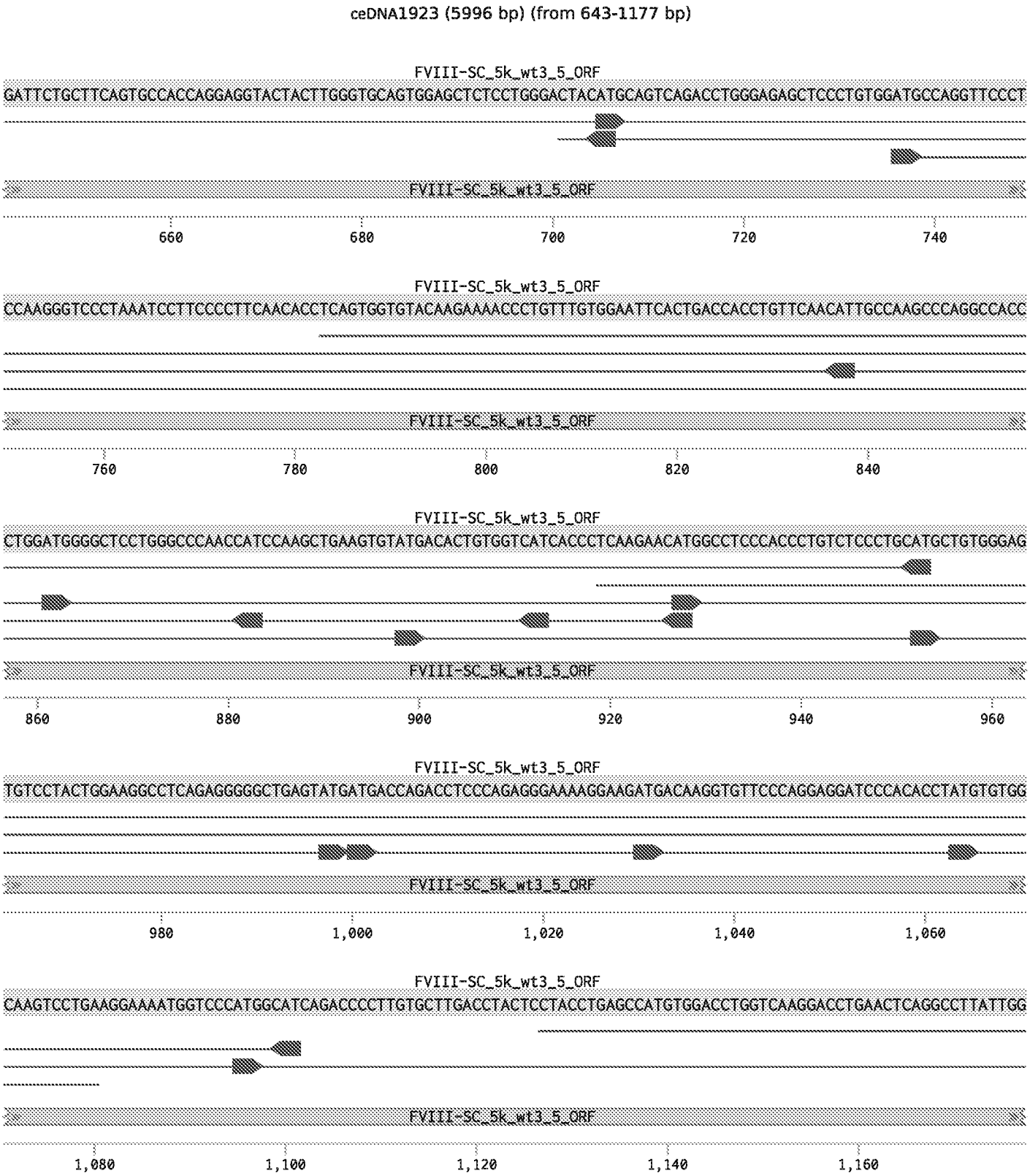
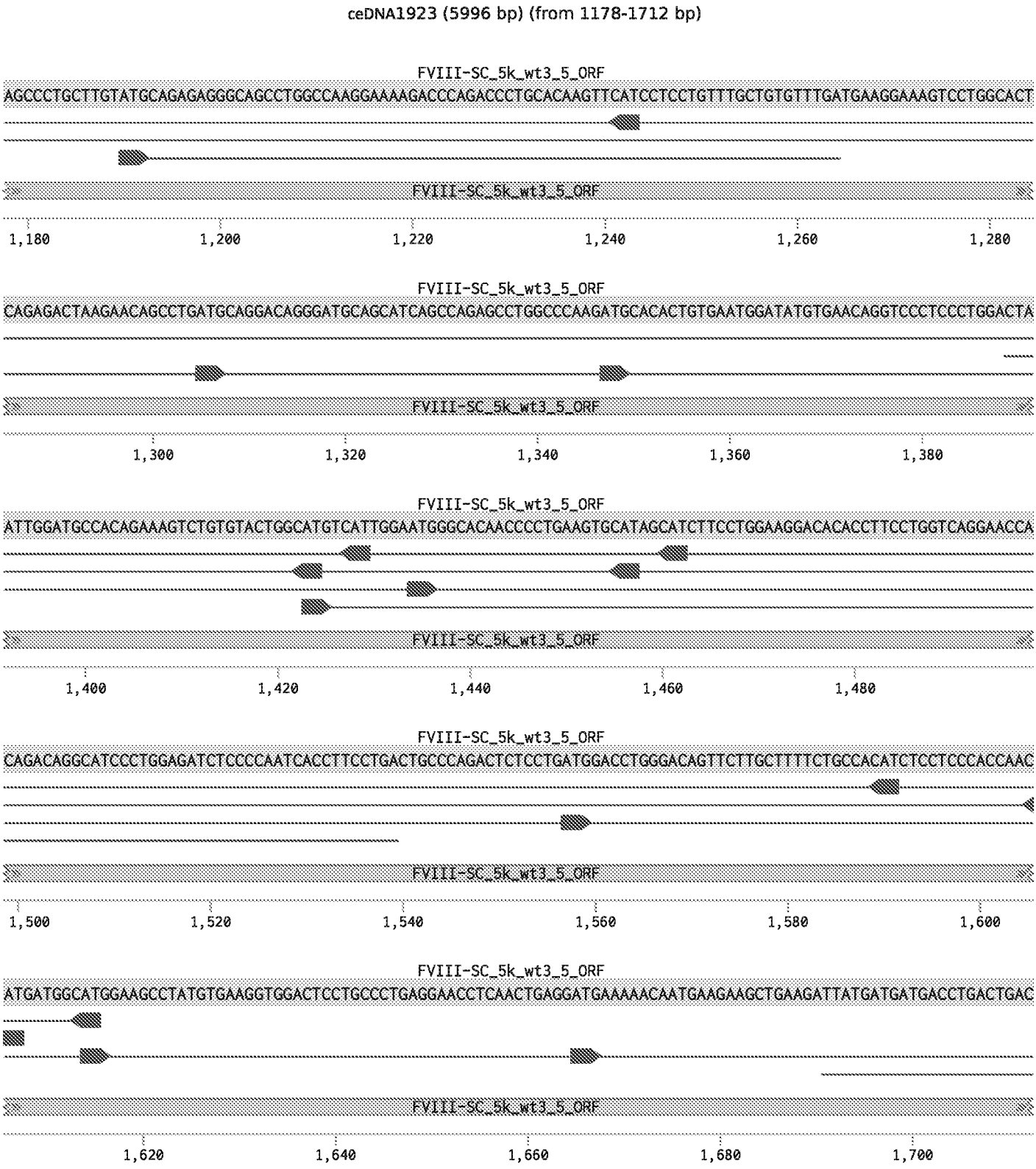
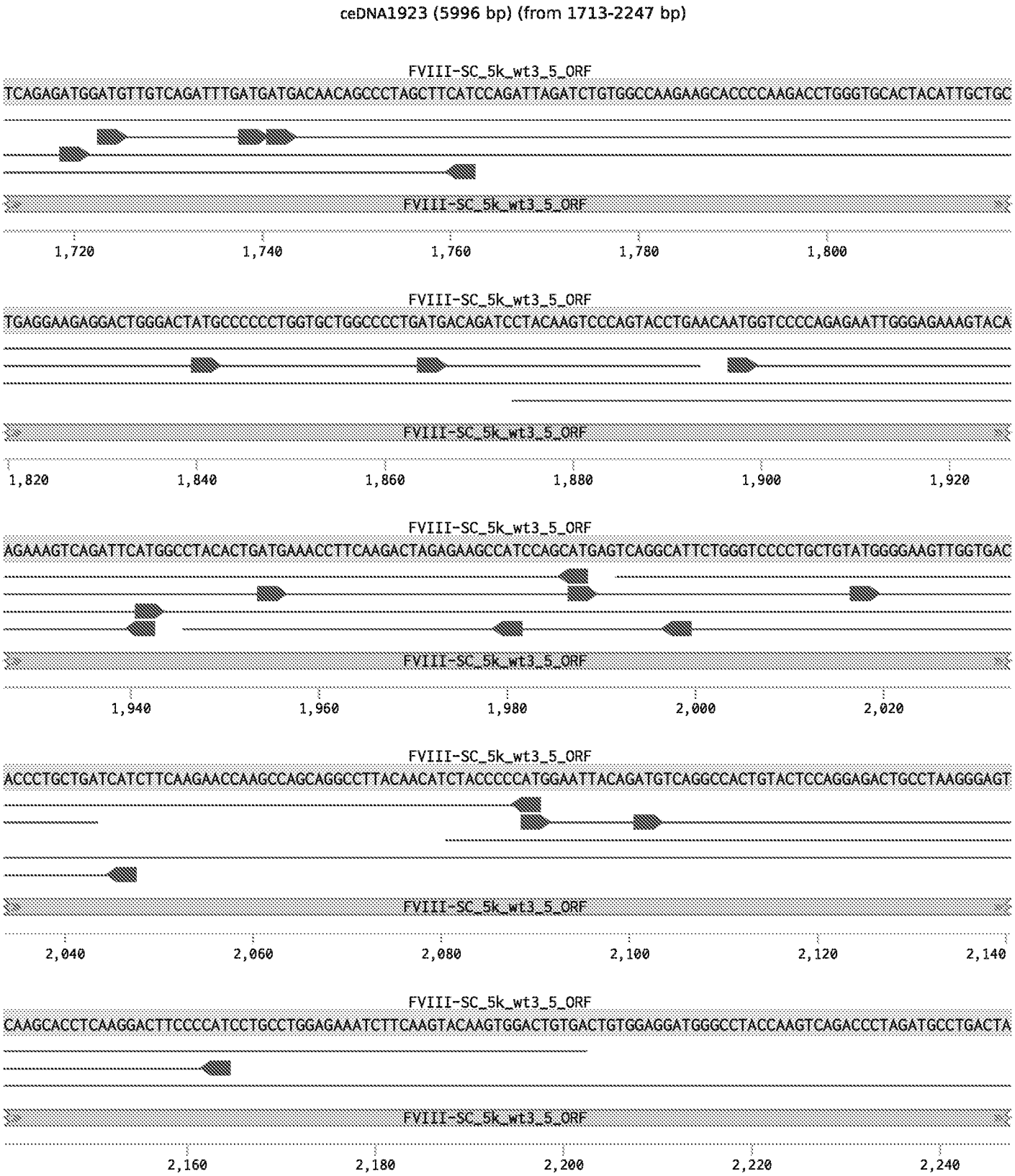

ceDNA1652 (6250 bp) (from 6100-6250 bp)
spacer_right-ITR_v1
right-ITR_v1
ATCATTAACTACACCTGCAGGAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGC
spacer_...ITR_v1
right-ITR_v1
6,100
6,120
6,140
6,160
6,180
6,200
right-ITR_v1
CCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGCTGCCTGCAGG
right-ITR_v1
6,210
6,220
6,230
6,240
6,250

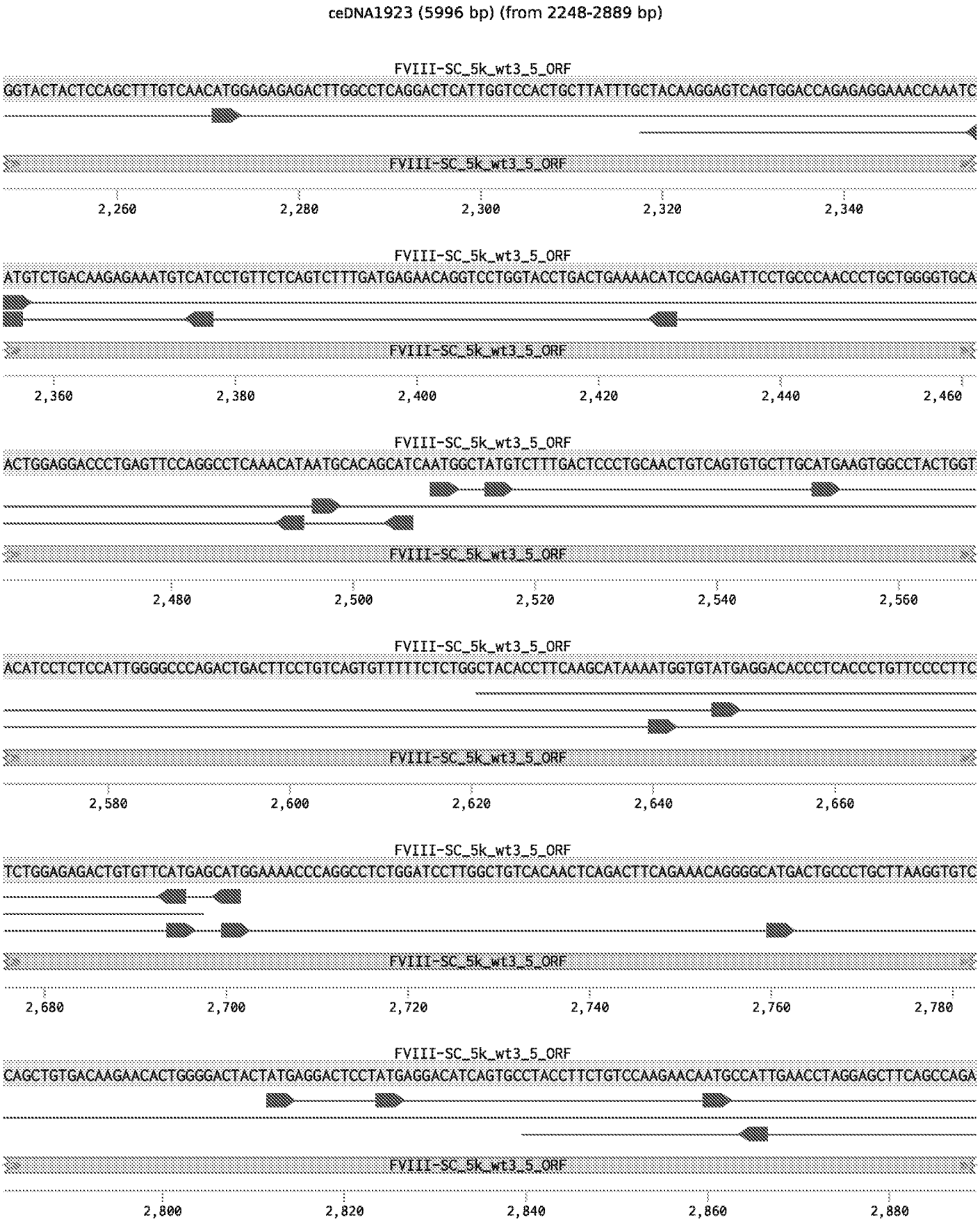
FIG. 7

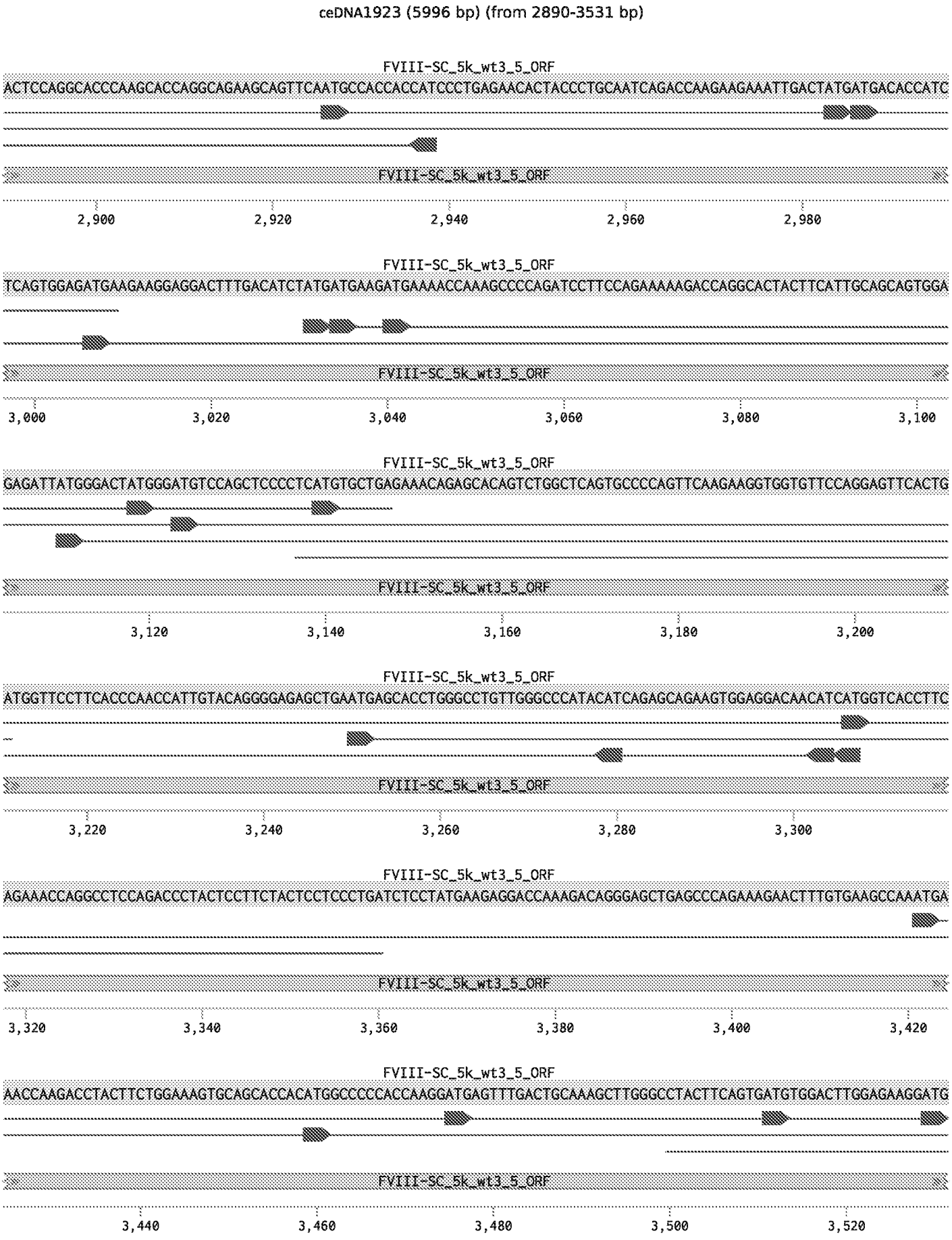

ceDNA1923 (5996 bp)
(from 1-642 bp)
left-ITR_v1
CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGA
left-ITR_v1
20
40
60
80
100
left-ITR_v1
spacer_left-ITR_v2.1
VandenDriessche_PromoterSet
GAGGGAGTGGCCAACTCCATCACTAGGGGTTCCTTGTAGTTAATGATTAACCCGCCATGCTACTTATCGCGGCCGCCGGGGGAGGCTGCTGGTGAATATTAACCAAG
left-ITR_v1
spacer_left-ITR_v2.1
VandenDriess...PromoterSet
120
140
160
180
200
VandenDriessche_PromoterSet
GTCACCCCAGTTATCGGAGGAGCAAACAGGGGCTAAGTCCACACGCGTGGTACCGTCTGTCTGCACATTTCGTAGAGCGAGTGTTCCGATACTCTAATCTCCCTAGG
Mous....5)
Mous....5)
VandenDriessche_PromoterSet
220
240
260
280
300
320
VandenDriessche_PromoterSet
CAAGGTTCATATTTGTGTAGGTTACTTATTCTCCTTTTGTTGACTAAGTCAATAATCAGAATCAGCAGGTTTGGAGTCAGCTTGGCAGGGATCAGCAGCCTGGGTTG
Mouse TTR 5pUTR (NM_013697.5)
Mouse TTR 5pUTR (NM_013697.5)
VandenDriessche_PromoterSet
340
360
380
400
420
VandenDriessche_PromoterSet
GAAGGAGGGGGTATAAAAGCCCCTTCACCAGGAGAAGCCGTCACACAGATCCACAAGCTCCTGAAGAGGTAAGGGTTTAAGGGATGGTTGGTTGGTGGGGTATTAAT
Mouse TTR 5pUTR (NM_013697.5)
Mouse TTR 5pUTR (NM_013697.5)
MVM Intron
VandenDriessche_PromoterSet
440
460
480
500
520
VandenDriessche_PromoterSet
FVIII-SC_5k_wt3_5_ORF
GTTTAATTACCTGGAGCACCTGCCTGAAATCACTTTTTTTCAGGTTGGTTTAAACGCCGCCACCATGCAGATTGAACTCTCCACCTGTTTCTTCCTGTGCCTGCTGA
MVM Intron
VandenDriessche_PromoterSet
FVIII-SC_5k_wt3_5_ORF
540
560
580
600
620
640

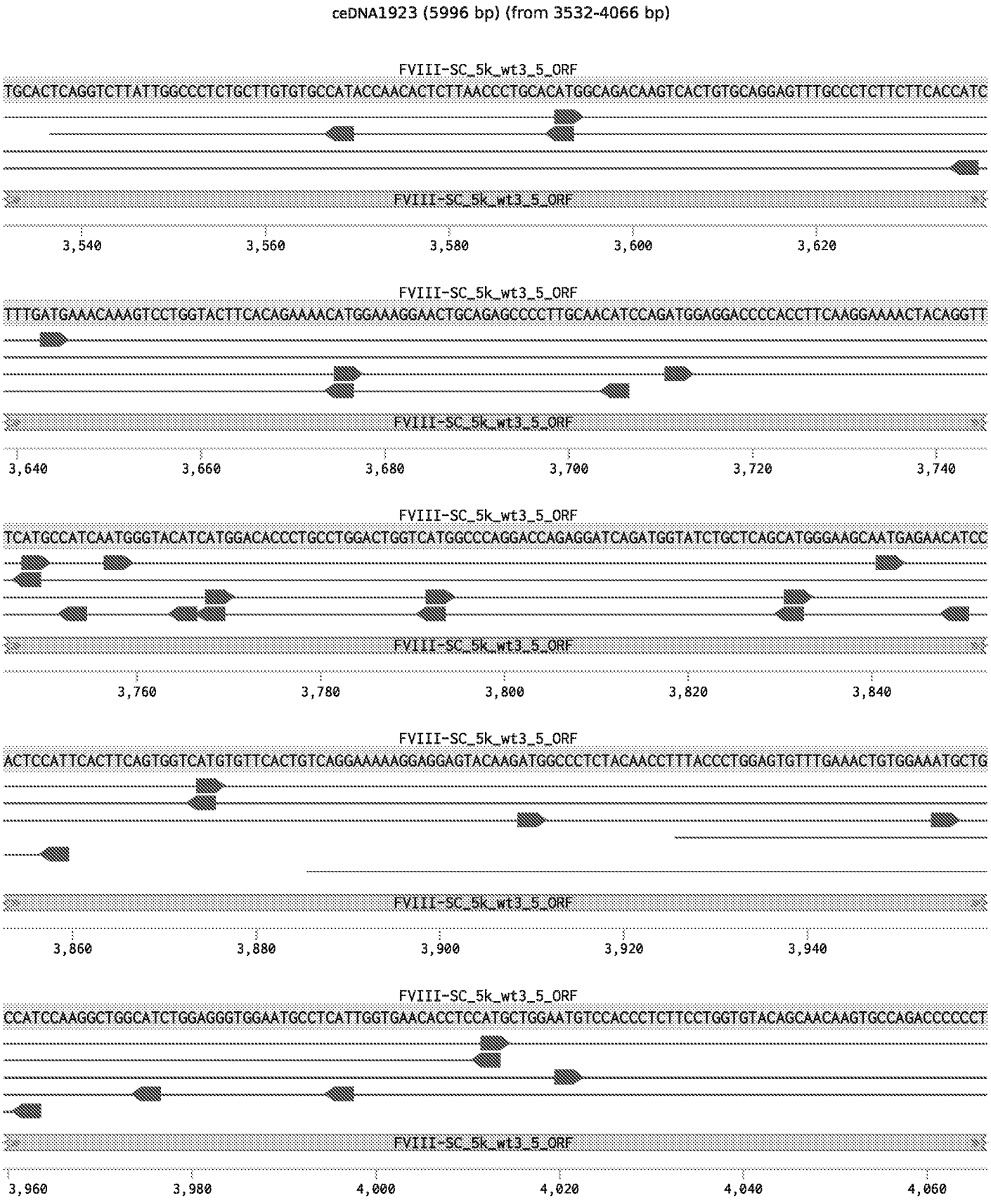

ceDNA1923 (5996 bp) (from 643-1177 bp)
FVIII-SC_5k_wt3_5_ORF
GATTCTGCTTCAGTGCCACCAGGAGGTACTACTTGGGTGCAGTGGAGCTCTCCTGGGACTACATGCAGTCAGACCTGGGAGAGCTCCCTGTGGATGCCAGGTTCCCT
FVIII-SC_5k_wt3_5_ORF
660
680
700
720
740
FVIII-SC_5k_wt3_5_ORF
CCAAGGGTCCCTAAATCCTTCCCCTTCAACACCTCAGTGGTGTACAAGAAAACCCTGTTTGTGGAATTCACTGACCACCTGTTCAACATTGCCAAGCCCAGGCCACC
FVIII-SC_5k_wt3_5_ORF
760
780
800
820
840
FVIII-SC_5k_wt3_5_ORF
CTGGATGGGGCTCCTGGGCCCAACCATCCAAGCTGAAGTGTATGACACTGTGGTCATCACCCTCAAGAACATGGCCTCCCACCCTGTCTCCCTGCATGCTGTGGGAG
FVIII-SC_5k_wt3_5_ORF
860
880
900
920
940
960
FVIII-SC_5k_wt3_5_ORF
TGTCCTACTGGAAGGCCTCAGAGGGGGCTGAGTATGATGACCAGACCTCCCAGAGGGAAAAGGAAGATGACAAGGTGTTCCCAGGAGGATCCCACACCTATGTGTGG
FVIII-SC_5k_wt3_5_ORF
980
1,000
1,020
1,040
1,060
FVIII-SC_5k_wt3_5_ORF
CAAGTCCTGAAGGAAAATGGTCCCATGGCATCAGACCCCTTGTGCTTGACCTACTCCTACCTGAGCCATGTGGACCTGGTCAAGGACCTGAACTCAGGCCTTATTGG
FVIII-SC_5k_wt3_5_ORF
1,080
1,100
1,120
1,140
1,160

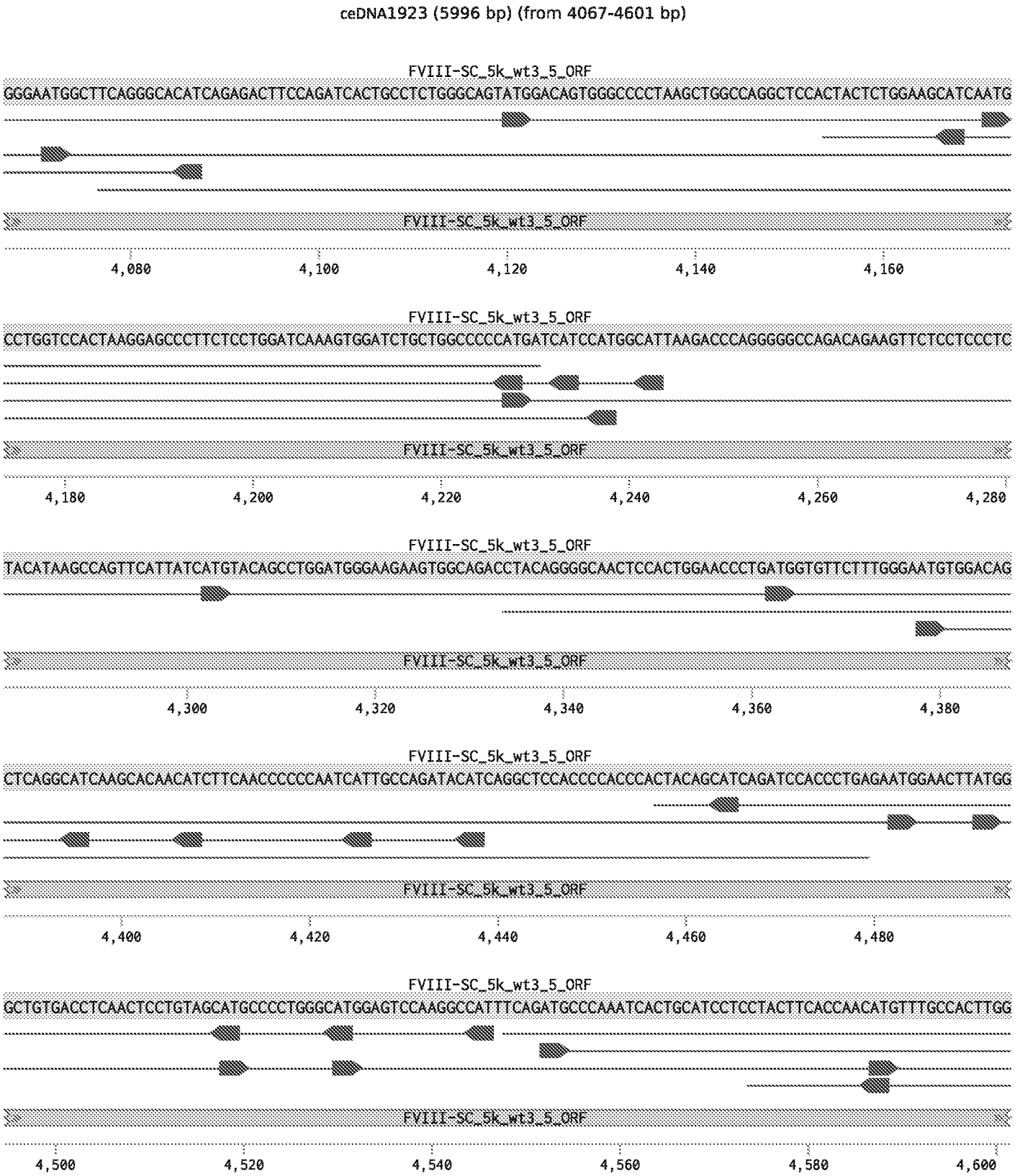

ceDNA1923 (5996 bp) (from 1178-1712 bp)
FVIII-SC_5k_wt3_5_ORF
AGCCCTGCTTGTATGCAGAGAGGGCAGCCTGGCCAAGGAAAAGACCCAGACCCTGCACAAGTTCATCCTCCTGTTTGCTGTGTTTGATGAAGGAAAGTCCTGGCACT
FVIII-SC_5k_wt3_5_ORF
1,180
1,200
1,220
1,240
1,260
1,280
FVIII-SC_5k_wt3_5_ORF
CAGAGACTAAGAACAGCCTGATGCAGGACAGGGATGCAGCATCAGCCAGAGCCTGGCCCAAGATGCACACTGTGAATGGATATGTGAACAGGTCCCTCCCTGGACTA
FVIII-SC_5k_wt3_5_ORF
1,300
1,320
1,340
1,360
1,380
FVIII-SC_5k_wt3_5_ORF
ATTGGATGCCACAGAAAGTCTGTGTACTGGCATGTCATTGGAATGGGCACAACCCCTGAAGTGCATAGCATCTTCCTGGAAGGACACACCTTCCTGGTCAGGAACCA
FVIII-SC_5k_wt3_5_ORF
1,400
1,420
1,440
1,460
1,480
FVIII-SC_5k_wt3_5_ORF
CAGACAGGCATCCCTGGAGATCTCCCCAATCACCTTCCTGACTGCCCAGACTCTCCTGATGGACCTGGGACAGTTCTTGCTTTTCTGCCACATCTCCTCCCACCAAC
FVIII-SC_5k_wt3_5_ORF
1,500
1,520
1,540
1,560
1,580
1,600
FVIII-SC_5k_wt3_5_ORF
ATGATGGCATGGAAGCCTATGTGAAGGTGGACTCCTGCCCTGAGGAACCTCAACTGAGGATGAAAAACAATGAAGAAGCTGAAGATTATGATGATGACCTGACTGAC
FVIII-SC_5k_wt3_5_ORF
1,620
1,640
1,660
1,680
1,700

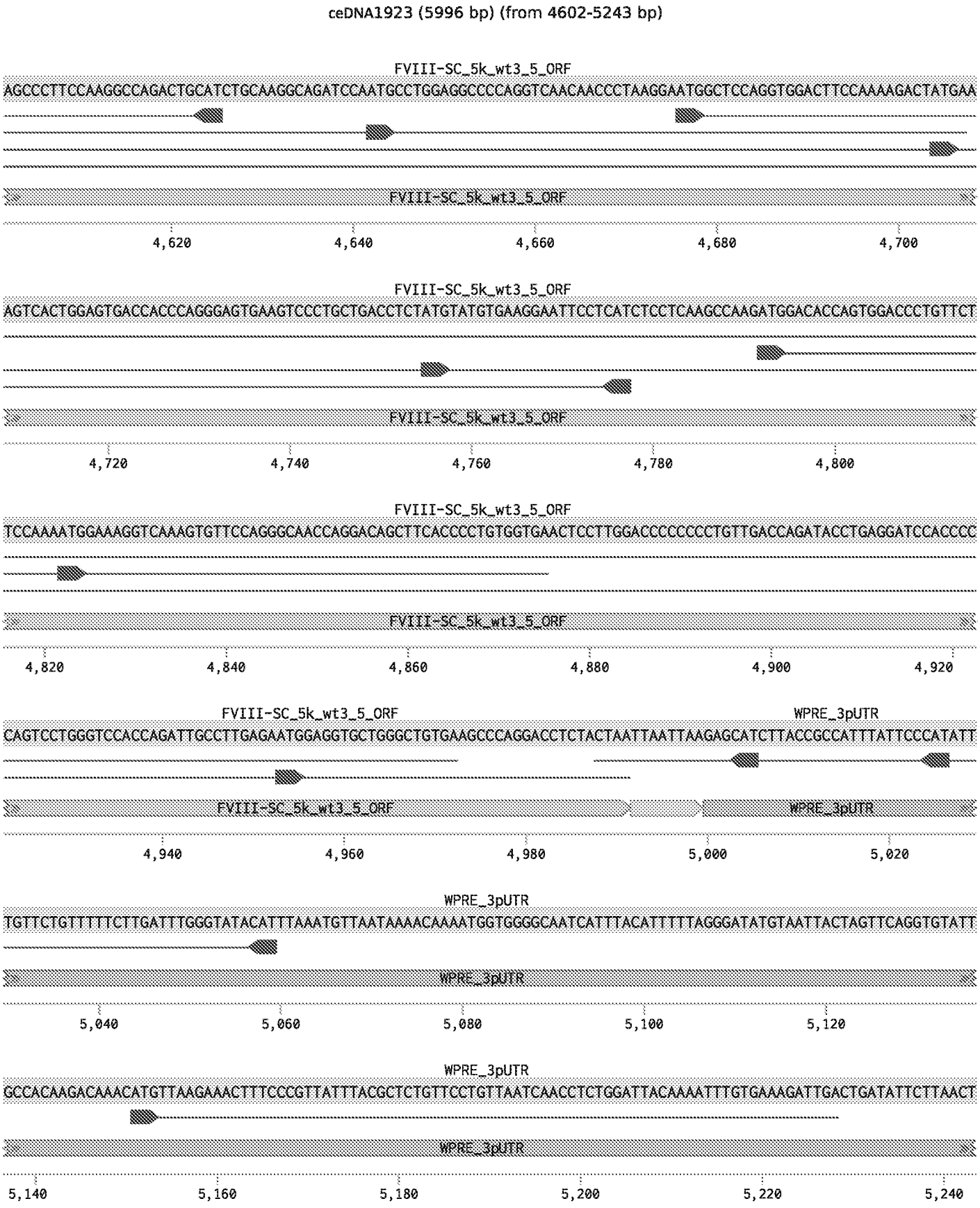

ceDNA1923 (5996 bp) (from 1713-2247 bp)
FVIII-SC_5k_wt3_5_ORF
TCAGAGATGGATGTTGTCAGATTTGATGATGACAACAGCCCTAGCTTCATCCAGATTAGATCTGTGGCCAAGAAGCACCCCAAGACCTGGGTGCACTACATTGCTGC
FVIII-SC_5k_wt3_5_ORF
1,720
1,740
1,760
1,780
1,800
FVIII-SC_5k_wt3_5_ORF
TGAGGAAGAGGACTGGGACTATGCCCCCCTGGTGCTGGCCCCTGATGACAGATCCTACAAGTCCCAGTACCTGAACAATGGTCCCCAGAGAATTGGGAGAAAGTACA
FVIII-SC_5k_wt3_5_ORF
1,820
1,840
1,860
1,880
1,900
1,920
FVIII-SC_5k_wt3_5_ORF
AGAAAGTCAGATTCATGGCCTACACTGATGAAACCTTCAAGACTAGAGAAGCCATCCAGCATGAGTCAGGCATTCTGGGTCCCCTGCTGTATGGGGAAGTTGGTGAC
FVIII-SC_5k_wt3_5_ORF
1,940
1,960
1,980
2,000
2,020
FVIII-SC_5k_wt3_5_ORF
ACCCTGCTGATCATCTTCAAGAACCAAGCCAGCAGGCCTTACAACATCTACCCCCATGGAATTACAGATGTCAGGCCACTGTACTCCAGGAGACTGCCTAAGGGAGT
FVIII-SC_5k_wt3_5_ORF
2,040
2,060
2,080
2,100
2,120
2,140
FVIII-SC_5k_wt3_5_ORF
CAAGCACCTCAAGGACTTCCCCATCCTGCCTGGAGAAATCTTCAAGTACAAGTGGACTGTGACTGTGGAGGATGGGCCTACCAAGTCAGACCCTAGATGCCTGACTA
FVIII-SC_5k_wt3_5_ORF
2,160
2,180
2,200
2,220
2,240

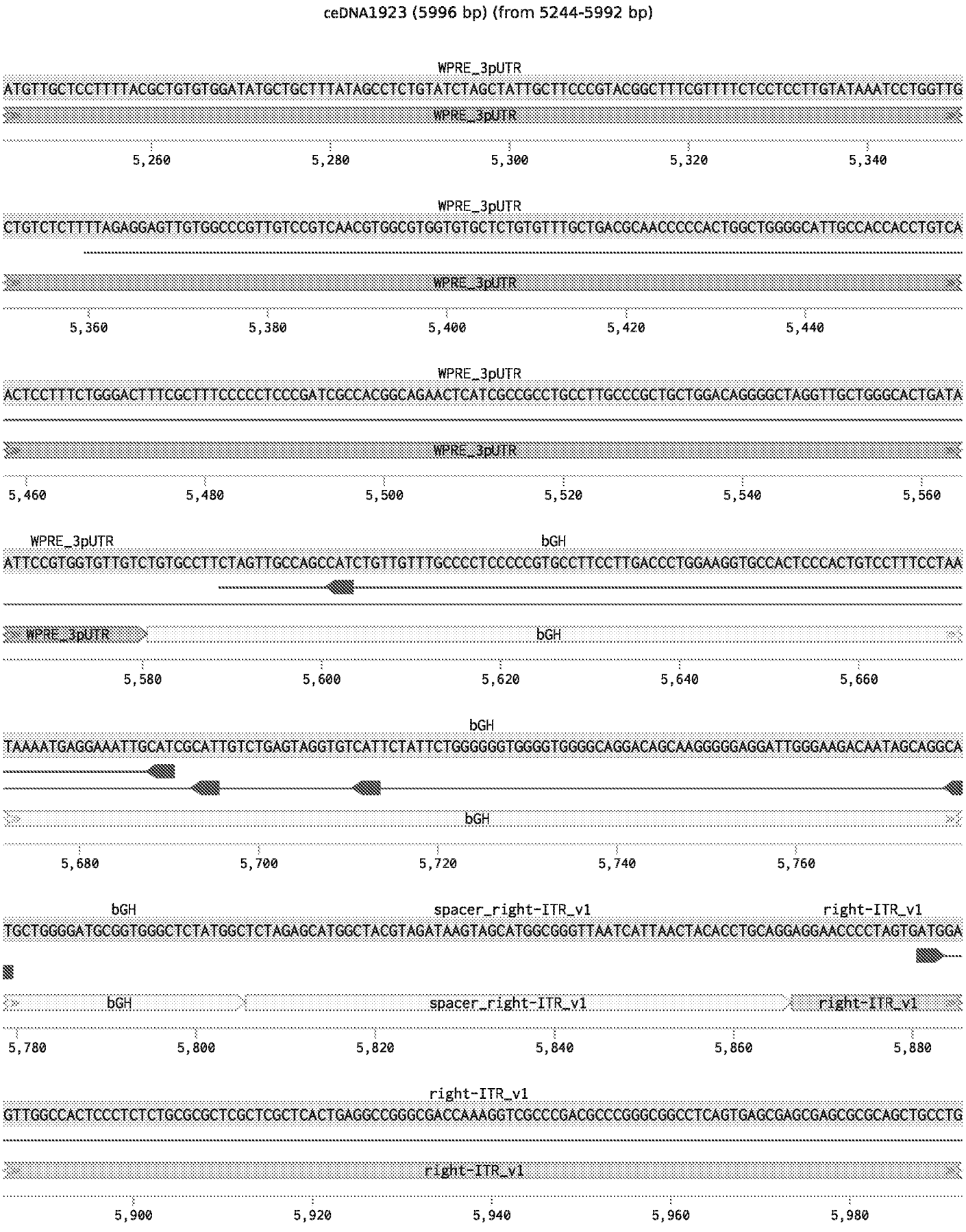

ceDNA1923 (5996 bp) (from 2248-2889 bp)
FVIII-SC_5k_wt3_5_ORF
GGTACTACTCCAGCTTTGTCAACATGGAGAGAGACTTGGCCTCAGGACTCATTGGTCCACTGCTTATTTGCTACAAGGAGTCAGTGGACCAGAGAGGAAACCAAATC
FVIII-SC_5k_wt3_5_ORF
2,260
2,280
2,300
2,320
2,340
FVIII-SC_5k_wt3_5_ORF
ATGTCTGACAAGAGAAATGTCATCCTGTTCTCAGTCTTTGATGAGAACAGGTCCTGGTACCTGACTGAAAACATCCAGAGATTCCTGCCCAACCCTGCTGGGGTGCA
FVIII-SC_5k_wt3_5_ORF
2,360
2,380
2,400
2,420
2,440
2,460
FVIII-SC_5k_wt3_5_ORF
ACTGGAGGACCCTGAGTTCCAGGCCTCAAACATAATGCACAGCATCAATGGCTATGTCTTTGACTCCCTGCAACTGTCAGTGTGCTTGCATGAAGTGGCCTACTGGT
FVIII-SC_5k_wt3_5_ORF
2,480
2,500
2,520
2,540
2,560
FVIII-SC_5k_wt3_5_ORF
ACATCCTCTCCATTGGGGCCCAGACTGACTTCCTGTCAGTGTTTTTCTCTGGCTACACCTTCAAGCATAAAATGGTGTATGAGGACACCCTCACCCTGTTCCCCTTC
FVIII-SC_5k_wt3_5_ORF
2,580
2,600
2,620
2,640
2,660
FVIII-SC_5k_wt3_5_ORF
TCTGGAGAGACTGTGTTCATGAGCATGGAAAACCCAGGCCTCTGGATCCTTGGCTGTCACAACTCAGACTTCAGAAACAGGGGCATGACTGCCCTGCTTAAGGTGTC
FVIII-SC_5k_wt3_5_ORF
2,680
2,700
2,720
2,740
2,760
2,780
FVIII-SC_5k_wt3_5_ORF
CAGCTGTGACAAGAACACTGGGGACTACTATGAGGACTCCTATGAGGACATCAGTGCCTACCTTCTGTCCAAGAACAATGCCATTGAACCTAGGAGCTTCAGCCAGA
FVIII-SC_5k_wt3_5_ORF
2,800
2,820
2,840
2,860
2,880


ceDNA1923 (5996 bp) (from 2890-3531 bp)
FVIII-SC_5k_wt3_5_ORF
ACTCCAGGCACCCAAGCACCAGGCAGAAGCAGTTCAATGCCACCACCATCCCTGAGAACACTACCCTGCAATCAGACCAAGAAGAAATTGACTATGATGACACCATC
FVIII-SC_5k_wt3_5_ORF
2,900
2,920
2,940
2,960
2,980
FVIII-SC_5k_wt3_5_ORF
TCAGTGGAGATGAAGAAGGAGGACTTTGACATCTATGATGAAGATGAAAACCAAAGCCCCAGATCCTTCCAGAAAAAGACCAGGCACTACTTCATTGCAGCAGTGGA
FVIII-SC_5k_wt3_5_ORF
3,000
3,020
3,040
3,060
3,080
3,100
FVIII-SC_5k_wt3_5_ORF
GAGATTATGGGACTATGGGATGTCCAGCTCCCCTCATGTGCTGAGAAACAGAGCACAGTCTGGCTCAGTGCCCCAGTTCAAGAAGGTGGTGTTCCAGGAGTTCACTG
FVIII-SC_5k_wt3_5_ORF
3,120
3,140
3,160
3,180
3,200
FVIII-SC_5k_wt3_5_ORF
ATGGTTCCTTCACCCAACCATTGTACAGGGGAGAGCTGAATGAGCACCTGGGCCTGTTGGGCCCATACATCAGAGCAGAAGTGGAGGACAACATCATGGTCACCTTC
FVIII-SC_5k_wt3_5_ORF
3,220
3,240
3,260
3,280
3,300
FVIII-SC_5k_wt3_5_ORF
AGAAACCAGGCCTCCAGACCCTACTCCTTCTACTCCTCCCTGATCTCCTATGAAGAGGACCAAAGACAGGGAGCTGAGCCCAGAAAGAACTTTGTGAAGCCAAATGA
FVIII-SC_5k_wt3_5_ORF
3,320
3,340
3,360
3,380
3,400
3,420
FVIII-SC_5k_wt3_5_ORF
AACCAAGACCTACTTCTGGAAAGTGCAGCACCACATGGCCCCCACCAAGGATGAGTTTGACTGCAAAGCTTGGGCCTACTTCAGTGATGTGGACTTGGAGAAGGATG
FVIII-SC_5k_wt3_5_ORF
3,440
3,460
3,480
3,500
3,520


ceDNA1923 (5996 bp) (from 3532-4066 bp)
FVIII-SC_5k_wt3_5_ORF
TGCACTCAGGTCTTATTGGCCCTCTGCTTGTGTGCCATACCAACACTCTTAACCCTGCACATGGCAGACAAGTCACTGTGCAGGAGTTTGCCCTCTTCTTCACCATC
FVIII-SC_5k_wt3_5_ORF
3,540
3,560
3,580
3,600
3,620
FVIII-SC_5k_wt3_5_ORF
TTTGATGAAACAAAGTCCTGGTACTTCACAGAAAACATGGAAAGGAACTGCAGAGCCCCTTGCAACATCCAGATGGAGGACCCCACCTTCAAGGAAAACTACAGGTT
FVIII-SC_5k_wt3_5_ORF
3,640
3,660
3,680
3,700
3,720
3,740
FVIII-SC_5k_wt3_5_ORF
TCATGCCATCAATGGGTACATCATGGACACCCTGCCTGGACTGGTCATGGCCCAGGACCAGAGGATCAGATGGTATCTGCTCAGCATGGGAAGCAATGAGAACATCC
FVIII-SC_5k_wt3_5_ORF
3,760
3,780
3,800
3,820
3,840
FVIII-SC_5k_wt3_5_ORF
ACTCCATTCACTTCAGTGGTCATGTGTTCACTGTCAGGAAAAAGGAGGAGTACAAGATGGCCCTCTACAACCTTTACCCTGGAGTGTTTGAAACTGTGGAAATGCTG
FVIII-SC_5k_wt3_5_ORF
3,860
3,880
3,900
3,920
3,940
FVIII-SC_5k_wt3_5_ORF
CCATCCAAGGCTGGCATCTGGAGGGTGGAATGCCTCATTGGTGAACACCTCCATGCTGGAATGTCCACCCTCTTCCTGGTGTACAGCAACAAGTGCCAGACCCCCCT
FVIII-SC_5k_wt3_5_ORF
3,960
3,980
4,000
4,020
4,040
4,060


ceDNA1923 (5996 bp) (from 4067-4601 bp)
FVIII-SC_5k_wt3_5_ORF
GGGAATGGCTTCAGGGCACATCAGAGACTTCCAGATCACTGCCTCTGGGCAGTATGGACAGTGGGCCCCTAAGCTGGCCAGGCTCCACTACTCTGGAAGCATCAATG
FVIII-SC_5k_wt3_5_ORF
4,080
4,100
4,120
4,140
4,160
FVIII-SC_5k_wt3_5_ORF
CCTGGTCCACTAAGGAGCCCTTCTCCTGGATCAAAGTGGATCTGCTGGCCCCCATGATCATCCATGGCATTAAGACCCAGGGGGCCAGACAGAAGTTCTCCTCCCTC
FVIII-SC_5k_wt3_5_ORF
4,180
4,200
4,220
4,240
4,260
4,280
FVIII-SC_5k_wt3_5_ORF
TACATAAGCCAGTTCATTATCATGTACAGCCTGGATGGGAAGAAGTGGCAGACCTACAGGGGCAACTCCACTGGAACCCTGATGGTGTTCTTTGGGAATGTGGACAG
FVIII-SC_5k_wt3_5_ORF
4,300
4,320
4,340
4,360
4,380
FVIII-SC_5k_wt3_5_ORF
CTCAGGCATCAAGCACAACATCTTCAACCCCCCAATCATTGCCAGATACATCAGGCTCCACCCCACCCACTACAGCATCAGATCCACCCTGAGAATGGAACTTATGG
FVIII-SC_5k_wt3_5_ORF
4,400
4,420
4,440
4,460
4,480
FVIII-SC_5k_wt3_5_ORF
GCTGTGACCTCAACTCCTGTAGCATGCCCCTGGGCATGGAGTCCAAGGCCATTTCAGATGCCCAAATCACTGCATCCTCCTACTTCACCAACATGTTTGCCACTTGG
FVIII-SC_5k_wt3_5_ORF
4,500
4,520
4,540
4,560
4,580
4,600


ceDNA1923 (5996 bp) (from 4602-5243 bp)
FVIII-SC_5k_wt3_5_ORF
AGCCCTTCCAAGGCCAGACTGCATCTGCAAGGCAGATCCAATGCCTGGAGGCCCCAGGTCAACAACCCTAAGGAATGGCTCCAGGTGGACTTCCAAAAGACTATGAA
FVIII-SC_5k_wt3_5_ORF
4,620
4,640
4,660
4,680
4,700
FVIII-SC_5k_wt3_5_ORF
AGTCACTGGAGTGACCACCCAGGGAGTGAAGTCCCTGCTGACCTCTATGTATGTGAAGGAATTCCTCATCTCCTCAAGCCAAGATGGACACCAGTGGACCCTGTTCT
FVIII-SC_5k_wt3_5_ORF
4,720
4,740
4,760
4,780
4,800
FVIII-SC_5k_wt3_5_ORF
TCCAAAATGGAAAGGTCAAAGTGTTCCAGGGCAACCAGGACAGCTTCACCCCTGTGGTGAACTCCTTGGACCCCCCCCCTGTTGACCAGATACCTGAGGATCCACCCC
FVIII-SC_5k_wt3_5_ORF
4,820
4,840
4,860
4,880
4,900
4,920
FVIII-SC_5k_wt3_5_ORF
WPRE_3pUTR
CAGTCCTGGGTCCACCAGATTGCCTTGAGAATGGAGGTGCTGGGCTGTGAAGCCCAGGACCTCTACTAATTAATTAAGAGCATCTTACCGCCATTTATTCCCATATT
FVIII-SC_5k_wt3_5_ORF
WPRE_3pUTR
4,940
4,960
4,980
5,000
5,020
WPRE_3pUTR
TGTTCTGTTTTTCTTGATTTGGGTATACATTTAAATGTTAATAAAACAAAATGGTGGGGCAATCATTTACATTTTTAGGGATATGTAATTACTAGTTCAGGTGTATT
WPRE_3pUTR
5,040
5,060
5,080
5,100
5,120
WPRE_3pUTR
GCCACAAGACAAACATGTTAAGAAACTTTCCCGTTATTTACGCTCTGTTCCTGTTAATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGATATTCTTAACT
WPRE_3pUTR
5,140
5,160
5,180
5,200
5,220
5,240


ceDNA1923 (5996 bp) (from 5244-5992 bp)
WPRE_3pUTR
ATGTTGCTCCTTTTACGCTGTGTGGATATGCTGCTTTATAGCCTCTGTATCTAGCTATTGCTTCCCGTACGGCTTTCGTTTTCTCCTCCTTGTATAAATCCTGGTTG
WPRE_3pUTR
5,260
5,280
5,300
5,320
5,340
WPRE_3pUTR
CTGTCTCTTTTAGAGGAGTTGTGGCCCGTTGTCCGTCAACGTGGCGTGGTGTGCTCTGTGTTTGCTGACGCAACCCCCACTGGCTGGGGCATTGCCACCACCTGTCA
WPRE_3pUTR
5,360
5,380
5,400
5,420
5,440
WPRE_3pUTR
ACTCCTTTCTGGGACTTTCGCTTTCCCCCTCCCGATCGCCACGGCAGAACTCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCTAGGTTGCTGGGCACTGATA
WPRE_3pUTR
5,460
5,480
5,500
5,520
5,540
5,560
WPRE_3pUTR
bGH
ATTCCGTGGTGTTGTCTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAA
WPRE_3pUTR
bGH
5,580
5,600
5,620
5,640
5,660
bGH
TAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCA
bGH
5,680
5,700
5,720
5,740
5,760
bGH
spacer_right-ITR_v1
right-ITR_v1
TGCTGGGGATGCGGTGGGCTCTATGGCTCTAGAGCATGGCTACGTAGATAAGTAGCATGGCGGGTTAATCATTAACTACACCTGCAGGAGGAACCCCTAGTGATGGA
bGH
spacer_right-ITR_v1
right-ITR_v1
5,780
5,800
5,820
5,840
5,860
5,880
right-ITR_v1
GTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGCTGCCTG
right-ITR_v1
5,900
5,920
5,940
5,960
5,980

ceDNA1923 (5996 bp) (from 5993-5996 bp)



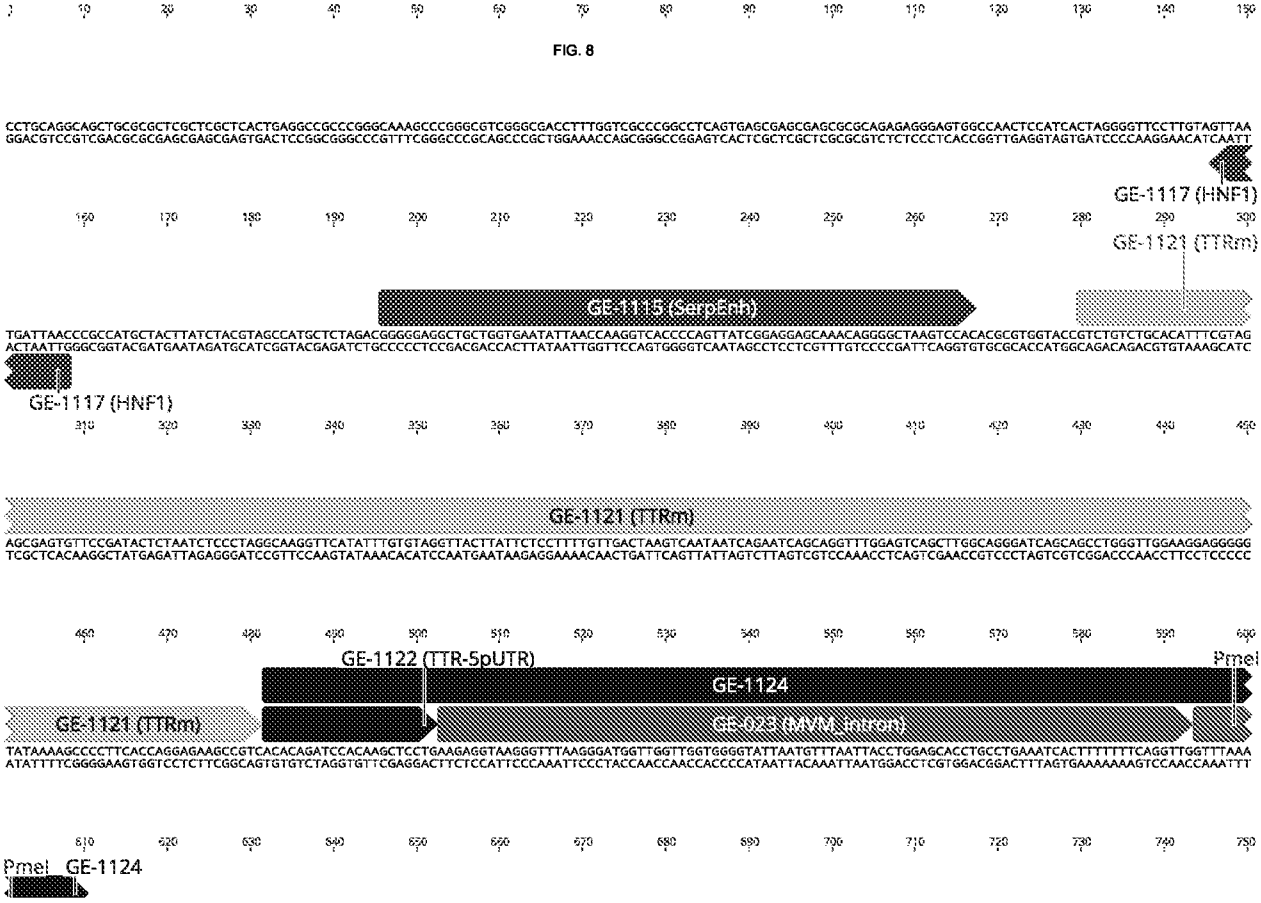
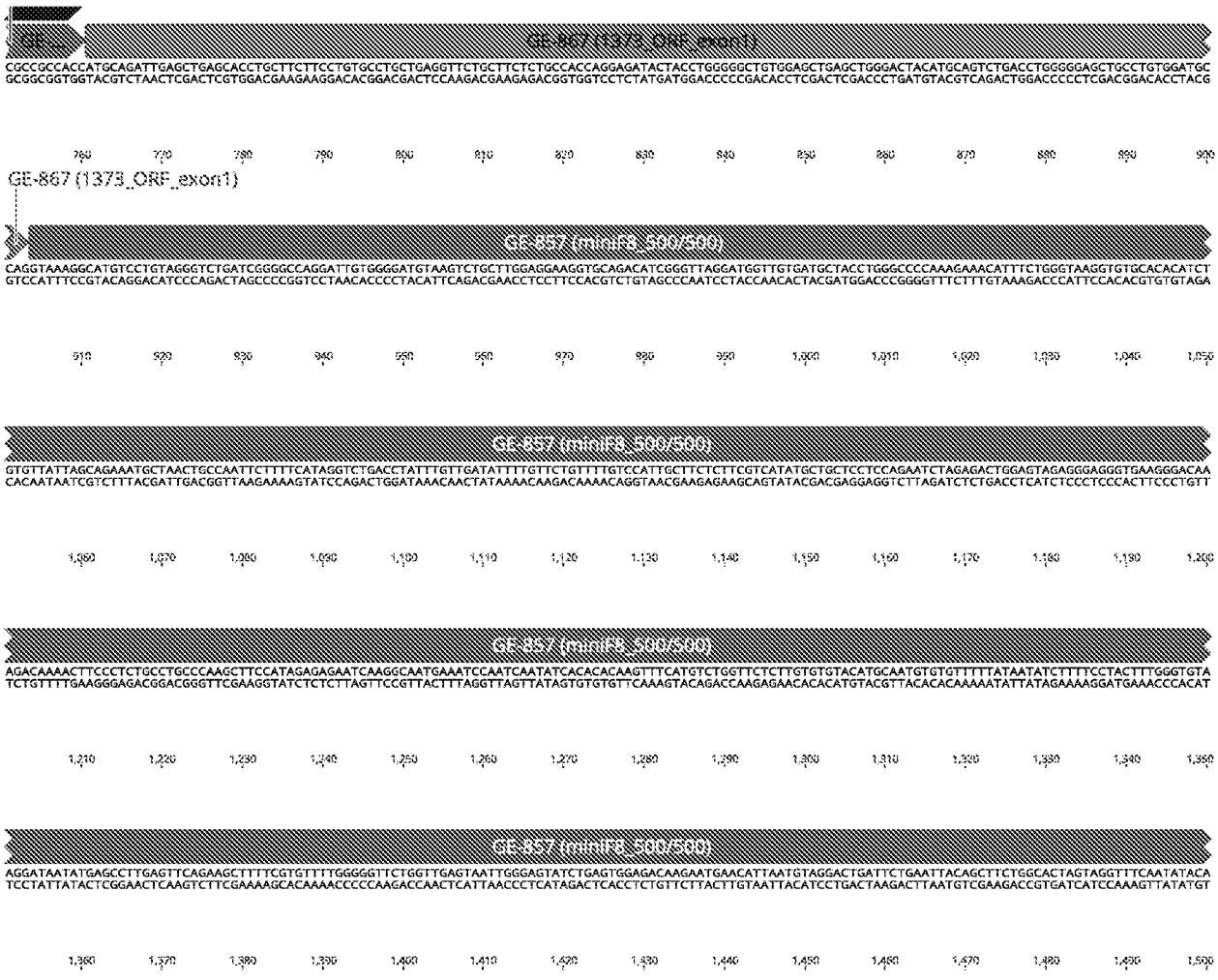
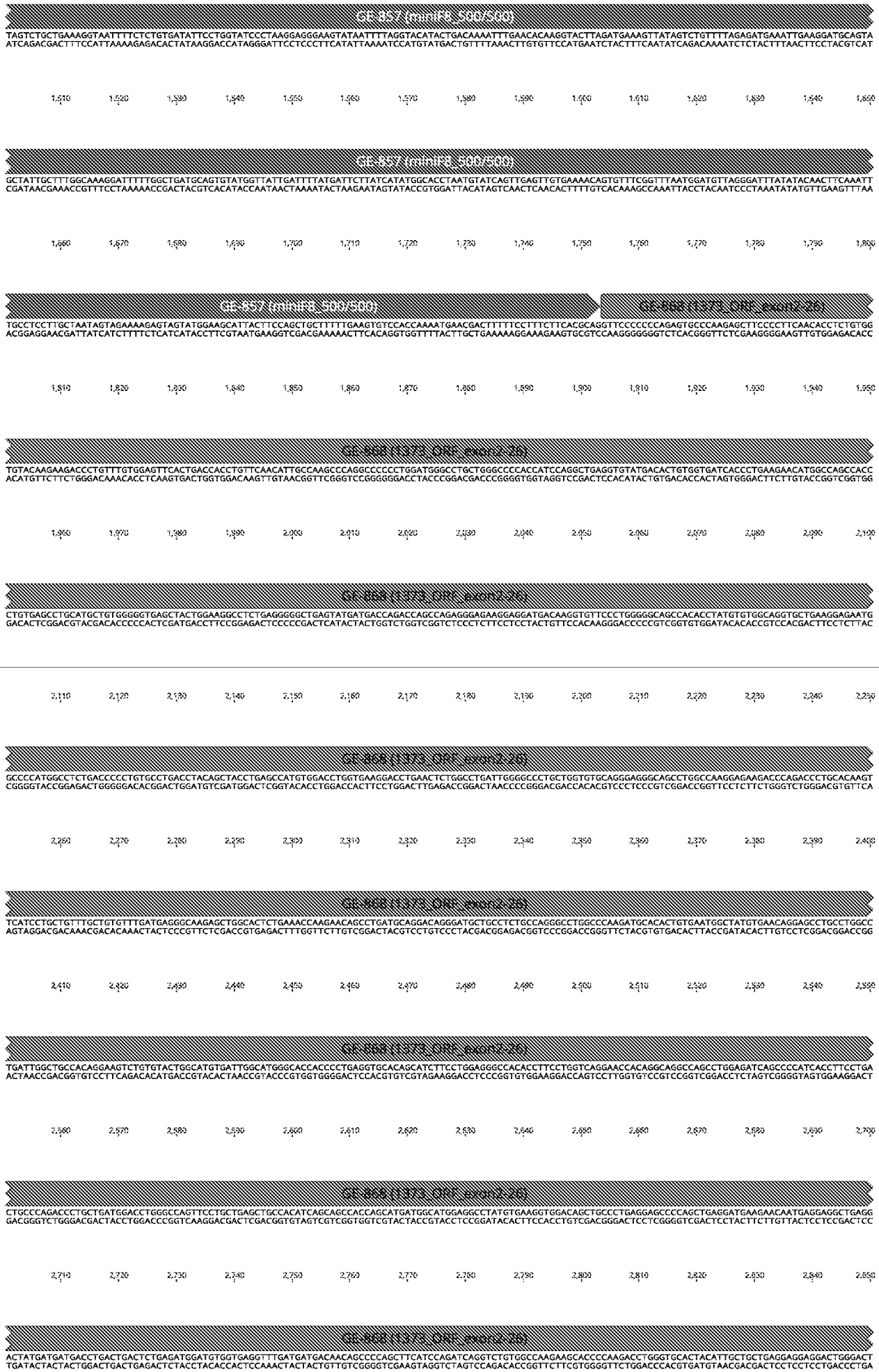

GE-1117 (HNF1)
GE-1121 (TTRm)
GE-1115 (SerpEnh)
GE-1117 (HNF1)
GE-1121 (TTRm)
GE-1122 (TTR-5pUTR)
PmeI
GE-1124
GE-1121 (TTRm)
GE-023 (MVM intron)
PmeI
GE-1124

FIG. 8


GE-867 (1373_ORF_exon1)
GE-867 (1373_ORF_exon1)
GE-857 (miniF8_500/500)
GE-857 (miniF8_500/500)
GE-857 (miniF8_500/500)
GE-857 (miniF8_500/500)


GE-857 (miniPB_500/500)
TAGTCTGCTGAAAGGTAATTTTCTCTGTGATATTCCTGGTATCCCTAAGGAGGGAAGTATAATTTTAGGTACATACTGACAAAATTTGAACACAAGGTACTTAGATGAAAGTTATAGTCTGTTTTAGAGATGAAATTGAAGGATGCAGTA
ATCAGACGACTTTCCATTAAAAGAGACACTATAAGGACCATAGGGATTCCTCCCTTCATATTAAAATCCATGTATGACTGTTTTAAACTTGTGTTCCATGAATCTACTTTCAATATCAGACAAAATCTCTACTTTAACTTCCTACGTCAT
1,510 1,520 1,530 1,540 1,550 1,560 1,570 1,580 1,590 1,600 1,610 1,620 1,630 1,640 1,650
GE-857 (miniPB_500/500)
GCTATTGCTTTGGCAAAGGATTTTTGGCTGATGCAGTGTATGGTTATTGATTTTATGATTCTTATCATATGGCACCTAATGTATCAGTTGAGTTGTGAAAACAGTGTTTCGGTTTAATGGATGTTAGGGATTTATATACAACTTCAAATT
CGATAACGAAACCGTTTCCTAAAAACCGACTACGTCACATACCAATAACTAAAATACTAAGAATAGTATACCGTGGATTACATAGTCAACTCAACACTTTTGTCACAAAGCCAAATTACCTACAATCCCTAAATATATGTTGAAGTTTAA
1,660 1,670 1,680 1,690 1,700 1,710 1,720 1,730 1,740 1,750 1,760 1,770 1,780 1,790 1,800
GE-857 (miniPB_500/500)
GE-868 (1373_ORF_exon2-26)
TGCCTCCTTGCTAATAGTAGAAAAGAGTAGTATGGAAGCATTACTTCCAGCTGCTTTTTGAAGTGTCCACCAAAATGAACGACTTTTTCCTTTCTTCACGCAGGTTCCCCCCCAGAGTGCCCAAGAGCTTCCCCTTCAACACCTCTGTGG
ACGGAGGAACGATTATCATCTTTTCTCATCATACCTTCGTAATGAAGGTCGACGAAAAACTTCACAGGTGGTTTTACTTGCTGAAAAAGGAAAGAAGTGCGTCCAAGGGGGGGTCTCACGGGTTCTCGAAGGGGAAGTTGTGGAGACACC
1,810 1,820 1,830 1,840 1,850 1,860 1,870 1,880 1,890 1,900 1,910 1,920 1,930 1,940 1,950
GE-868 (1373_ORF_exon2-26)
TGTACAAGAAGACCCTGTTTGTGGAGTTCACTGACCACCTGTTCAACATTGCCAAGCCCAGGCCCCCCTGGATGGGCCTGCTGGGCCCCACCATCCAGGCTGAGGTGTATGACACTGTGGTGATCACCCTGAAGAACATGGCCAGCCACC
ACATGTTCTTCTGGGACAAACACCTCAAGTGACTGGTGGACAAGTTGTAACGGTTCGGGTCCGGGGGGACCTACCCGGACGACCCGGGGTGGTAGGTCCGACTCCACATACTGTGACACCACTAGTGGGACTTCTTGTACCGGTCGGTGG
1,960 1,970 1,980 1,990 2,000 2,010 2,020 2,030 2,040 2,050 2,060 2,070 2,080 2,090 2,100
GE-868 (1373_ORF_exon2-26)
CTGTGAGCCTGCATGCTGTGGGGGTGAGCTACTGGAAGGCCTCTGAGGGGGCTGAGTATGATGACCAGACCAGCCAGAGGGAGAAGGAGGATGACAAGGTGTTCCCTGGGGGCAGCCACACCTATGTGTGGCAGGTGCTGAAGGAGAATG
GACACTCGGACGTACGACACCCCCACTCGATGACCTTCCGGAGACTCCCCCGACTCATACTACTGGTCTGGTCGGTCTCCCTCTTCCTCCTACTGTTCCACAAGGGACCCCCGTCGGTGTGGATACACACCGTCCACGACTTCCTCTTAC
2,110 2,120 2,130 2,140 2,150 2,160 2,170 2,180 2,190 2,200 2,210 2,220 2,230 2,240 2,250
GE-868 (1373_ORF_exon2-26)
GCCCCATGGCCTCTGACCCCCTGTGCCTGACCTACAGCTACCTGAGCCATGTGGACCTGGTGAAGGACCTGAACTCTGGCCTGATTGGGGCCCTGCTGGTGTGCAGGGAGGGCAGCCTGGCCAAGGAGAAGACCCAGACCCTGCACAAGT
CGGGGTACCGGAGACTGGGGGACACGGACTGGATGTCGATGGACTCGGTACACCTGGACCACTTCCTGGACTTGAGACCGGACTAACCCCGGGACGACCACACGTCCCTCCCGTCGGACCGGTTCCTCTTCTGGGTCTGGGACGTGTTCA
2,260 2,270 2,280 2,290 2,300 2,310 2,320 2,330 2,340 2,350 2,360 2,370 2,380 2,390 2,400
GE-868 (1373_ORF_exon2-26)
TCATCCTGCTGTTTGCTGTGTTTGATGAGGGCAAGAGCTGGCACTCTGAAACCAAGAACAGCCTGATGCAGGACAGGGATGCTGCCTCTGCCAGGGCCTGGCCCAAGATGCACACTGTGAATGGCTATGTGAACAGGAGCCTGCCTGGCC
AGTAGGACGACAAACGACACAAACTACTCCCGTTCTCGACCGTGAGACTTTGGTTCTTGTCGGACTACGTCCTGTCCCTACGACGGAGACGGTCCCGGACCGGGTTCTACGTGTGACACTTACCGATACACTTGTCCTCGGACGGACCGG
2,410 2,420 2,430 2,440 2,450 2,460 2,470 2,480 2,490 2,500 2,510 2,520 2,530 2,540 2,550
GE-868 (1373_ORF_exon2-26)
TGATTGGCTGCCACAGGAAGTCTGTGTACTGGCATGTGATTGGCATGGGCACCACCCCTGAGGTGCACAGCATCTTCCTGGAGGGCCACACCTTCCTGGTCAGGAACCACAGGCAGGCCAGCCTGGAGATCAGCCCCATCACCTTCCTGA
ACTAACCGACGGTGTCCTTCAGACACATGACCGTACACTAACCGTACCCGTGGTGGGGACTCCACGTGTCGTAGAAGGACCTCCCGGTGTGGAAGGACCAGTCCTTGGTGTCCGTCCGGTCGGACCTCTAGTCGGGGTAGTGGAAGGACT
2,560 2,570 2,580 2,590 2,600 2,610 2,620 2,630 2,640 2,650 2,660 2,670 2,680 2,690 2,700
GE-868 (1373_ORF_exon2-26)
CTGCCCAGACCCTGCTGATGGACCTGGGCCAGTTCCTGCTGAGCTGCCACATCAGCAGCCACCAGCATGATGGCATGGAGGCCTATGTGAAGGTGGACAGCTGCCCTGAGGAGCCCCAGCTGAGGATGAAGAACAATGAGGAGGCTGAGG
GACGGGTCTGGGACGACTACCTGGACCCGGTCAAGGACGACTCGACGGTGTAGTCGTCGGTGGTCGTACTACCGTACCTCCGGATACACTTCCACCTGTCGACGGGACTCCTCGGGGTCGACTCCTACTTCTTGTTACTCCTCCGACTCC
2,710 2,720 2,730 2,740 2,750 2,760 2,770 2,780 2,790 2,800 2,810 2,820 2,830 2,840 2,850
GE-868 (1373_ORF_exon2-26)
ACTATGATGATGACCTGACTGACTCTGAGATGGATGTGGTGAGGTTTGATGATGACAACAGCCCCAGCTTCATCCAGATCAGGTCTGTGGCCAAGAAGCACCCCAAGACCTGGGTGCACTACATTGCTGCTGAGGAGGAGGACTGGGACT
TGATACTACTACTGGACTGACTGAGACTCTACCTACACCACTCCAAACTACTACTGTTGTCGGGGTCGAAGTAGGTCTAGTCCAGACACCGGTTCTTCGTGGGGTTCTGGACCCACGTGATGTAACGACGACTCCTCCTCCTGACCCTGA

2,880 2,870 2,880 2,890 2,900 2,910 2,920 2,930 2,940 2,950 2,960 2,970 2,980 2,990 3,000

GE-868 (1373_ORF_exon2-26)

ATGCCCCCCTGGTGCTGGCCCCTGATGACAGGAGCTACAAGAGCCAGTACCTGAACAATGGCCCCCAGAGGATTGGCAGGAAGTACAAGAAGGTCAGGTTCATGGCCTACACTGATGAAACCTTCAAGACCAGGGAGGCCATCCAGCATG
TACGGGGGGACCACGACCGGGGACTACTGTCCTCGATGTTCTCGGTCATGGACTTGTTACCGGGGGTCTCCTAACCGTCCTTCATGTTCTTCCAGTCCAAGTACCGGATGTGACTACTTTGGAAGTTCTGGTCCCTCCGGTAGGTCGTAC

3,010 3,020 3,030 3,040 3,050 3,060 3,070 3,080 3,090 3,100 3,110 3,120 3,130 3,140 3,150

GE-868 (1373_ORF_exon2-26)

AGTCTGGCATCCTGGGCCCCCTGCTGTATGGGGAGGTGGGGGACACCCTGCTGATCATCTTCAAGAACCAGGCCAGCAGGCCCTACAACATCTACCCCCATGGCATCACTGATGTGAGGCCCCTGTACAGCAGGAGGCTGCCCAAGGGGG
TCAGACCGTAGGACCCGGGGGACGACATACCCCTCCACCCCCTGTGGGACGACTAGTAGAAGTTCTTGGTCCGGTCGTCCGGGATGTTGTAGATGGGGGTACCGTAGTGACTACACTCCGGGGACATGTCGTCCTCCGACGGGTTCCCCC

3,160 3,170 3,180 3,190 3,200 3,210 3,220 3,230 3,240 3,250 3,260 3,270 3,280 3,290 3,300

GE-868 (1373_ORF_exon2-26)

TGAAGCACCTGAAGGACTTCCCCATCCTGCCTGGGGAGATCTTCAAGTACAAGTGGACTGTGACTGTGGAGGATGGCCCCACCAAGTCTGACCCCAGGTGCCTGACCAGATACTACAGCAGCTTTGTGAACATGGAGAGGGACCTGGCCT
ACTTCGTGGACTTCCTGAAGGGGTAGGACGGACCCCTCTAGAAGTTCATGTTCACCTGACACTGACACCTCCTACCGGGGTGGTTCAGACTGGGGTCCACGGACTGGTCTATGATGTCGTCGAAACACTTGTACCTCTCCCTGGACCGGA

3,310 3,320 3,330 3,340 3,350 3,360 3,370 3,380 3,390 3,400 3,410 3,420 3,430 3,440 3,450

GE-868 (1373_ORF_exon2-26)

CTGGCCTGATTGGCCCCCTGCTGATCTGCTACAAGGAGTCTGTGGACCAGAGGGGCAACCAGATCATGTCTGACAAGAGGAATGTGATCCTGTTCTCTGTGTTTGATGAGAACAGGAGCTGGTACCTGACTGAGAACATCCAGAGGTTCC
GACCGGACTAACCGGGGGACGACTAGACGATGTTCCTCAGACACCTGGTCTCCCCGTTGGTCTAGTACAGACTGTTCTCCTTACACTAGGACAAGAGACACAAACTACTCTTGTCCTCGACCATGGACTGACTCTTGTAGGTCTCCAAGG

3,460 3,470 3,480 3,490 3,500 3,510 3,520 3,530 3,540 3,550 3,560 3,570 3,580 3,590 3,600

GE-868 (1373_ORF_exon2-26)

TGCCCAACCCTGCTGGGGTGCAGCTGGAGGACCCTGAGTTCCAGGCCAGCAACATCATGCACAGCATCAATGGCTATGTGTTTGACAGCCTGCAGCTGTCTGTGTGCCTGCATGAGGTGGCCTACTGGTACATCCTGAGCATTGGGGCCC
ACGGGTTGGGACGACCCCACGTCGACCTCCTGGGACTCAAGGTCCGGTCGTTGTAGTACGTGTCGTAGTTACCGATACACAAACTGTCGGACGTCGACAGACACACGGACGTACTCCACCGGATGACCATGTAGGACTCGTAACCCCGGG

3,610 3,620 3,630 3,640 3,650 3,660 3,670 3,680 3,690 3,700 3,710 3,720 3,730 3,740 3,750

GE-868 (1373_ORF_exon2-26)

AGACTGACTTCCTGTCTGTTCTTCTCTGGCTACACCTTCAAGCACAAGATGGTGTATGAGGACACCCTGACCCTGTTCCCCTTCTCTGGGGAGACTGTGTTCATGAGCATGGAGAACCCTGGCCTGTGGATTCTGGGCTGCCACAACT
TCTGACTGAAGGACAGACACACAAGAAGAGACCGATGTGGAAGTTCGTGTTCTACCACATACTCCTGTGGGACTGGGACAAGGGGAAGAGACCCCTCTGACACAAGTACTCGTACCTCTTGGGACCGGACACCTAAGACCCGACGGTGTTGA

3,760 3,770 3,780 3,790 3,800 3,810 3,820 3,830 3,840 3,850 3,860 3,870 3,880 3,890 3,900

GE-868 (1373_ORF_exon2-26)

CTGACTTCAGGAACAGGGGCATGACTGCCCTGCTGAAAGTCTCCAGCTGTGACAAGAACACTGGGGACTACTATGAGGACAGCTATGAGGACATCTCTGCCTACCTGCTGAGCAAGAACAATGCCATTGAGCCCAGGAGCTTCAGCCAGA
GACTGAAGTCCTTGTCCCCGTACTGACGGGACGACTTTCAGAGGTCGACACTGTTCTTGTGACCCCTGATGATACTCCTGTCGATACTCCTGTAGAGACGGATGGACGACTCGTTCTTGTTACGGTAACTCGGGTCCTCGAAGTCGGTCT

3,910 3,920 3,930 3,940 3,950 3,960 3,970 3,980 3,990 4,000 4,010 4,020 4,030 4,040 4,050

GE-868 (1373_ORF_exon2-26)

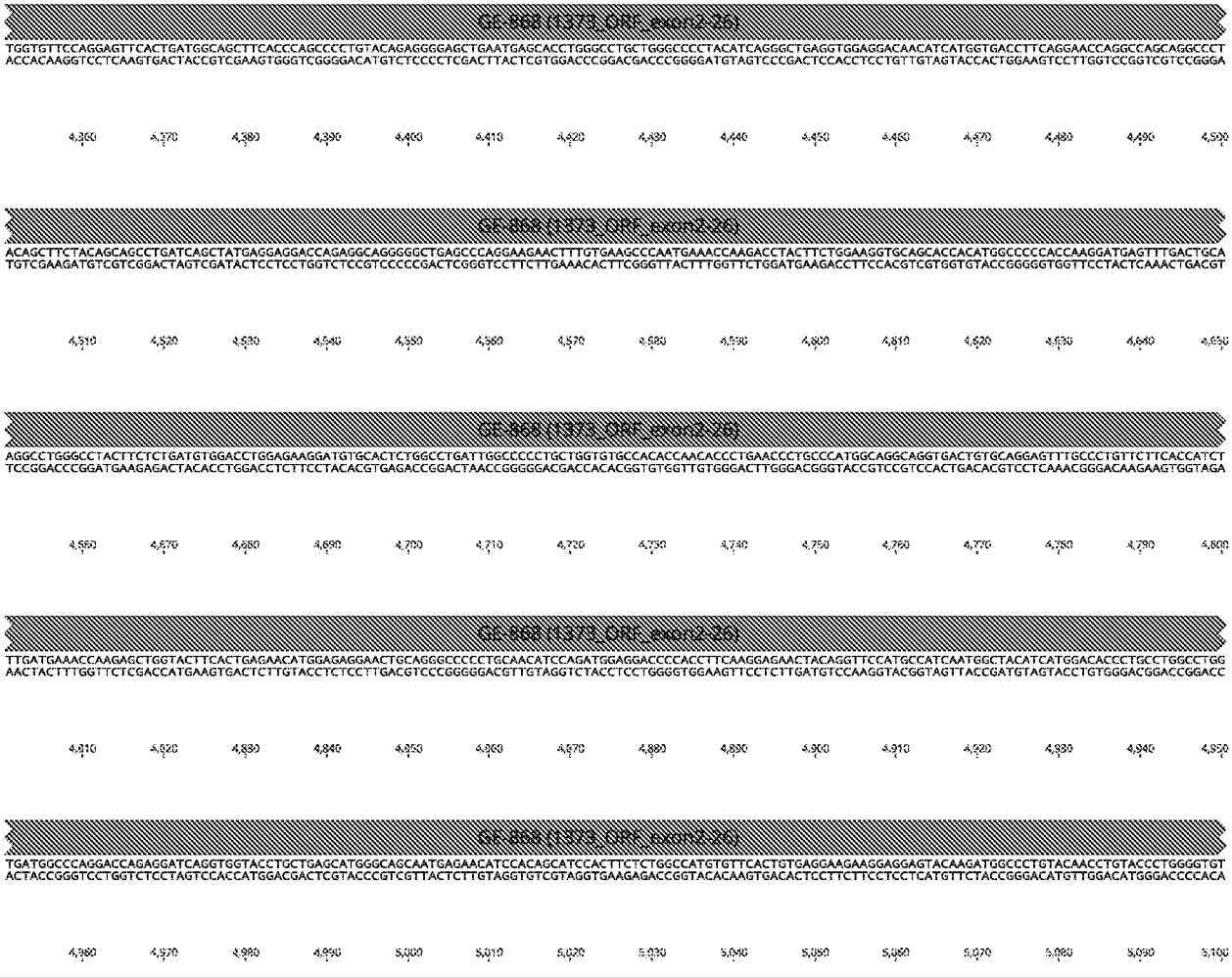
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TATCGTCCGTGGGGTCGTGGTCCGTCTTCGTCAAGTTACGGTGGTGGTAGGGTCTCTTATGGTGGGACGTCAGACTGGTCCTCCTCTAACTGATACTACTGTGGTAGAGACACCTCTACTTCTTCCTCCTGAAACTGTAGATGCTGCTCC

4,060 4,070 4,080 4,090 4,100 4,110 4,120 4,130 4,140 4,150 4,160 4,170 4,180 4,190 4,200

GE-868 (1373_ORF_exon2-26)

ACGAGAACCAGAGCCCCAGGAGCTTCCAGAAGAAGACCAGGCACTACTTCATTGCTGCTGTGGAGAGGCTGTGGGACTATGGCATGAGCAGCAGCCCCCATGTGCTGAGGAACAGGGCCCAGTCTGGCTCTGTGCCCCAGTTCAAGAAGG
TGCTCTTGGTCTCGGGGTCCTCGAAGGTCTTCTTCTGGTCCGTGATGAAGTAACGACGACACCTCTCCGACACCCTGATACCGTACTCGTCGTCGGGGGTACACGACTCCTTGTCCCGGGTCAGACCGAGACACGGGGTCAAGTTCTTCC

4,210 4,220 4,230 4,240 4,250 4,260 4,270 4,280 4,290 4,300 4,310 4,320 4,330 4,340 4,350


GE-868 (1373_ORF_exon2-26)
TGGTGTTCCAGGAGTTCACTGATGGCAGCTTCACCCAGCCCCTGTACAGAGGGGAGCTGAATGAGCACCTGGGCCTGCTGGGCCCCTACATCAGGGCTGAGGTGGAGGACAACATCATGGTGACCTTCAGGAACCAGGCCAGCAGGCCCT
ACCACAAGGTCCTCAAGTGACTACCGTCGAAGTGGGTCGGGGACATGTCTCCCCTCGACTTACTCGTGGACCCGGACGACCCGGGGATGTAGTCCCGACTCCACCTCCTGTTGTAGTACCACTGGAAGTCCTTGGTCCGGTCGTCCGGGA
4,360 4,370 4,380 4,390 4,400 4,410 4,420 4,430 4,440 4,450 4,460 4,470 4,480 4,490 4,500
GE-868 (1373_ORF_exon2-26)
ACAGCTTCTACAGCAGCCTGATCAGCTATGAGGAGGACCAGAGGCAGGGGGCTGAGCCCAGGAAGAACTTTGTGAAGCCCAATGAAACCAAGACCTACTTCTGGAAGGTGCAGCACCACATGGCCCCCACCAAGGATGAGTTTGACTGCA
TGTCGAAGATGTCGTCGGACTAGTCGATACTCCTCCTGGTCTCCGTCCCCCGACTCGGGTCCTTCTTGAAACACTTCGGGTTACTTTGGTTCTGGATGAAGACCTTCCACGTCGTGGTGTACCGGGGGTGGTTCCTACTCAAACTGACGT
4,510 4,520 4,530 4,540 4,550 4,560 4,570 4,580 4,590 4,600 4,610 4,620 4,630 4,640 4,650
GE-868 (1373_ORF_exon2-26)
AGGCCTGGGCCTACTTCTCTGATGTGGACCTGGAGAAGGATGTGCACTCTGGCCTGATTGGCCCCCTGCTGGTGTGCCACACCAACACCCTGAACCCTGCCCATGGCAGGCAGGTGACTGTGCAGGAGTTTGCCCTGTTCTTCACCATCT
TCCGGACCCGGATGAAGAGACTACACCTGGACCTCTTCCTACACGTGAGACCGGACTAACCGGGGGACGACCACACGGTGTGGTTGTGGGACTTGGGACGGGTACCGTCCGTCCACTGACACGTCCTCAAACGGGACAAGAAGTGGTAGA
4,660 4,670 4,680 4,690 4,700 4,710 4,720 4,730 4,740 4,750 4,760 4,770 4,780 4,790 4,800
GE-868 (1373_ORF_exon2-26)
TTGATGAAACCAAGAGCTGGTACTTCACTGAGAACATGGAGAGGAACTGCAGGGCCCCCTGCAACATCCAGATGGAGGACCCCACCTTCAAGGAGAACTACAGGTTCCATGCCATCAATGGCTACATCATGGACACCCTGCCTGGCCTGG
AACTACTTTGGTTCTCGACCATGAAGTGACTCTTGTACCTCTCCTTGACGTCCCGGGGGACGTTGTAGGTCTACCTCCTGGGGTGGAAGTTCCTCTTGATGTCCAAGGTACGGTAGTTACCGATGTAGTACCTGTGGGACGGACCGGACC
4,810 4,820 4,830 4,840 4,850 4,860 4,870 4,880 4,890 4,900 4,910 4,920 4,930 4,940 4,950
GE-868 (1373_ORF_exon2-26)
TGATGGCCCAGGACCAGAGGATCAGGTGGTACCTGCTGAGCATGGGCAGCAATGAGAACATCCACAGCATCCACTTCTCTGGCCATGTGTTCACTGTGAGGAAGAAGGAGGAGTACAAGATGGCCCTGTACAACCTGTACCCTGGGGTGT
ACTACCGGGTCCTGGTCTCCTAGTCCACCATGGACGACTCGTACCCGTCGTTACTCTTGTAGGTGTCGTAGGTGAAGAGACCGGTACACAAGTGACACTCCTTCTTCCTCCTCATGTTCTACCGGGACATGTTGGACATGGGACCCCACA
4,960 4,970 4,980 4,990 5,000 5,010 5,020 5,030 5,040 5,050 5,060 5,070 5,080 5,090 5,100
GE-868 (1373_ORF_exon2-26)
TTGAGACTGTGGAGATGCTGCCCAGCAAGGCTGGCATCTGGAGGGTGGAGTGCCTGATTGGGGAGCACCTGCATGCTGGCATGAGCACCCTGTTCCTGGTGTACAGCAACAAGTGCCAGACCCCCCTGGGCATGGCCTCTGGCCACATCA
AACTCTGACACCTCTACGACGGGTCGTTCCGACCGTAGACCTCCCACCTCACGGACTAACCCCTCGTGGACGTACGACCGTACTCGTGGGACAAGGACCACATGTCGTTGTTCACGGTCTGGGGGGACCCGTACCGGAGACCGGTGTAGT
5,110 5,120 5,130 5,140 5,150 5,160 5,170 5,180 5,190 5,200 5,210 5,220 5,230 5,240 5,250
GE-868 (1373_ORF_exon2-26)
GGGACTTCCAGATCACTGCCTCTGGCCAGTATGGCCAGTGGGCCCCCAAGCTGGCCAGGCTGCACTACTCTGGCAGCATCAATGCCTGGAGCACCAAGGAGCCCTTCAGCTGGATCAAGGTGGACCTGCTGGCCCCCATGATCATCCATG
CCCTGAAGGTCTAGTGACGGAGACCGGTCATACCGGTCACCCGGGGGTTCGACCGGTCCGACGTGATGAGACCGTCGTAGTTACGGACCTCGTGGTTCCTCGGGAAGTCGACCTAGTTCCACCTGGACGACCGGGGGTACTAGTAGGTAC
5,260 5,270 5,280 5,290 5,300 5,310 5,320 5,330 5,340 5,350 5,360 5,370 5,380 5,390 5,400
GE-868 (1373_ORF_exon2-26)
GCATCAAGACCCAGGGGGCCAGGCAGAAGTTCAGCAGCCTGTACATCAGCCAGTTCATCATCATGTACAGCCTGGATGGCAAGAAGTGGCAGACCTACAGGGGCAACAGCACTGGCACCCTGATGGTGTTCTTTGGCAATGTGGACAGCT
CGTAGTTCTGGGTCCCCCGGTCCGTCTTCAAGTCGTCGGACATGTAGTCGGTCAAGTAGTAGTACATGTCGGACCTACCGTTCTTCACCGTCTGGATGTCCCCGTTGTCGTGACCGTGGGACTACCACACAAGAAACCGTTACACCTGTCGA
5,410 5,420 5,430 5,440 5,450 5,460 5,470 5,480 5,490 5,500 5,510 5,520 5,530 5,540 5,550
GE-868 (1373_ORF_exon2-26)
CTGGCATCAAGCACAACATCTTCAACCCCCCCATCATTGCCAGATACATCAGGCTGCACCCCACCCACTACAGCATCAGGAGCACCCTGAGGATGGAGCTGATGGGCTGTGACCTGAACAGCTGCAGCATGCCCCTGGGCATGGAGAGCA
GACCGTAGTTCGTGTTGTAGAAGTTGGGGGGGTAGTAACGGTCTATGTAGTCCGACGTGGGGTGGGTGATGTCGTAGTCCTCGTGGGACTCCTACCTCGACTACCCGACACTGGACTTGTCGACGTCGTACGGGGACCCGTACCTCTCGT
5,560 5,570 5,580 5,590 5,600 5,610 5,620 5,630 5,640 5,650 5,660 5,670 5,680 5,690 5,700
GE-868 (1373_ORF_exon2-26)
AGGCCATCTCTGATGCCCAGATCACTGCCAGCAGCTACTTCACCAACATGTTTGCCACCTGGAGCCCCAGCAAGGCCAGGCTGCACCTGCAGGGCAGGAGCAATGCCTGGAGGCCCCAGGTCAACAACCCCAAGGAGTGGCTGCAGGTGG
TCCGGTAGAGACTACGGGTCTAGTGACGGTCGTCGATGAAGTGGTTGTACAAACGGTGGACCTCGGGGTCGTTCCGGTCCGACGTGGACGTCCCGTCCTCGTTACGGACCTCCGGGGTCCAGTTGTTGGGGTTCCTCACCGACGTCCACC

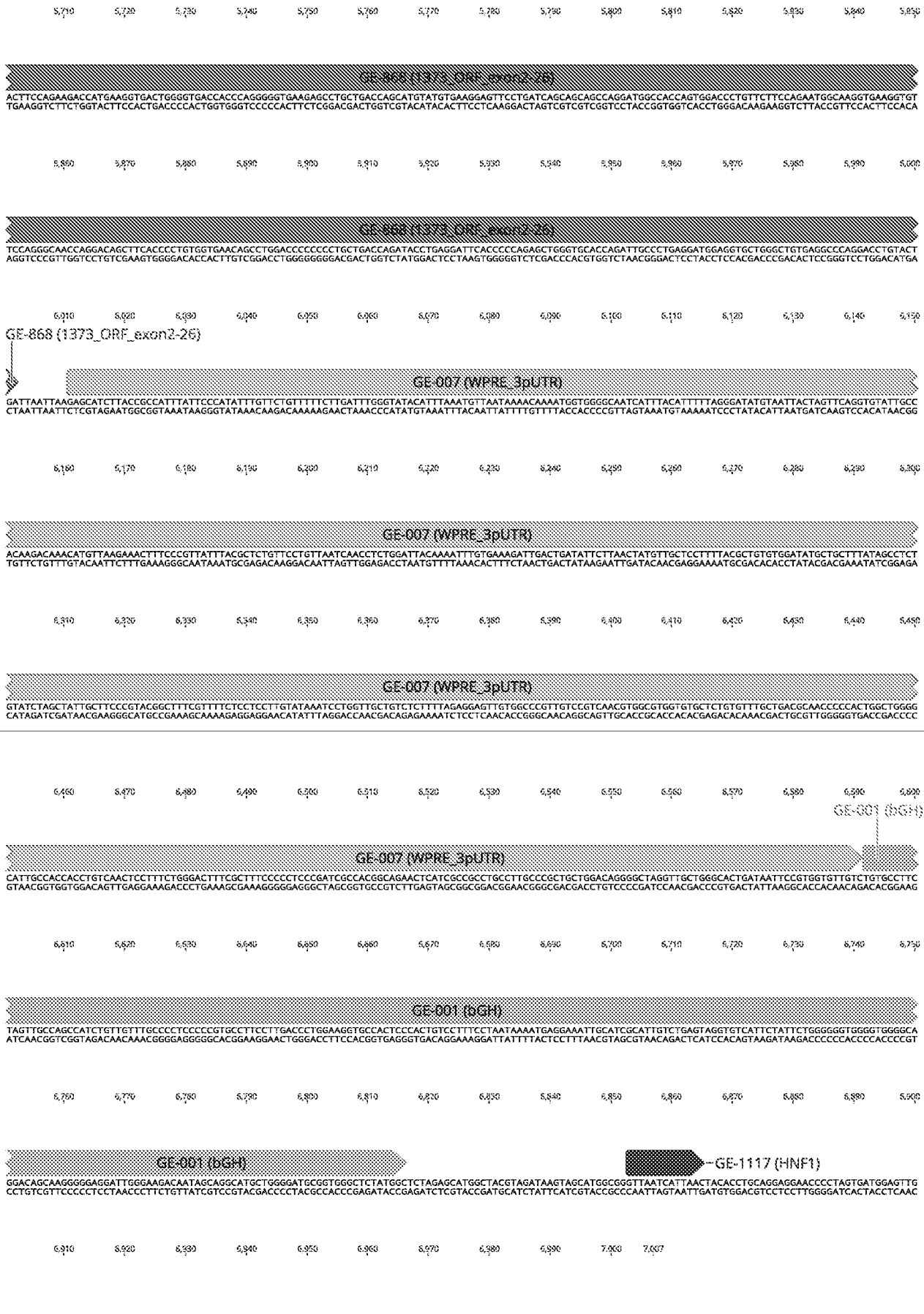

5,710 5,720 5,730 5,740 5,750 5,760 5,770 5,780 5,790 5,800 5,810 5,820 5,830 5,840 5,850
GE-868 (1373_ORF_exon2-26)
ACTTCCAGAAGACCATGAAGGTGACTGGGGTGACCACCCAGGGGGTGAAGAGCCTGCTGACCAGCATGTATGTGAAGGAGTTCCTGATCAGCAGCAGCCAGGATGGCCACCAGTGGACCCTGTTCTTCCAGAATGGCAAGGTGAAGGTGT
TGAAGGTCTTCTGGTACTTCCACTGACCCCACTGGTGGGTCCCCCACTTCTCGGACGACTGGTCGTACATACACTTCCTCAAGGACTAGTCGTCGTCGGTCCTACCGGTGGTCACCTGGGACAAGAAGGTCTTACCGTTCCACTTCCACA
5,860 5,870 5,880 5,890 5,900 5,910 5,920 5,930 5,940 5,950 5,960 5,970 5,980 5,990 6,000
GE-868 (1373_ORF_exon2-26)
TCCAGGGCAACCAGGACAGCTTCACCCCTGTGGTGAACAGCCTGGACCCCCCCCTGCTGACCAGATACCTGAGGATTCACCCCCAGAGCTGGGTGCACCAGATTGCCCTGAGGATGGAGGTGCTGGGCTGTGAGGCCCAGGACCTGTACT
AGGTCCCGTTGGTCCTGTCGAAGTGGGGACACCACTTGTCGGACCTGGGGGGGGGACGACTGGTCTATGGACTCCTAAGTGGGGGTCTCGACCCACGTGGTCTAACGGGACTCCTACCTCCACGACCCGACACTCCGGGTCCTGGACATGA
6,010 6,020 6,030 6,040 6,050 6,060 6,070 6,080 6,090 6,100 6,110 6,120 6,130 6,140 6,150
GE-868 (1373_ORF_exon2-26)
GE-007 (WPRE_3pUTR)
GATTAATTAAGAGCATCTTACCGCCATTTATTCCCATATTTGTTCTGTTTTTCTTGATTTGGGTATACATTTAAATGTTAATAAAACAAAATGGTGGGGCAATCATTTACATTTTTAGGGATATGTAATTACTAGTTCAGGTGTATTGCC
CTAATTAATTCTCGTAGAATGGCGGTAAATAAGGGTATAAACAAGACAAAAAGAACTAAACCCATATGTAAATTTACAATTATTTTGTTTTACCACCCCGTTAGTAAATGTAAAAATCCCTATACATTAATGATCAAGTCCACATAACGG
6,160 6,170 6,180 6,190 6,200 6,210 6,220 6,230 6,240 6,250 6,260 6,270 6,280 6,290 6,300
GE-007 (WPRE_3pUTR)
ACAAGACAAACATGTTAAGAAACTTTCCCGTTATTTACGCTCTGTTCCTGTTAATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGATATTCTTAACTATGTTGCTCCTTTTACGCTGTGTGGATATGCTGCTTTATAGCCTCT
TGTTCTGTTTGTACAATTCTTTGAAAGGGCAATAAATGCGAGACAAGGACAATTAGTTGGAGACCTAATGTTTTAAACACTTTCTAACTGACTATAAGAATTGATACAACGAGGAAAATGCGACACACCTATACGACGAAATATCGGAGA
6,310 6,320 6,330 6,340 6,350 6,360 6,370 6,380 6,390 6,400 6,410 6,420 6,430 6,440 6,450
GE-007 (WPRE_3pUTR)
GTATCTAGCTATTGCTTCCCGTACGGCTTTCGTTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTCTTTTAGAGGAGTTGTGGCCCGTTGTCCGTCAACGTGGCGTGGTGTGCTCTGTGTTTGCTGACGCAACCCCCACTGGCTGGGG
CATAGATCGATAACGAAGGGCATGCCGAAAGCAAAAGAGGAGGAAACATATTTAGGACCAACGACAGAGAAAATCTCCTCAACACCGGGCAACAGGCAGTTGCACCGCACCACACGAGACACAAACGACTGCGTTGGGGGTGACCGACCCC
6,460 6,470 6,480 6,490 6,500 6,510 6,520 6,530 6,540 6,550 6,560 6,570 6,580 6,590 6,600
GE-001 (bGH)
GE-007 (WPRE_3pUTR)
CATTGCCACCACCTGTCAACTCCTTTCTGGGACTTTCGCTTTCCCCCTCCGATCGCCACGGCAGAACTCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCTAGGTTGCTGGGCACTGATAATTCCGTGGTGTTGTCTGTGCCTTC
GTAACGGTGGTGGACAGTTGAGGAAAGACCCTGAAAGCGAAAGGGGGAGGGCTAGCGGTGCCGTCTTGAGTAGCGGCGGACGGAACGGGCGACGACCTGTCCCCGATCCAACGACCCGTGACTATTAAGGCACCACAACAGACACGGAAG
6,610 6,620 6,630 6,640 6,650 6,660 6,670 6,680 6,690 6,700 6,710 6,720 6,730 6,740 6,750
GE-001 (bGH)
TAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCA
ATCAACGGTCGGTAGACAACAAACGGGGAGGGGGCACGGAAGGAACTGGGACCTTCCACGGTGAGGGTGACAGGAAAGGATTATTTTACTCCTTTAACGTAGCGTAACAGACTCATCCACAGTAAGATAAGACCCCCCACCCCACCCCGT
6,760 6,770 6,780 6,790 6,800 6,810 6,820 6,830 6,840 6,850 6,860 6,870 6,880 6,890 6,900
GE-001 (bGH)
GE-1117 (HNF1)
GGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGCTCTAGAGCATGGCTACGTAGATAAGTAGCATGGCGGGTTAATCATTAACTACACCTGCAGGAGGAACCCCTAGTGATGGAGTTG
CCTGTCGTTCCCCCTCCTAACCCTTCTGTTATCGTCCGTACGACCCCTACGCCACCCGAGATACCGAGATCTCGTACCGATGCATCTATTCATCGTACCGCCCAATTAGTAATTGATGTGGACGTCCTCCTTGGGGATCACTACCTCAAC
6,910 6,920 6,930 6,940 6,950 6,960 6,970 6,980 6,990 7,000 7,007
GCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGCTGCCTGCAGG
CGGTGAGGGAGAGACGCGCGAGCGAGCGAGTGACTCCGGCCCGCTGGTTTCCAGCGGGCTGCGGGCCCGCCGGAGTCACTCGCTCGCTCGCGCGTCGACGGACGTCC

FIG. 9

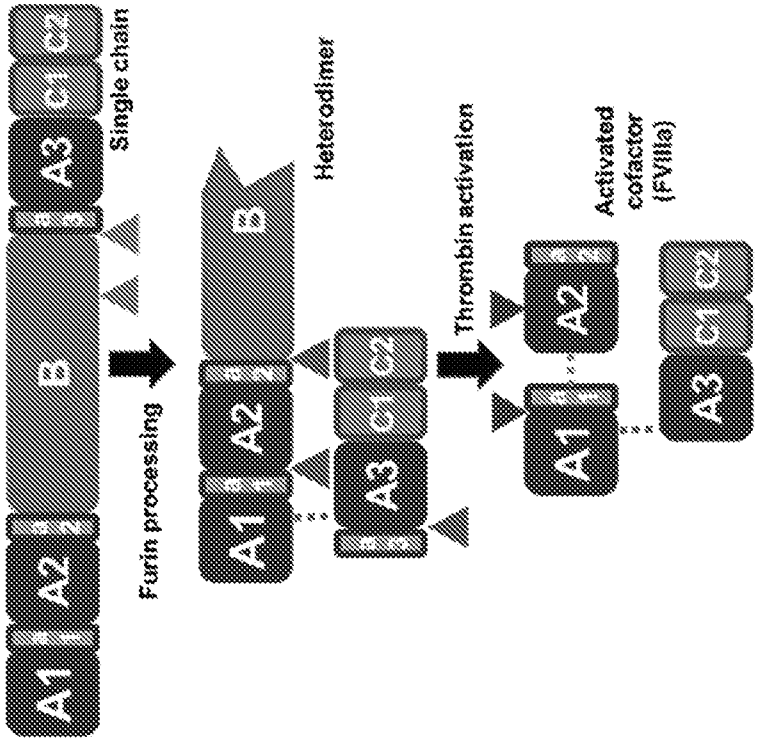

A1
A2
B
A3
C1
C2
Single chain
Furin processing
Heterodimer
Thrombin activation
Activated cofactor (FVIIIa)

FIG. 10

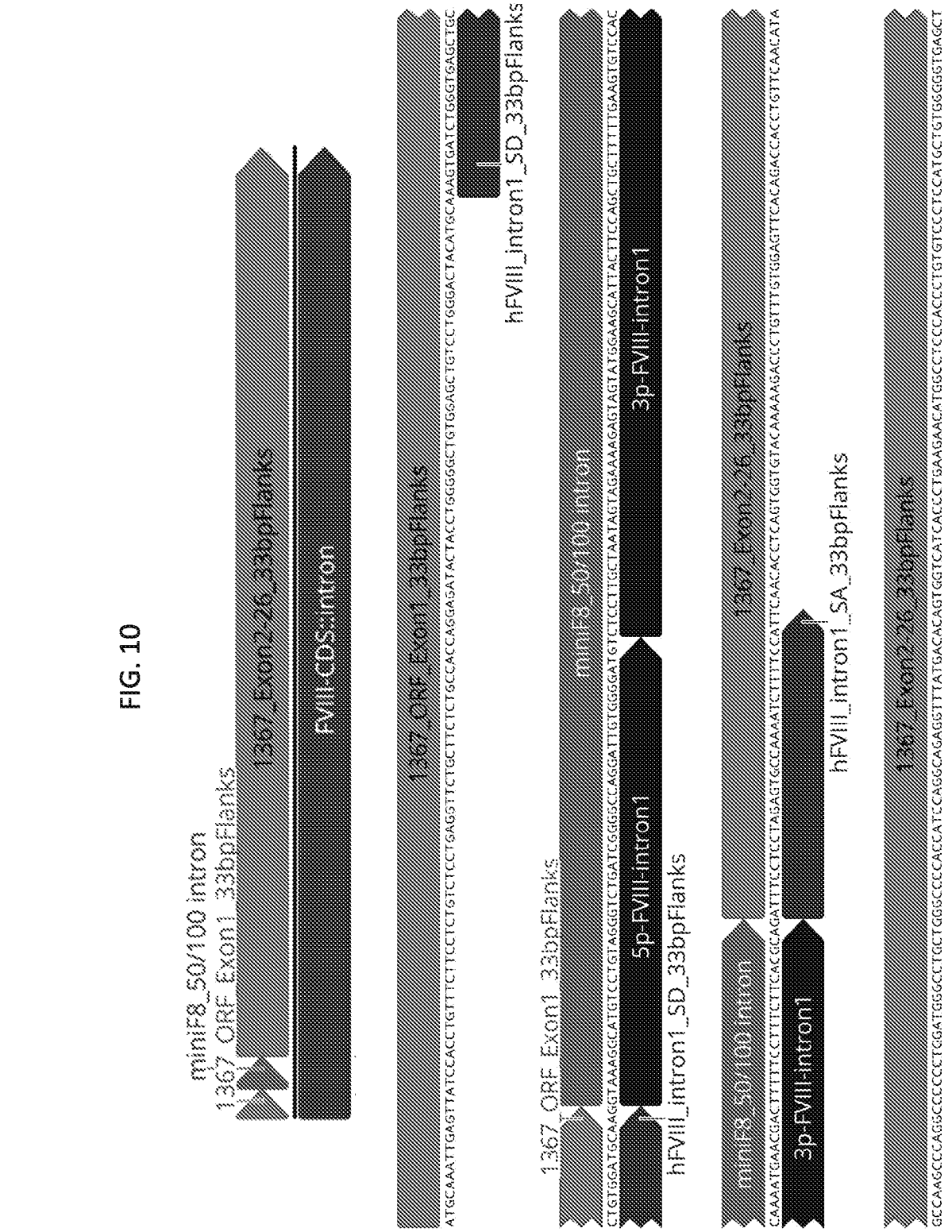

A)
miniF8_50/100 intron
1367_ORF_Exon1_33bpFlanks
1367_Exon2-26_33bpFlanks
FVIII-CDS-intron
B)
1367_ORF_Exon1_33bpFlanks
ATGCAAATTGAGTTATCCACCTGTTTCTTCCTCTGTCTCCTGAGGTTCTGCTTCTCTGCCACCAGGAGATACTACCTGGGGGCTGTGGAGCTGTCCTGGGACTACATGCAAAGTGATCTGGGTGAGCTGC
hFVIII_intron1_SD_33bpFlanks
1367_ORF_Exon1_33bpFlanks
CTGTGGATGCAAGGTAAAGGCATGTCCTGTAGGGTCTGATCGGGGCCAGGATTGTGGGGATGTCTCCTTGCTAATAGTAGAAAAGAGTAGTATGGAAGCATTACTTCCAGCTGCTTTTTGAAGTGTCCAC
5p-FVIII-intron1
3p-FVIII-intron1
hFVIII_intron1_SD_33bpFlanks
miniF8_50/100 intron
1367_Exon2-26_33bpFlanks
CAAAATCAACGACTTTTTCCTTTCTTCTTCACGCCAGATTTTCCTTTCCATTTCAACACCTCAGTGGTGTACAAAAAAGACCCTGTTTGTGGAGTTCACAGACCACCTGTTCAACATA
3p-FVIII-intron1
hFVIII_intron1_SA_33bpFlanks
1367_Exon2-26_33bpFlanks
GCCAAGCCCAGGCCCCCCTGGATGGGCCTGCTGGGCCCCACCATCCAGGCAGAGGTTTATGACACAGTGGTCATCACCCTGAAGAACATGGCCTCCCACCCTGTGTCCCTCCATGCTGTGGGGGTGAGCT

FIG. 11

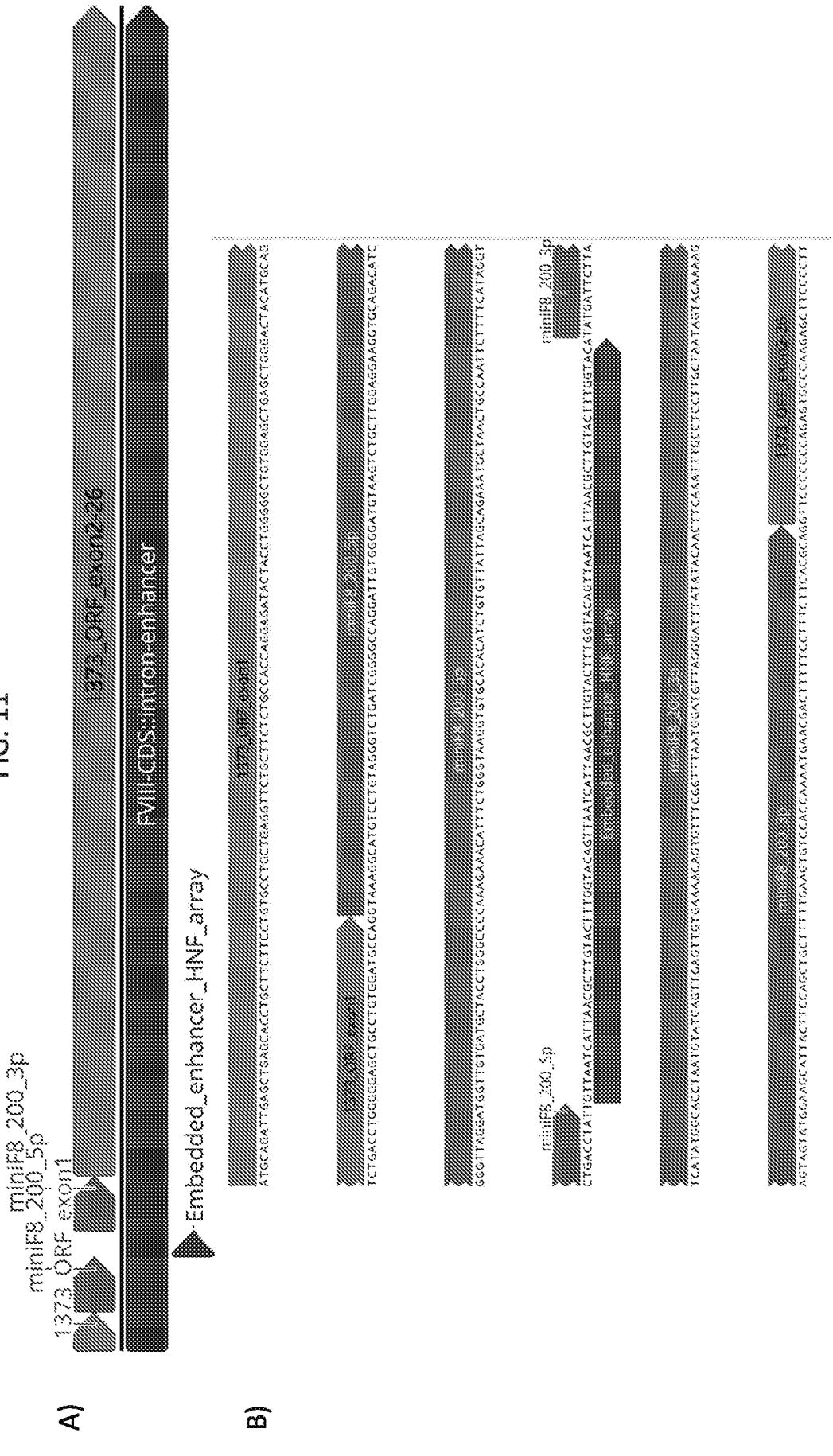

A)
miniF8_200_3p
miniF8_200_5p
1373_ORF_exon1
1373_ORF_exon2-26
FVIII-CDS-intron-enhancer
Embedded_enhancer_HNF_array
B)
1373_ORF_exon1
miniF8_200_3p
miniF8_200_5p
Embedded_enhancer_HNF_array
1373_ORF_exon2-26

FIG. 12

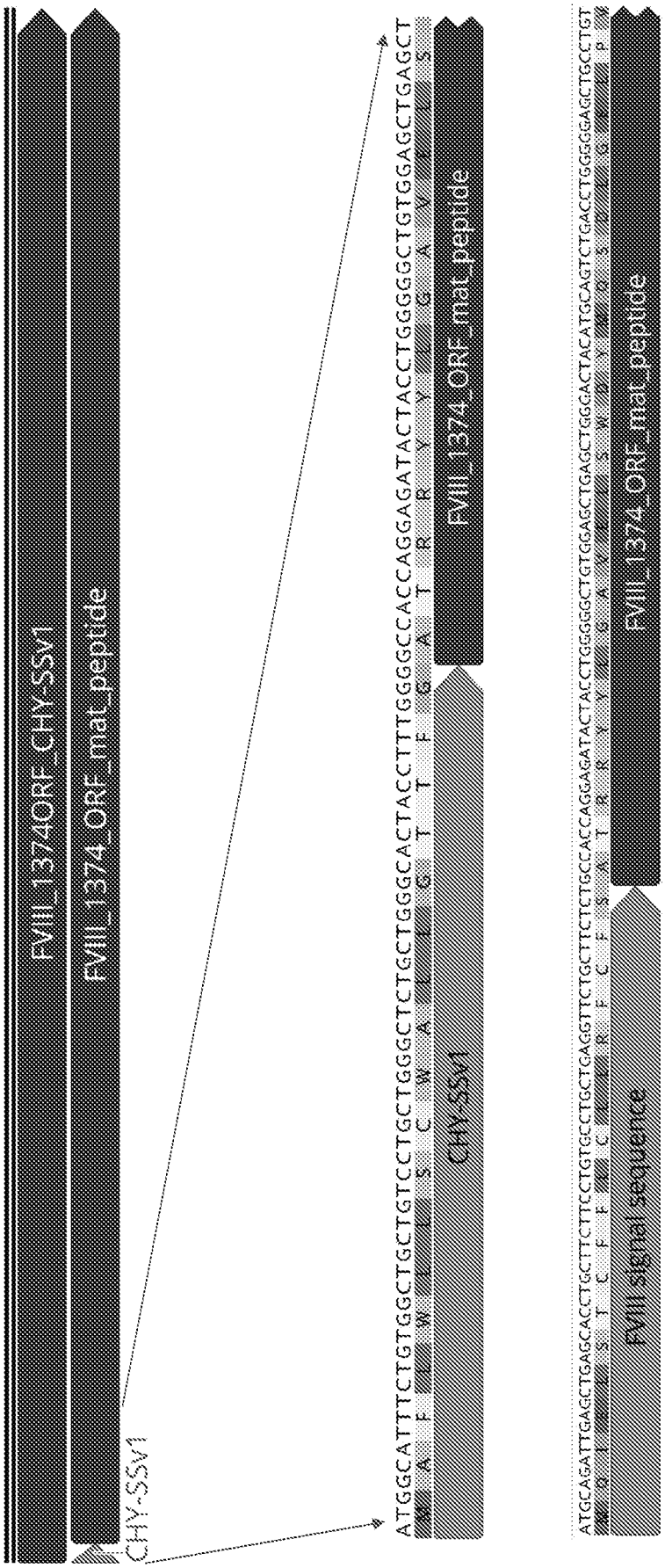

FVIII_1374ORF_CHY-SSv1
FVIII_1374_ORF_mat_peptide
CHY-SSv1
FVIII_1374_ORF_mat_peptide
CHY-SSv1
FVIII signal sequence
FVIII_1374_ORF_mat_peptide

FIG. 13

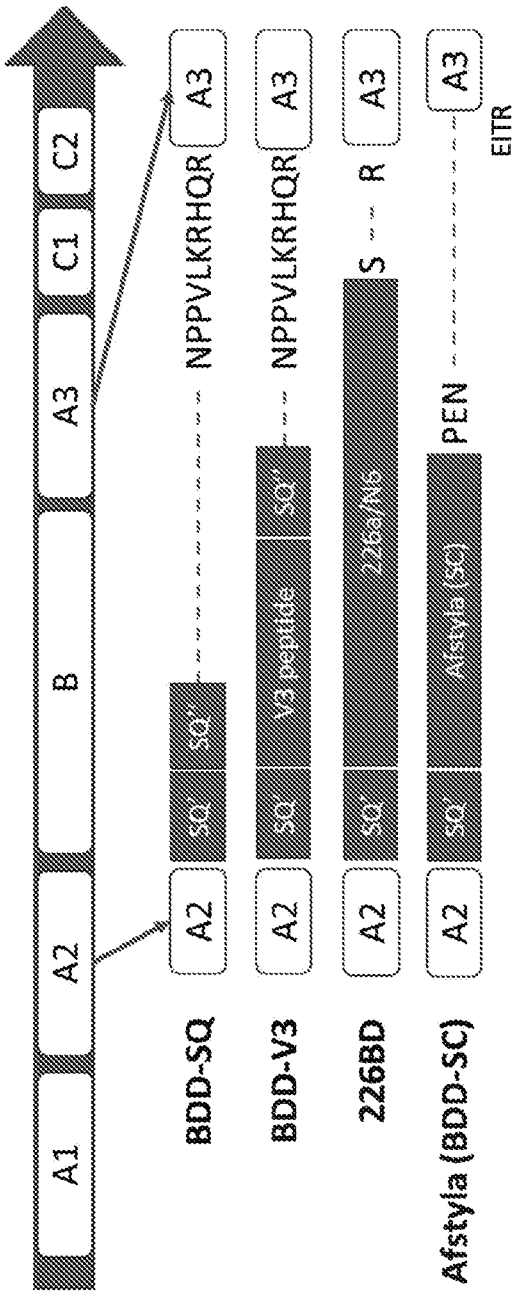
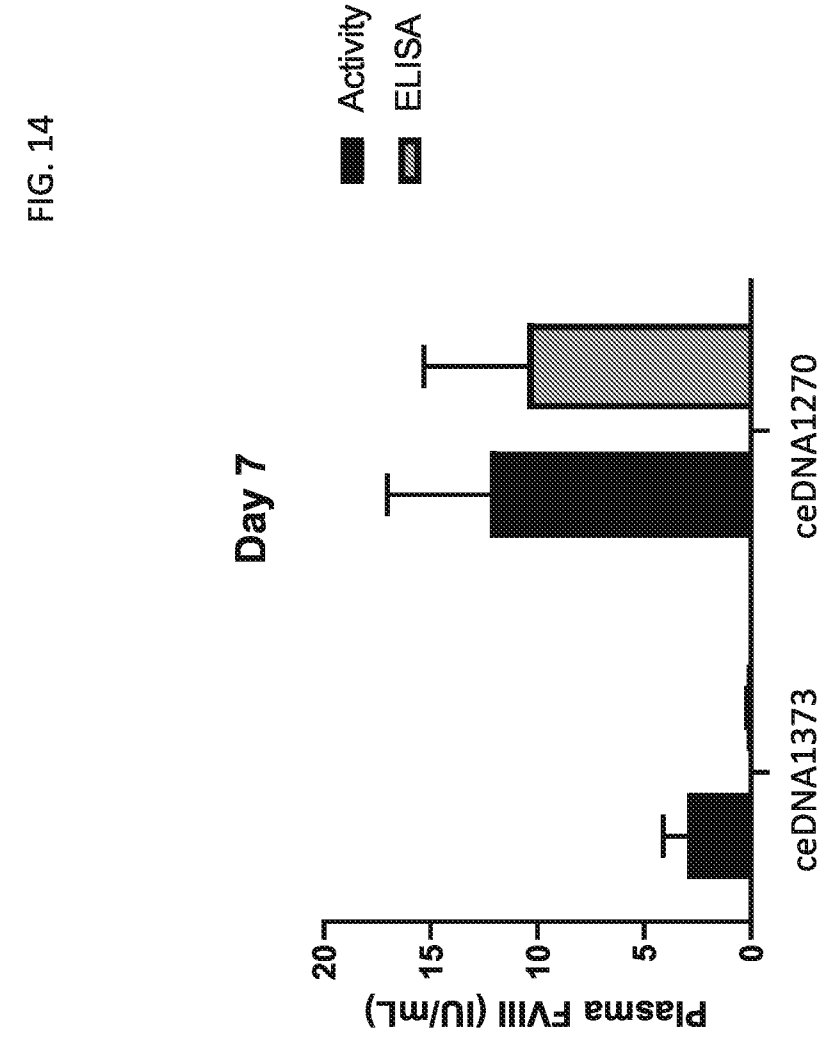
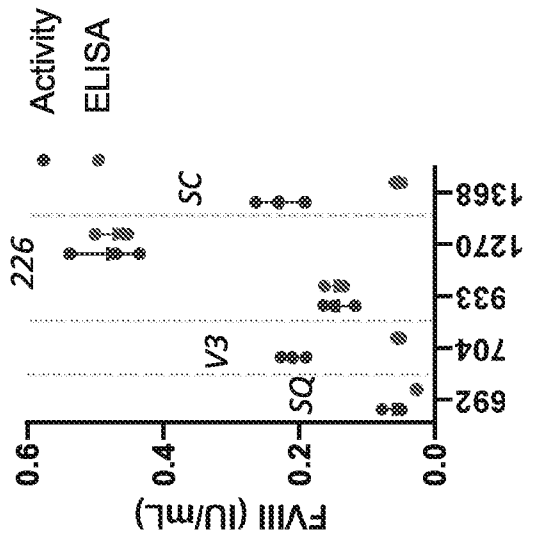

A1
A2
B
A3
C1
C2
BDD-SQ
BDD-V3
226BD
Afstyla (BDD-SC)
A2
SQ
SQ'
V3 peptide
SQ''
226/Ab
Afstyla (SQ)
NPPVLKRHQR
S
R
PEN
EITR
A3

FIG. 14


Activity
ELISA
Day 7
Plasma FVIII (IU/mL)
0
5
10
15
20
ceDNA1373
ceDNA1270


Activity
ELISA
FVIII (IU/mL)
0.0
0.2
0.4
0.6
692
704
933
1270
1368
226
V3
SQ
SC

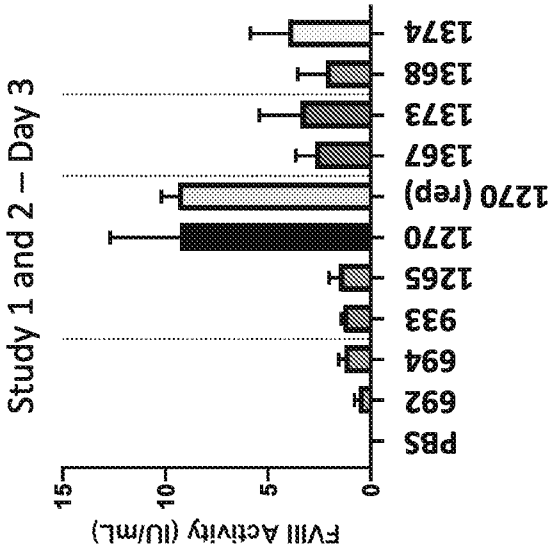

Study 1 and 2 – Day 3
FVIII Activity (IU/mL)
15
10
5
0
PBS
692
694
933
1265
1270
1270 (rep)
1367
1373
1368
1374

FIG. 15

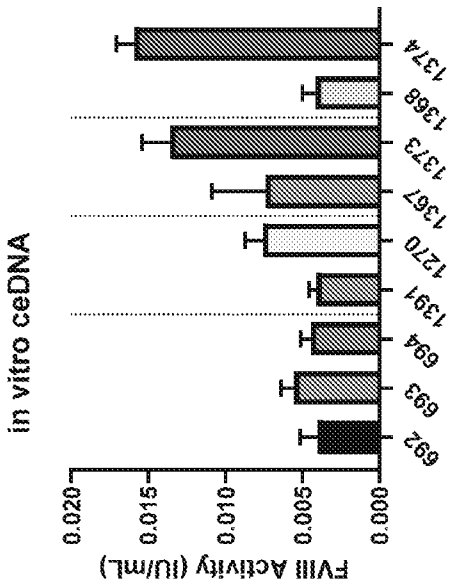
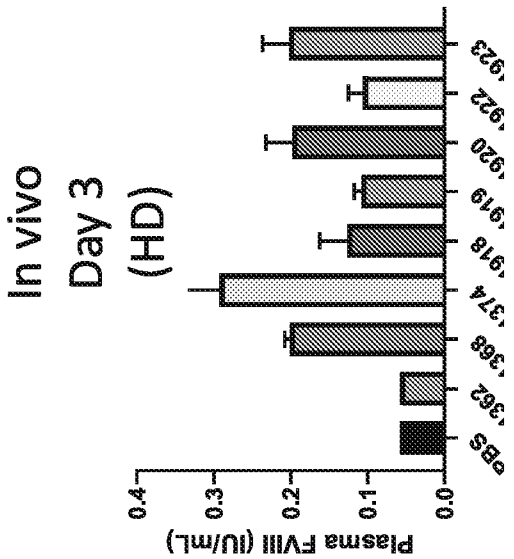
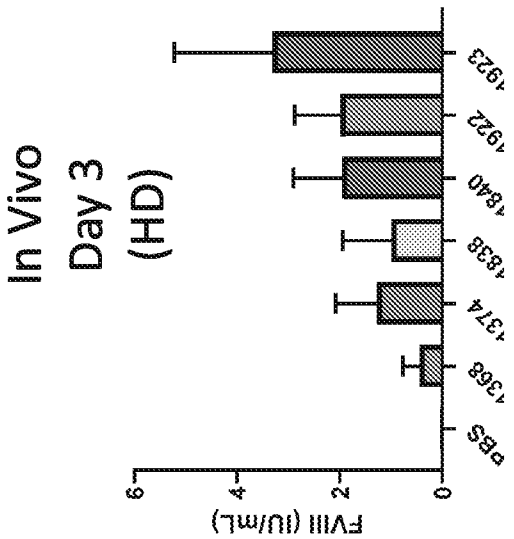

in vitro ceDNA
FVIII Activity (IU/mL)
0.020
0.015
0.010
0.005
0.000
692
693
694
1391
1270
1367
1373
1368
1374

FIG. 16

Strain	Group	Treatment	Dosing Route (Female)	Injection Volume (Female) ?	Injection/Infusion Duration	Bolus Vol. (ml/kg)	Bolus (mg/kg)	Treatment Regimen, N
FVIII KO (B6;129S-F8<tm1Kaz>/J)	5	LNP 0933	226/N6	Yes	300 mg/kg, 10 ml/kg, Day 0 30 min. pre-dose & 5 hr post-dose	5	2.0	Once on Day 0 by IV
	6	LNP 1270	226/N6	Yes				
	7	LNP 1367	SC	Yes				
	8	LNP 1368	SC	No				

FIG. 17


In vivo
Day 3
(HD)
Plasma FVIII (IU/mL)
0.0
0.1
0.2
0.3
0.4
vBS
362
368
374
918
919
920
922
923


In Vivo
Day 3
(HD)
FVIII (IU/mL)
0
2
4
6
vBS
368
374
838
840
922
923

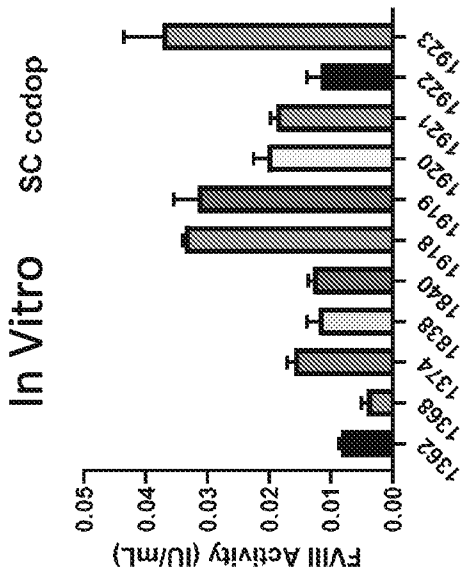

In Vitro
SC codop
FVIII Activity (IU/mL)
0.00
0.01
0.02
0.03
0.04
0.05
362
368
374
838
840
918
919
920
921
922
923

FIG. 18

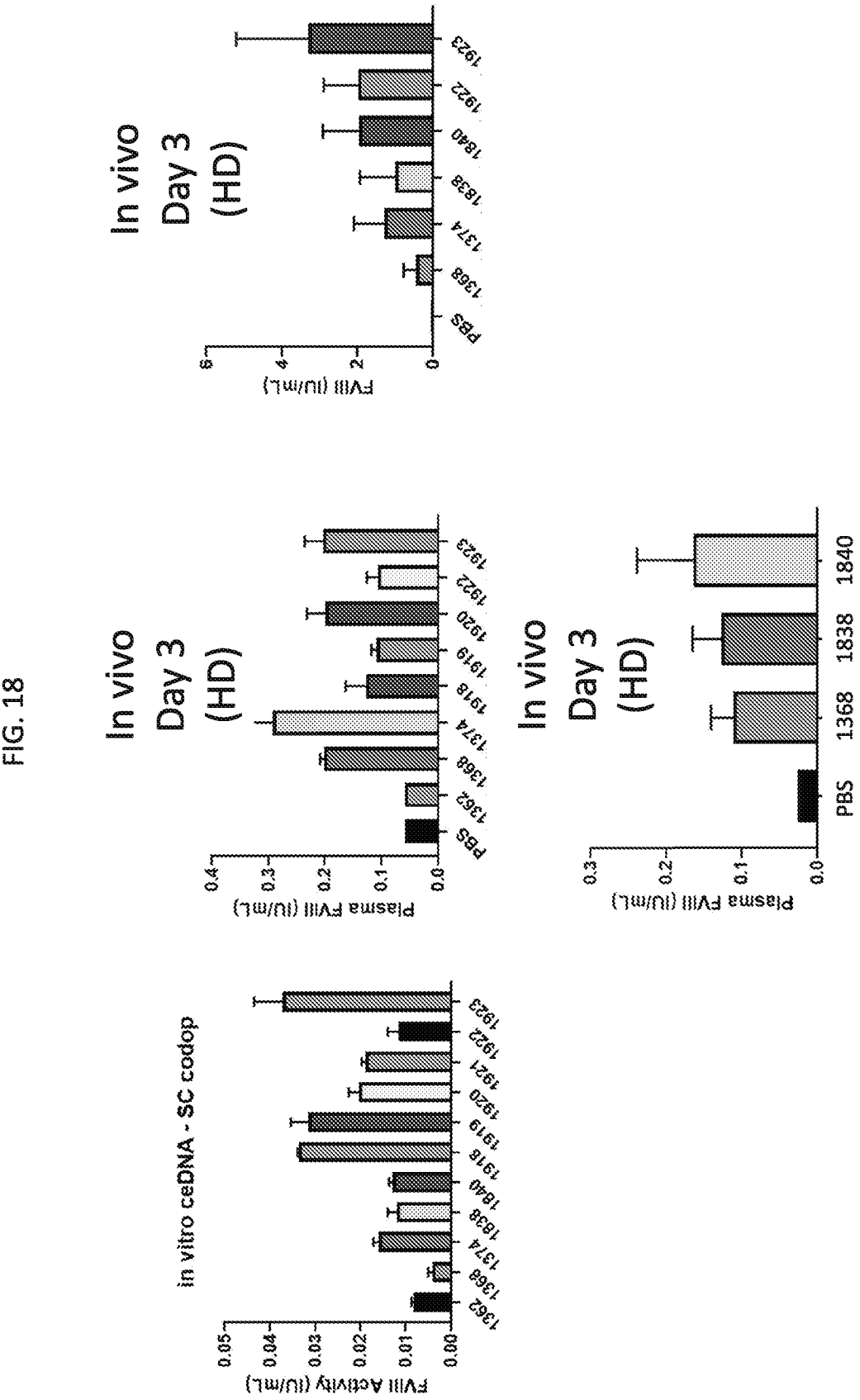

In vivo
Day 3
(HD)
FVIII (IU/mL)
PBS
1368
1374
1838
1840
1922
1923
In vivo
Day 3
(HD)
Plasma FVIII (IU/mL)
PBS
1362
1368
1374
1838
1840
1918
1919
1920
1922
1923
In vivo
Day 3
(HD)
Plasma FVIII (IU/mL)
PBS
1368
1838
1840
in vitro ceDNA - SC codop
FVIII Activity (IU/mL)
1362
1368
1374
1838
1840
1918
1919
1920
1921
1922
1923

FIG. 19

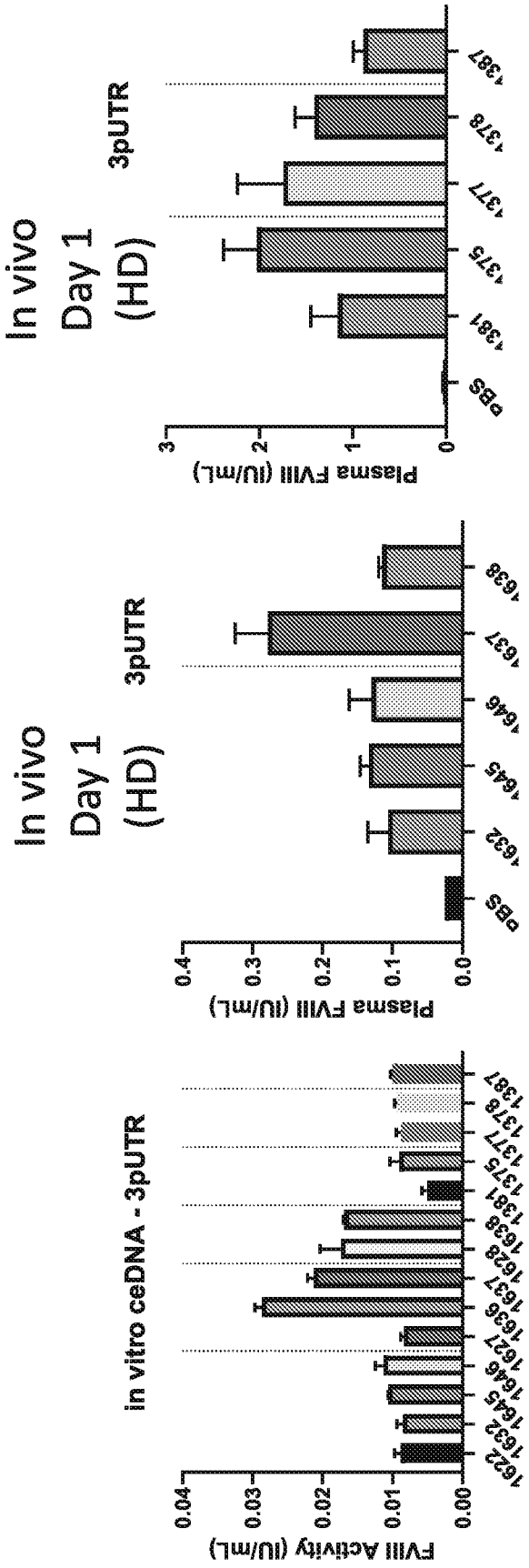

In vivo
Day 1
(HD)
3pUTR
Plasma FVIII (IU/mL)
0
1
2
3
pBS
1381
1375
1377
1378
1387
In vivo
Day 1
(HD)
3pUTR
Plasma FVIII (IU/mL)
0.0
0.1
0.2
0.3
0.4
pBS
1632
1645
1646
1637
1638
in vitro ceDNA - 3pUTR
FVIII Activity (IU/mL)
0.00
0.01
0.02
0.03
0.04
1622
1632
1645
1646
1627
1636
1637
1628
1638
1381
1375
1377
1378
1387

FIG. 20

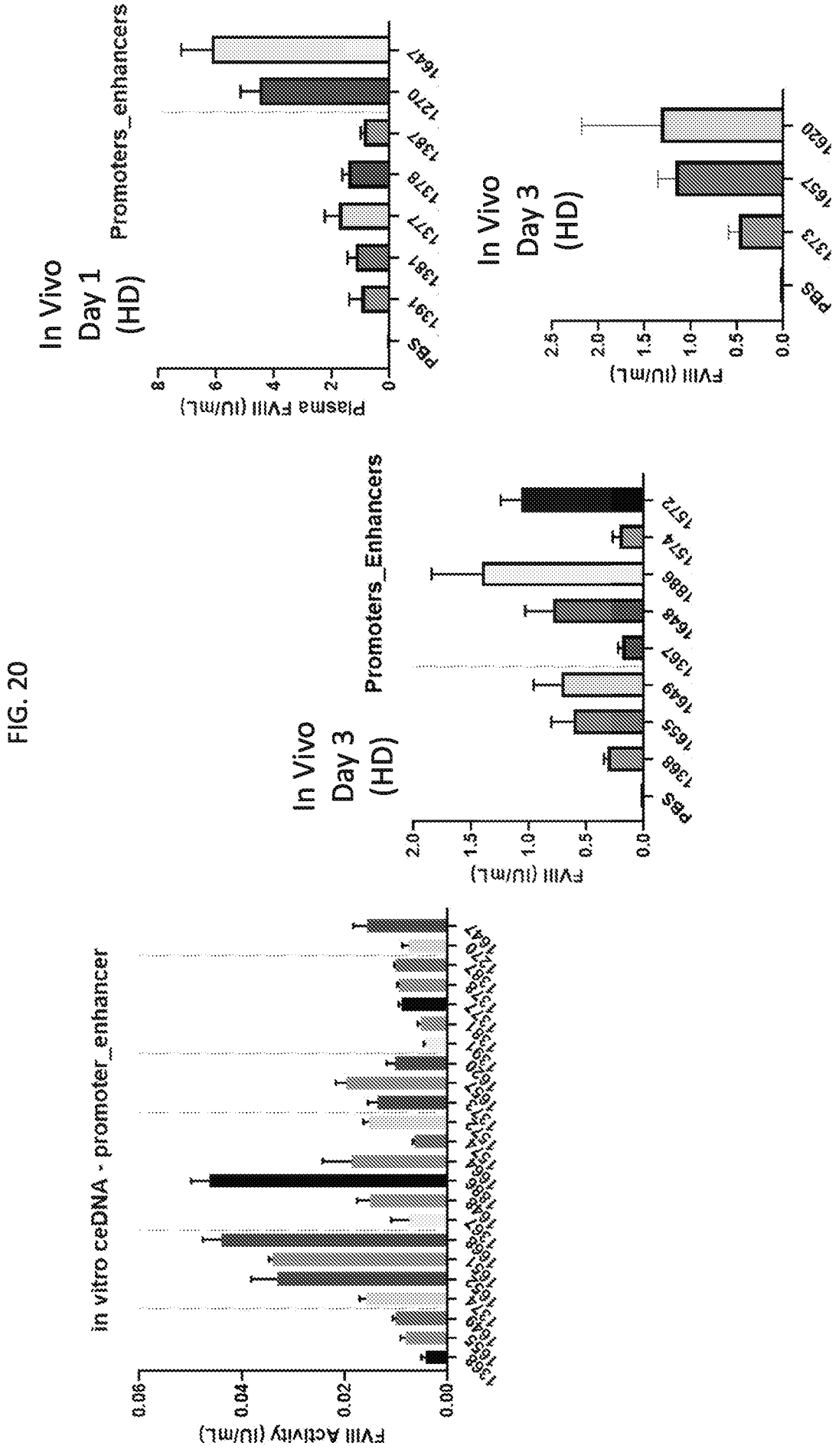
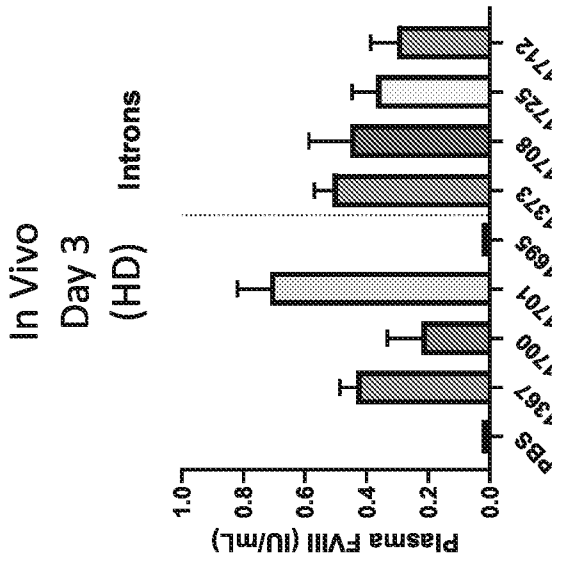

in vitro ceDNA - promoter_enhancer
FVIII Activity (IU/mL)
0.00
0.02
0.04
0.06
In Vivo
Day 3
(HD)
Promoters_Enhancers
FVIII (IU/mL)
0.0
0.5
1.0
1.5
2.0
PBS
1368
1655
1649
1367
1648
1886
1574
1572
In Vivo
Day 3
(HD)
FVIII (IU/mL)
0.0
0.5
1.0
1.5
2.0
2.5
PBS
1373
1657
1620
In Vivo
Day 1
(HD)
Promoters_enhancers
Plasma FVIII (IU/mL)
0
2
4
6
8
PBS
1391
1381
1377
1378
1387
1270
1647

FIG. 21


In Vivo
Day 3
(HD)
Introns
Plasma FVIII (IU/mL)
1.0
0.8
0.6
0.4
0.2
0.0
PBS
1367
1700
1701
1695
1373
1708
1725
1712

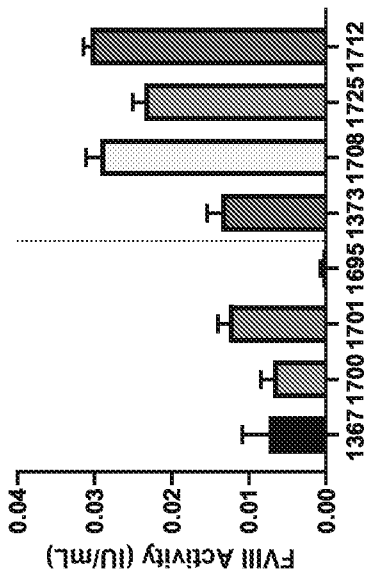
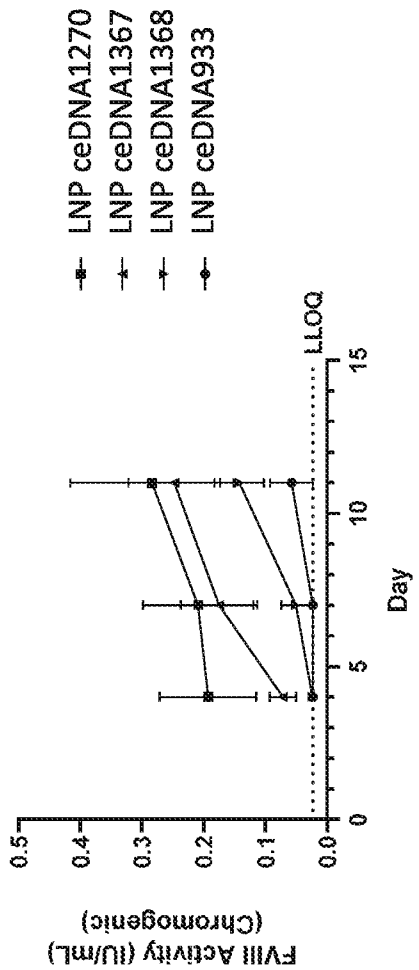

in vitro ceDNA - introns
FVIII Activity (IU/mL)
0.04
0.03
0.02
0.01
0.00
1367
1700
1701
1695
1373
1708
1725
1712

FIG. 22


LNP ceDNA1270
LNP ceDNA1367
LNP ceDNA1368
LNP ceDNA933
LLOQ
FVIII Activity (IU/mL)
(Chromogenic)
0.5
0.4
0.3
0.2
0.1
0.0
0
5
10
15
Day

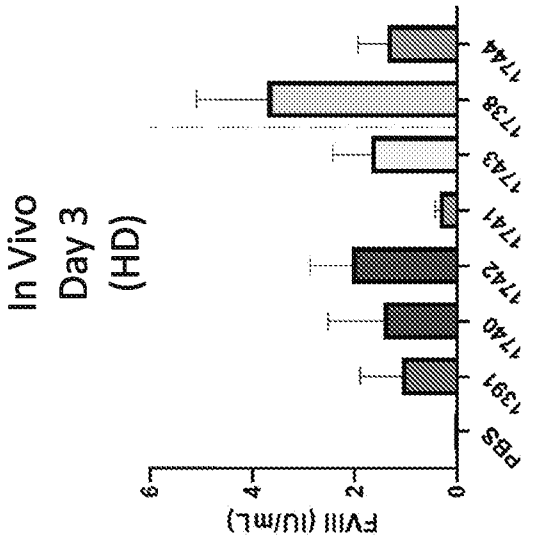

In Vivo
Day 3
(HD)
FVIII (IU/mL)
0
2
4
6
PBS
1391
1740
1742
1741
1743
1738
1744

FIG. 23

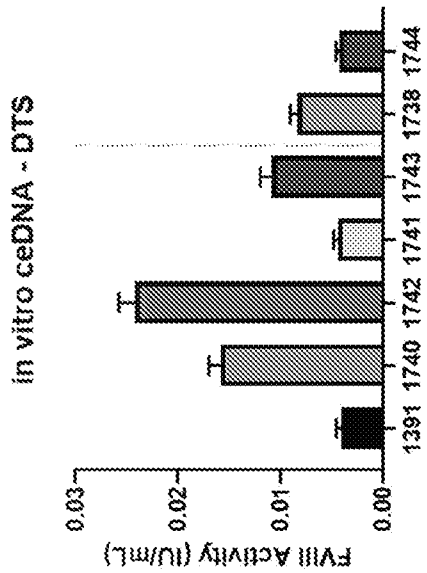
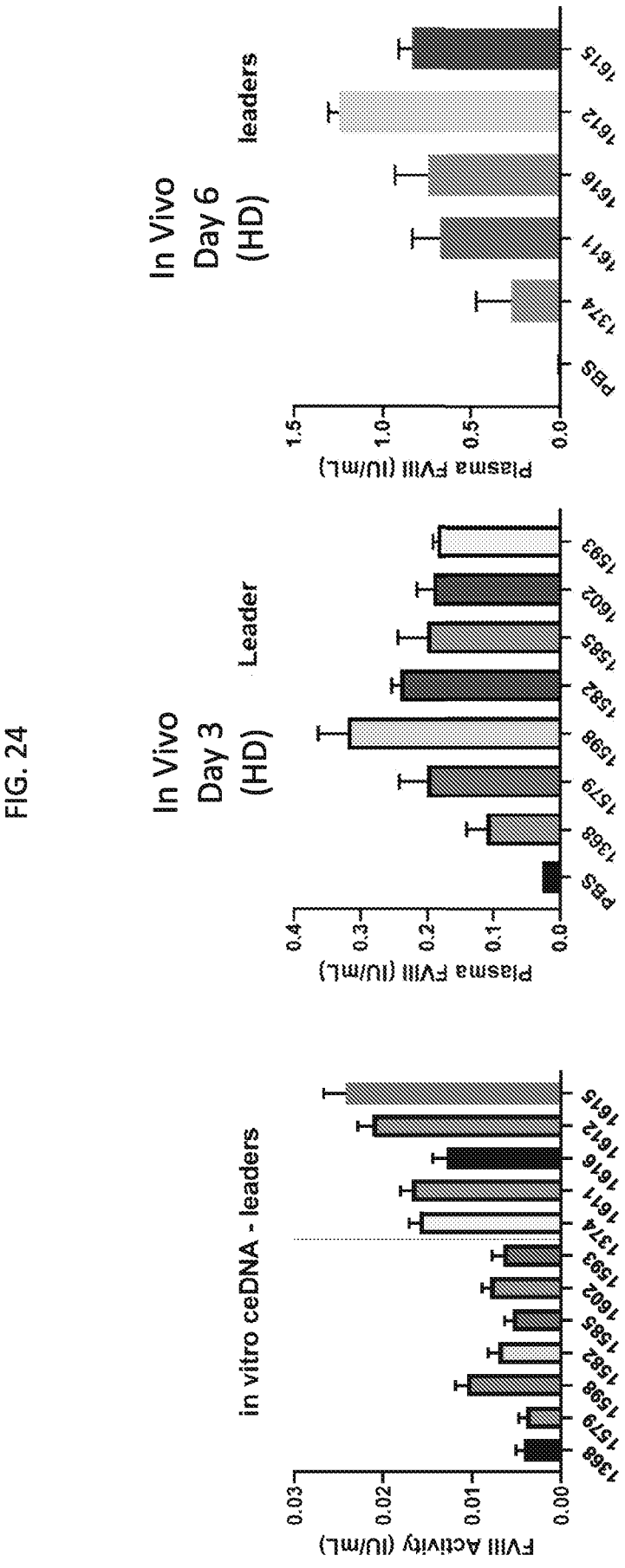

in vitro ceDNA - DTS
FVIII Activity (IU/mL)
0.00
0.01
0.02
0.03
1391
1740
1742
1741
1743
1738
1744

FIG. 24


in vitro ceDNA - leaders
FVIII Activity (IU/mL)
0.03
0.02
0.01
0.00
1368
1579
1598
1582
1585
1602
1593
1374
1611
1616
1612
1615
In Vivo
Day 3
(HD)
Leader
Plasma FVIII (IU/mL)
0.4
0.3
0.2
0.1
0.0
PBS
1368
1579
1598
1582
1585
1602
1593
In Vivo
Day 6
(HD)
leaders
Plasma FVIII (IU/mL)
1.5
1.0
0.5
0.0
PBS
1374
1611
1616
1612
1615

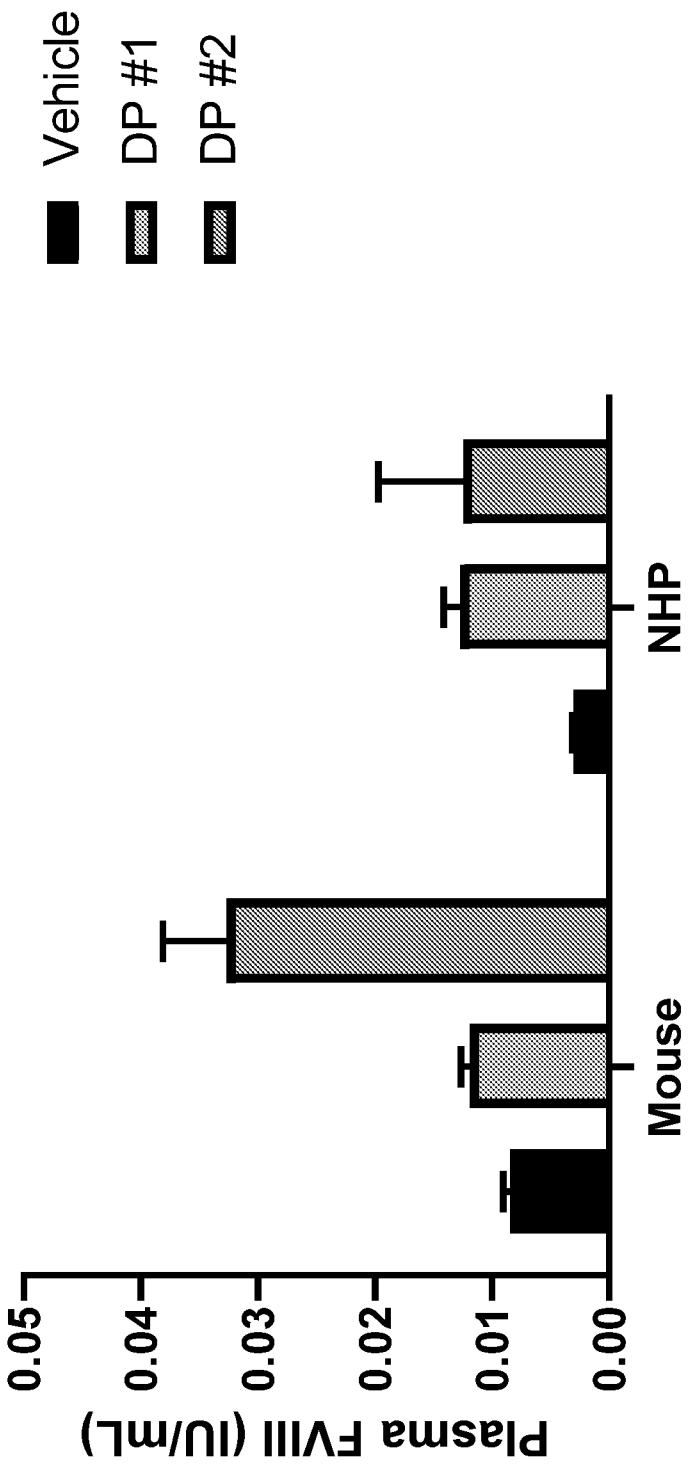

Vehicle
DP #1
DP #2
Plasma FVIII (IU/mL)
0.05
0.04
0.03
0.02
0.01
0.00
Mouse
NHP

FIG. 25

FIG. 26

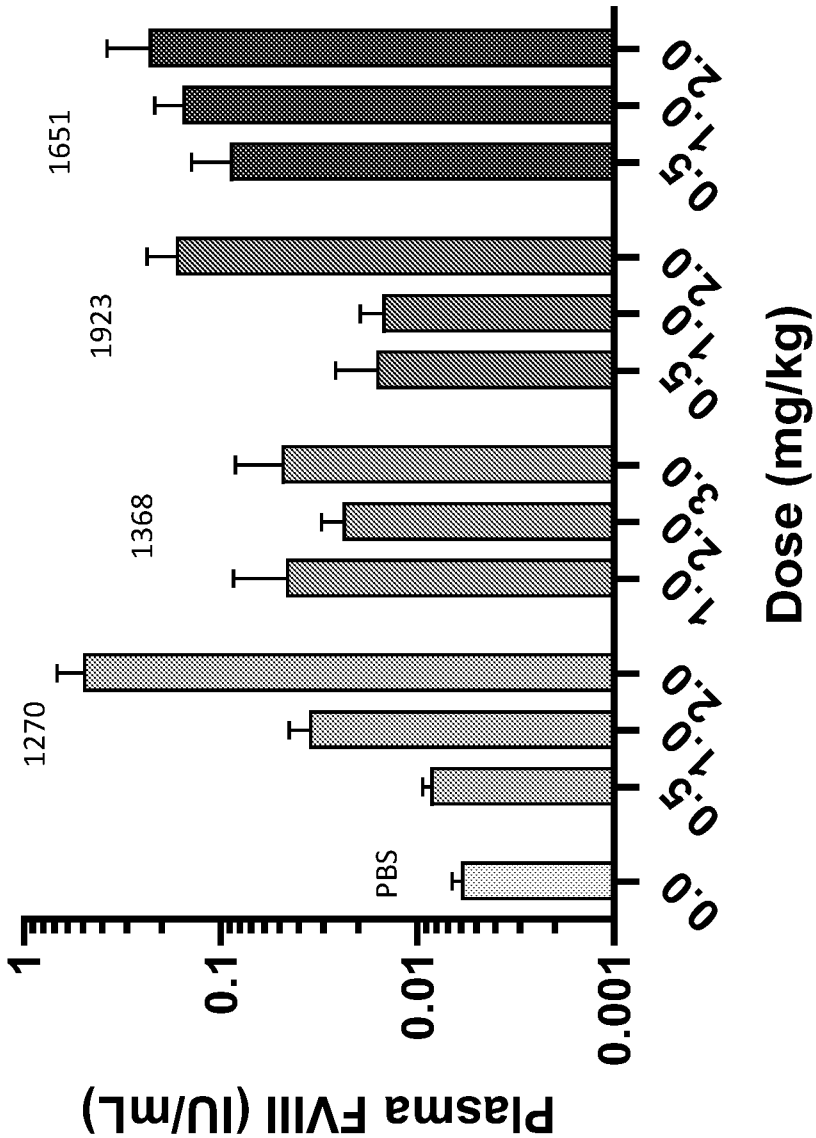

Plasma FVIII (IU/mL)
1
0.1
0.01
0.001
PBS
1270
1368
1923
1651
0.0
0.5
1.0
2.0
0.5
1.0
2.0
0.5
1.0
2.0
0.5
1.0
2.0
Dose (mg/kg)

FIG. 27

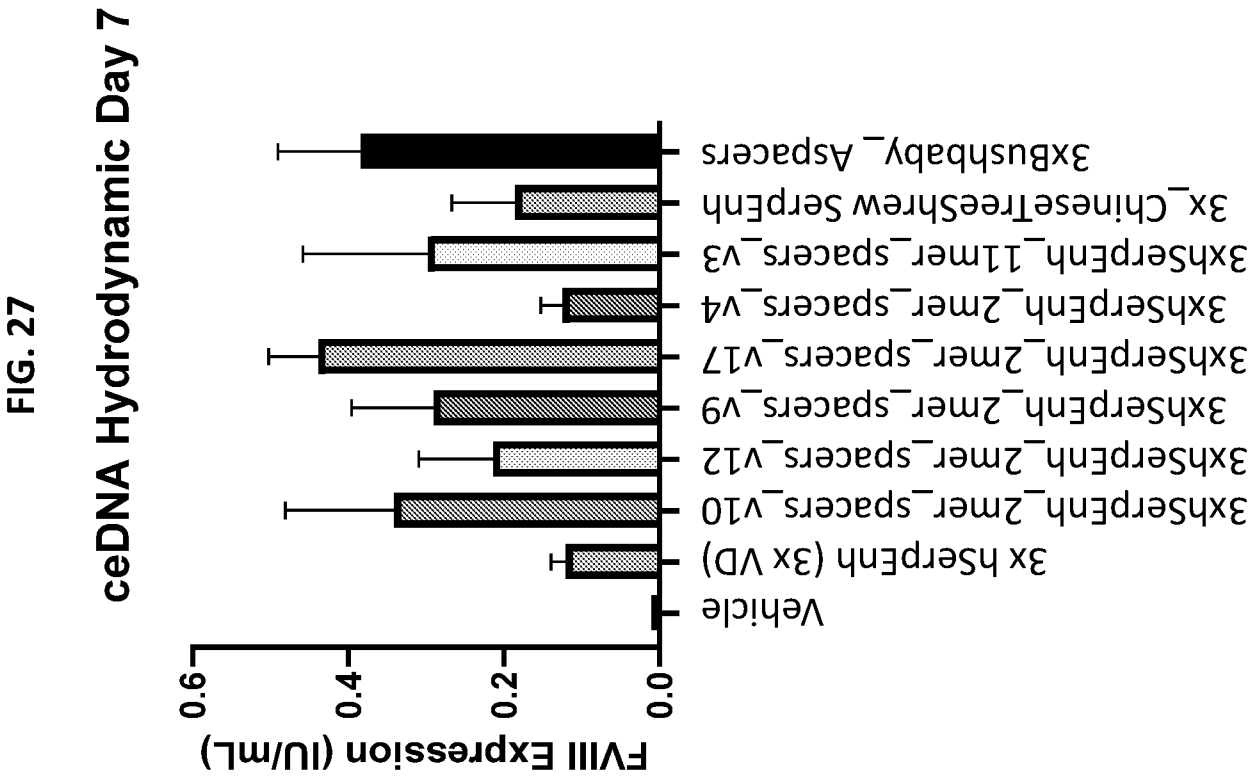
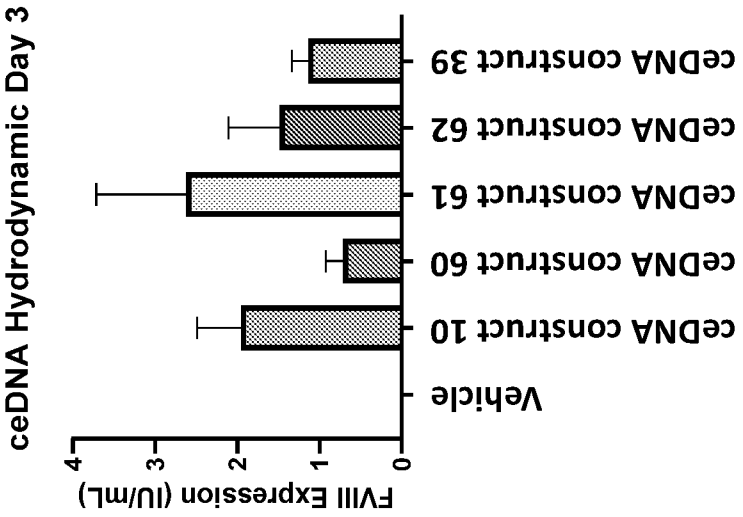

ceDNA Hydrodynamic Day 7
FVIII Expression (IU/mL)
0.0
0.2
0.4
0.6
Vehicle
3x hSerpEnh (3x VD)
3xhSerpEnh_2mer_spacers_v10
3xhSerpEnh_2mer_spacers_v12
3xhSerpEnh_2mer_spacers_v9
3xhSerpEnh_2mer_spacers_v17
3xhSerpEnh_2mer_spacers_v4
3xhSerpEnh_11mer_spacers_v3
3x_ChineseTreeShrew SerpEnh
3xBushbaby_ Aspacers

FIG. 28


ceDNA Hydrodynamic Day 3
FVIII Expression (IU/mL)
0
1
2
3
4
Vehicle
ceDNA construct 10
ceDNA construct 60
ceDNA construct 61
ceDNA construct 62
ceDNA construct 39

SEQUENCE LISTING

<110> GENERATION BIO INC.
KEENAN, Jessica Lynn

<120> NON-VIRAL DNA VECTORS AND USES THEREOF FOR EXPRESSING FVIII THERAPEUTICS

<130> 131698-08220

<140>
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<150> 63/132,838
<151> 2020-12-31

<150> 63/079,349
<151> 2020-09-16

<160> 641

<170> PatentIn version 3.5

<210> 1
<211> 5065
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 1
cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccgggcaaag cccgggcgtc 60

gggcgacctt tggtcgcccg gcctcagtga gcgagcgagc gcgcagagag ggagtggcca 120

actccatcac taggggttcc ttgtagttaa tgattaaccc gccatgctac ttatctacgt 180

agccatgctc tagagtttgc tgcttgcaat gtttgcccat tttagggtgg acacaggacg 240

ctgtggtttc tgagccaggg ggcgactcag atcccagcca gtggacttag cccctgtttg 300

ctcctccgat aactggggtg accttggtta atattcacca gcagcctccc ccgttgcccc 360

tctggatcca ctgcttaaat acggacgagg acagggccct gtctcctcag cttcaggcac 420

caccactgac ctgggacagt gaatcgccac catgcagatt gagctgagca cctgcttctt 480

cctgtgcctg ctgaggttct gcttctctgc caccaggaga tactacctgg gggctgtgga 540

gctgagctgg gactacatgc agtctgacct gggggagctg cctgtggatg ccaggttccc 600

ccccagagtg cccaagagct tccccttcaa cacctctgtg gtgtacaaga agaccctgtt 660

tgtggagttc actgaccacc tgttcaacat tgccaagccc aggccccccct ggatgggcct 720

gctgggcccc accatccagg ctgaggtgta tgacactgtg gtgatcaccc tgaagaacat 780

ggccagccac cctgtgagcc tgcatgctgt gggggtgagc tactggaagg cctctgaggg 840

ggctgagtat gatgaccaga ccagccagag ggagaaggag gatgacaagg tgttccctgg 900

gggcagccac acctatgtgt ggcaggtgct gaaggagaat ggccccatgg cctctgaccc 960

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polynucleotide

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polynucleotide

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polynucleotide

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polynucleotide

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polynucleotide

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polynucleotide

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polynucleotide

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cagatcatgt ctgacaagag gaatgtgatc ctgttctcag tgtttgatga gaacagatcc 1800

tggtacctca ctgagaacat ccagaggttc ctgcccaacc cagcaggggt gcagctggag 1860

gacccagagt tccaggcctc caacatcatg cactccatca atggctatgt ctttgactcc 1920

ctgcagctgt cagtgtgcct gcatgaggtg gcctactggt acatcctctc cattggagcc 1980

cagactgact tcctgtcagt cttcttcagt ggctacacct tcaagcacaa gatggtctat 2040

gaggacactc tgaccctgtt ccccttctct ggggagactg tgttcatgtc catggagaac 2100

cctggactgt ggatcctggg ctgccacaac agtgacttca ggaacagggg catgactgcc 2160

ctgctgaagg tctccagctg tgacaagaac actggggact actatgagga ctcctatgag 2220

gacatctctg cctacctgct gtccaagaac aatgccattg agcccaggtc cttctcccag 2280

aacagcagac acccctccac caggcagaag cagttcaatg caaccaccat ccctgaaaat 2340

accaccctgc agagtgacca ggaggagatt gactatgatg acaccatctc tgtggagatg 2400

aagaaggagg actttgacat ctatgatgag gatgagaacc agagccctag aagcttccag 2460

aagaagacca gacactactt cattgctgca gtggaaagac tgtgggacta tgggatgagc 2520

tcctcccccc atgtgctgag gaacagggcc cagtctggct ctgtgcccca gttcaagaag 2580

gtggtgttcc aggagttcac tgatggctcc ttcacccagc ccctgtacag gggagagctg 2640

aatgagcacc tgggcctgct gggcccctac atcagggctg aggtggagga caatatcatg 2700

gtgaccttca gaaaccaggc ctccagacct tactccttct acagcagcct gatctcctat 2760

gaggaggacc agagacaggg ggctgagccc aggaagaact ttgtgaagcc caatgagacc 2820

aagacctact tctggaaggt gcagcaccac atggccccca ccaaggatga gtttgactgc 2880

aaggcctggg cctacttctc tgatgtggac ctggagaagg atgtgcactc tggcctgatt 2940

ggcccctgc tggtgtgcca caccaacacc ctgaaccctg cccatggcag gcaggtgact 3000

gtgcaggagt ttgccctgtt cttcaccatc tttgatgaga ccaagagctg gtacttcact 3060

gagaacatgg agaggaactg cagggccccc tgcaacatcc agatggagga ccccaccttc 3120

aaggagaact acaggttcca tgccatcaat ggctacatca tggacaccct gcctggcctg 3180

gtgatggccc aggaccagag gatcaggtgg tacctgctga gcatggggag caatgagaac 3240

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gctggaatct ggagggtgga gtgcctgatt ggggagcacc tgcatgctgg catgagcacc 3420

ctgttcctgg tgtacagcaa caagtgccag acccccctgg gcatggcctc tggccacatc 3480

agagacttcc agatcacagc ctcaggccag tatggccagt gggcccccaa gctggccagg 3540

ctgcactact ctggcagcat caatgcctgg tccaccaagg agcccttcag ctggatcaag 3600

gtggacctcc tggcccccat gatcatccat ggcatcaaga cccaggggggc caggcagaag 3660

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cagacctaca gaggcaacag cacaggcacc ctgatggtgt tctttggcaa tgtggacagc 3780

tctgggatca agcacaacat cttcaacccc cccatcattg ccaggtacat cagactgcac 3840

cccacccact actccatcag gtccaccctg agaatggagc tgatgggctg tgacctgaac 3900

agctgcagca tgcccctggg catggagagc aaggccatct ctgatgccca gatcacagcc 3960

agcagctact tcaccaacat gtttgccacc tggtccccca gcaaggccag gctgcacctg 4020

cagggcagga gcaatgcctg gaggccccag gtgaacaacc ccaaggagtg gctgcaggtg 4080

gacttccaga agaccatgaa ggtgacaggg gtgaccaccc agggggtgaa gtccctgctg 4140

accagcatgt atgtgaagga gttcctgatc agcagctccc aggatggcca ccagtggacc 4200

ctgttcttcc agaatggcaa ggtgaaggtg ttccagggca accaggacag cttcaccccct 4260

gtggtgaact ccctggaccc ccccctgctg accagatacc tgaggatcca cccccagtcc 4320

tgggtgcacc agattgccct gagaatggag gtgctgggct gtgaggccca ggacctgtac 4380

tga 4383

<210> 184
<211> 436
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 184

gttaatcatt aacgcttgta ctttggtaca gttaatcatt aacgcttgta ctttggtaca 60

gttaatcatt aacgcttgta ctttggtaca atcgatgcga gaacttgtgc ctccccgtgt 120

tcctgctctt tgtccctctg tcctacttag actaatattt gccttgggta ctgcaaacag 180

gaaatggggg agggacagga gtagggcgga gggtagttaa gaatgtggat ccccgtctgt 240

ctgcacattt cgtagagcga gtgttccgat actctaatct ccctaggcaa ggttcatatt 300

tgtgtaggtt acttattctc cttttgttga ctaagtcaat aatcagaatc agcaggtttg 360

gagtcagctt ggcagggatc agcagcctgg gttggaagga gggggtataa aagccccttc 420

accaggagaa gccgtc 436

<210> 185
<211> 773
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 185
gttaatcatt aacgcttgta ctttggtaca gttaatcatt aacgcttgta ctttggtaca 60

gttaatcatt aacgcttgta ctttggtaca atcgatgcga gaacttgtgc ctccccgtgt 120

tcctgctctt tgtccctctg tcctacttag actaatattt gccttgggta ctgcaaacag 180

gaaatggggg agggacagga gtagggcgga gggtagttaa gaatgtcaat tgggggggagg 240

ctgctggtga atattaacca aggtcacccc agttatcgga ggagcaaaca ggggctaagt 300

ccaccggggg aggctgctgg tgaatattaa ccaaggtcac cccagttatc ggaggagcaa 360

acaggggcta agtccaccgg gggaggctgc tggtgaatat taaccaaggt caccccagtt 420

atcggaggag caaacagggg ctaagtccac ggtacccact gggaggatgt tgagtaagat 480

ggaaaactac tgatgaccct tgcagagaca gagtattagg acatgtttga acaggggccg 540

ggcgatcagc aggtagcact agaggatccc cgtctgtctg cacatttcgt agagcgagtg 600

ttccgatact ctaatctccc taggcaaggt tcatatttgt gtaggttact tattctcctt 660

ttgttgacta agtcaataat cagaatcagc aggtttggag tcagcttggc agggatcagc 720

agcctgggtt ggaaggaggg ggtataaaag ccccttcacc aggagaagcc gtc 773

<210> 186
<211> 431
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 186
aacaggttag tttgactagc gttaatcatt aacgttaatc attaacgtta atcattaacg 60

ttaatcatta acgttaatca ttaacatcga tgcgagaact tgtgcctccc cgtgttcctg 120

ctctttgtcc ctctgtccta cttagactaa tatttgcctt gggtactgca aacaggaaat 180

gggggaggga caggagtagg gcggagggta gttaagaatg tggatccccg tctgtctgca 240

catttcgtag agcgagtgtt ccgatactct aatctcccta ggcaaggttc atatttgtgt 300

aggttactta ttctccttttt gttgactaag tcaataatca gaatcagcag gtttggagtc 360

agcttggcag ggatcagcag cctgggttgg aaggaggggg tataaaagcc ccttcaccag 420

gagaagccgt c 431

<210> 187
<211> 768
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 187
aacaggttag tttgactagc gttaatcatt aacgttaatc attaacgtta atcattaacg 60

ttaatcatta acgttaatca ttaacatcga tgcgagaact tgtgcctccc cgtgttcctg 120

ctctttgtcc ctctgtccta cttagactaa tatttgcctt gggtactgca aacaggaaat 180

gggggaggga caggagtagg gcggagggta gttaagaatg tcaattgggg ggaggctgct 240

ggtgaatatt aaccaaggtc accccagtta tcggaggagc aaacaggggc taagtccacc 300

gggggaggct gctggtgaat attaaccaag gtcaccccag ttatcggagg agcaaacagg 360

ggctaagtcc accgggggag gctgctggtg aatattaacc aaggtcaccc cagttatcgg 420

aggagcaaac aggggctaag tccacggtac ccactgggag gatgttgagt aagatggaaa 480

actactgatg acccttgcag agacagagta ttaggacatg tttgaacagg ggccgggcga 540

tcagcaggta gcactagagg atccccgtct gtctgcacat ttcgtagagc gagtgttccg 600

atactctaat ctccctaggc aaggttcata tttgtgtagg ttacttattc tccttttgtt 660

gactaagtca ataatcagaa tcagcaggtt tggagtcagc ttggcaggga tcagcagcct 720

gggttggaag gaggggggtat aaaagcccct tcaccaggag aagccgtc 768

<210> 188
<211> 540
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 188
gttaatcatt aacgcttgta ctttggtaca gttaatcatt aacgcttgta ctttggtaca 60

gttaatcatt aacgcttgta ctttggtaca atcgatgcga gaacttgtgc ctccccgtgt 120

tcctgctctt tgtccctctg tcctacttag actaatattt gccttgggta ctgcaaacag 180

gaaatggggg agggacagga gtagggcgga gggtagcccg gggattctgc agtgagagca 240

gagggccagc taagtggtac tctcccagag actgtctgac tcacgccacc ccctccacct 300

tggacacagg acgctgtggt ttctgagcca ggtacaatga ctcctttcgg taagtgcagt 360

ggaagctgta cactgcccag gcaaagcgtc cgggcagcgt aggcgggcga ctcagatccc 420

agccagtgga cttagcccct gtttgctcct ccgataactg gggtgacctt ggttaatatt 480

caccagcagc ctcccccgtt gcccctctgg atccactgct taaatacgga cgaggacagg 540

<210> 189
<211> 624
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 189
agcaacagat cattctatgg gttaatcatt aacaacaggt taggcttgta ctttggtaca 60

ttggctccta gttaatcatt aacttagcaa tgtgcttgta ctttggtaca tagttaccta 120

gttaatcatt aacaaacagt atggcttgta ctttggtaca agcaacagat cattctatgg 180

gcgagaactt gtgcctcccc gtgttcctgc tctttgtccc tctgtcctac ttagactaat 240

atttgccttg ggtactgcaa acaggaaatg ggggagggac aggagtaggg cggagggtag 300

caattggatt ctgcagtgag agcagagggc cagctaagtg gtactctccc agagactgtc 360

tgactcacgc caccccctcc accttggaca caggacgctg tggtttctga gccaggtaca 420

atgactcctt tcggtaagtg cagtggaagc tgtacactgc ccaggcaaag cgtccgggca 480

gcgtaggcgg gcgactcaga tcccagccag tggacttagc ccctgtttgc tcctccgata 540

actggggtga ccttggttaa tattcaccag cagcctcccc cgttgcccct ctggatccac 600

tgcttaaata cggacgagga cagg 624

<210> 190
<211> 951
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 190
aggctcagag gcacacagga gtttctgggc tcaccctgcc cccttccaac ccctcagttc 60

ccatcctcca gcagctgttt gtgtgctgcc tctgaagtcc acactgaaca aacttcagcc 120

tactcatgtc cctaaaatgg gcaaacattg caagcagcaa acagcaaaca cacagccctc 180

cctgcctgct gaccttggag ctggggcaga ggtcagagac ctctctgggc ccatgccacc 240

tccaacatcc actcgacccc ttggaatttc ggtggagagg agcagaggtt gtcctggcgt 300

ggtttaggta gtgtgagagg gcttaagagc aacagatcat tctatgggtt aatcattaac 360

aacaggttag gcttgtactt tggtacattg gctcctagtt aatcattaac ttagcaatgt 420

gcttgtactt tggtacatag ttacctagtt aatcattaac aaacagtatg gcttgtactt 480

tggtacaagc aacagatcat tctatgggcg agaacttgtg cctccccgtg ttcctgctct 540

ttgtccctct gtcctactta gactaatatt tgccttgggt actgcaaaca ggaaatgggg 600

gagggacagg agtagggcgg agggtagcaa ttggattctg cagtgagagc agagggccag 660

ctaagtggta ctctcccaga gactgtctga ctcacgccac cccctccacc ttggacacag 720

gacgctgtgg tttctgagcc aggtacaatg actcctttcg gtaagtgcag tggaagctgt 780

acactgccca ggcaaagcgt ccgggcagcg taggcgggcg actcagatcc cagccagtgg 840

acttagcccc tgtttgctcc tccgataact ggggtgacct tggttaatat tcaccagcag 900

cctcccccgt tgcccctctg gatccactgc ttaaatacgg acgaggacag g 951

<210> 191
<211> 535
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 191
aacaggttag tttgactagc gttaatcatt aacgttaatc attaacgtta atcattaacg 60

ttaatcatta acgttaatca ttaacatcga tgcgagaact tgtgcctccc cgtgttcctg 120

ctctttgtcc ctctgtccta cttagactaa tatttgcctt gggtactgca aacaggaaat 180

gggggaggga caggagtagg gcggagggta gcccggggat tctgcagtga gagcagaggg 240

ccagctaagt ggtactctcc cagagactgt ctgactcacg ccacccccctc caccttggac 300

acaggacgct gtggtttctg agccaggtac aatgactcct ttcggtaagt gcagtggaag 360

ctgtacactg cccaggcaaa gcgtccgggc agcgtaggcg ggcgactcag atcccagcca 420

gtggacttag cccctgtttg ctcctccgat aactggggtg accttggtta atattcacca 480

gcagcctccc ccgttgcccc tctggatcca ctgcttaaat acggacgagg acagg 535

<210> 192
<211> 579
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 192
agcaacagat cattctatgg gttaatcatt aacaacaggt taggttaatc attaacttgg 60

ctcctagtta atcattaaca aacagtatgg ttaatcatta acaaattctg gggttaatca 120

ttaactagtt acctagcgag aacttgtgcc tccccgtgtt cctgctcttt gtccctctgt 180

cctacttaga ctaatatttg ccttgggtac tgcaaacagg aaatgggggа gggacaggag 240

tagggcggag ggtagcaatt ggattctgca gtgagagcag agggccagct aagtggtact 300

ctcccagaga ctgtctgact cacgccaccc cctccacctt ggacacagga cgctgtggtt 360

tctgagccag gtacaatgac tcctttcggt aagtgcagtg gaagctgtac actgcccagg 420

caaagcgtcc gggcagcgta ggcgggcgac tcagatccca gccagtggac ttagcccctg 480

tttgctcctc cgataactgg ggtgaccttg gttaatattc accagcagcc tcccccgttg 540

cccctctgga tccactgctt aaatacggac gaggacagg 579

<210> 193
<211> 906
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 193
aggctcagag gcacacagga gtttctgggc tcaccctgcc cccttccaac ccctcagttc 60

ccatcctcca gcagctgttt gtgtgctgcc tctgaagtcc acactgaaca aacttcagcc 120

tactcatgtc cctaaaatgg gcaaacattg caagcagcaa acagcaaaca cacagccctc 180

cctgcctgct gaccttggag ctggggcaga ggtcagagac ctctctgggc ccatgccacc 240

tccaacatcc actcgacccc ttggaatttc ggtggagagg agcagaggtt gtcctggcgt 300

ggtttaggta gtgtgagagg gcttaagagc aacagatcat tctatgggtt aatcattaac 360

aacaggttag gttaatcatt aacttggctc ctagttaatc attaacaaac agtatggtta 420

atcattaaca aattctgggg ttaatcatta actagttacc tagcgagaac ttgtgcctcc 480

ccgtgttcct gctctttgtc cctctgtcct acttagacta atatttgcct tgggtactgc 540

aaacaggaaa tgggggaggg acaggagtag ggcggagggt agcaattgga ttctgcagtg 600

agagcagagg gccagctaag tggtactctc ccagagactg tctgactcac gccacccccct 660

ccaccttgga cacaggacgc tgtggtttct gagccaggta caatgactcc tttcggtaag 720

tgcagtggaa gctgtacact gcccaggcaa agcgtccggg cagcgtaggc gggcgactca 780

gatcccagcc agtggactta gcccctgttt gctcctccga taactggggt gaccttggtt 840

aatattcacc agcagcctcc cccgttgccc ctctggatcc actgcttaaa tacggacgag 900

gacagg 906

<210> 194
<211> 286
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 194
gggggaggct gctggtgaat attaaccaag gtcaccccag ttatcggagg agcaaacagg 60

ggctaagtcc acacgcgtgg taccgtctgt ctgcacattt cgtagagcga gtgttccgat 120

actctaatct ccctaggcaa ggttcatatt tgtgtaggtt acttattctc cttttgttga 180

ctaagtcaat aatcagaatc agcaggtttg gagtcagctt ggcagggatc agcagcctgg 240

gttggaagga gggggtataa aagccccttc accaggagaa gccgtc 286

<210> 195
<211> 541
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 195
gggggaggct gctggtgaat attaaccaag gtcaccccag ttatcggagg agcaaacagg 60

ggctaagtcc accgggggag gctgctggtg aatattaacc aaggtcaccc cagttatcgg 120

aggagcaaac aggggctaag tccaccgggg gaggctgctg gtgaatatta accaaggtca 180

ccccagttat cggaggagca aacaggggct aagtccacgg tacccactgg gaggatgttg 240

agtaagatgg aaaactactg atgacccttg cagagacaga gtattaggac atgtttgaac 300

aggggccggg cgatcagcag gtagctctag aggatccccg tctgtctgca catttcgtag 360

agcgagtgtt ccgatactct aatctcccta ggcaaggttc atatttgtgt aggttactta 420

ttctcctttt gttgactaag tcaataatca gaatcagcag gtttggagtc agcttggcag 480

ggatcagcag cctgggttgg aaggaggggg tataaaagcc ccttcaccag gagaagccgt 540

c 541

<210> 196
<211> 452
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 196
cggtacgcgg gggaggctgc tggtgaatat taaccaaggt caccccagtt atcggaggag 60

caaacagggg ctaagtccac cgggggaggc tgctggtgaa tattaaccaa ggtcacccca 120

gttatcggag gagcaaacag gggctaagtc caccgggggа ggctgctggt gaatattaac 180

caaggtcacc ccagttatcg gaggagcaaa caggggctaa gtccactgta caacgcgtga 240

attcgctagc gtctgtctgc acatttcgta gagcgagtgt tccgatactc taatctccct 300

aggcaaggtt catatttgtg taggttactt attctccttt tgttgactaa gtcaataatc 360

agaatcagca ggtttggagt cagcttggca gggatcagca gcctgggttg gaaggagggg 420

gtataaaagc cccttcacca ggagaagccg tc 452

<210> 197
<211> 1125
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 197
aggctcagag gcacacagga gtttctgggc tcaccctgcc cccttccaac ccctcagttc 60

ccatcctcca gcagctgttt gtgtgctgcc tctgaagtcc acactgaaca aacttcagcc 120

tactcatgtc cctaaaatgg gcaaacattg caagcagcaa acagcaaaca cacagccctc 180

cctgcctgct gaccttggag ctggggcaga ggtcagagac ctctctgggc ccatgccacc 240

tccaacatcc actcgacccc ttggaatttc ggtggagagg agcagaggtt gtcctggcgt 300

ggtttaggta gtgtgagagg gtccgggttc aaaaccactt gctgggtggg gagtcgtcag 360

taagtggcta tgccccgacc ccgaagcctg tttccccatc tgtacaatgg aaatgataaa 420

gacgcccatc tgatagggtt tttgtggcaa ataaacattt ggtttttttg ttttgttttg 480

ttttgttttt tgagatggag gtttgctctg tcgcccaggc tggagtgcag tgacacaatc 540

tcatctcacc acaaccttcc cctgcctcag cctcccaagt agctgggatt acaagcatgt 600

gccaccacac ctggctaatt ttctattttt agtagagacg ggtttctcca tgttggtcag 660

cctcagcctc ccaagtaact gggattacag gcctgtgcca ccacacccgg ctaatttttt 720

ctatttttga cagggacggg gtttcaccat gttggtcagg ctggtctaga ggtactggat 780

cttgctacca gtggaacagc cactaaggat tctgcagtga gagcagaggg ccagctaagt 840

ggtactctcc cagagactgt ctgactcacg ccacccccctc caccttggac acaggacgct 900

gtggtttctg agccaggtac aatgactcct ttcggtaagt gcagtggaag ctgtacactg 960

cccaggcaaa gcgtccgggc agcgtaggcg ggcgactcag atcccagcca gtggacttag 1020

cccctgtttg ctcctccgat aactggggtg accttggtta atattcacca gcagcctccc 1080

ccgttgcccc tctggatcca ctgcttaaat acggacgagg acagg 1125

<210> 198
<211> 72
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 198
gggggaggct gctggtgaat attaaccaag gtcaccccag ttatcggagg agcaaacagg 60

ggctaagtcc ac 72

<210> 199
<211> 100
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 199
cactgggagg atgttgagta agatggaaaa ctactgatga cccttgcaga gacagagtat 60

taggacatgt ttgaacaggg gccgggcgat cagcaggtag 100

<210> 200
<211> 13

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 200
gttaatcatt aac 13

<210> 201
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 201
gcttgtactt tggtaca 17

<210> 202
<211> 770
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 202
aggctcagag gcacacagga gtttctgggc tcaccctgcc cccttccaac ccctcagttc 60

ccatcctcca gcagctgttt gtgtgctgcc tctgaagtcc acactgaaca aacttcagcc 120

tactcatgtc cctaaaatgg gcaaacattg caagcagcaa acagcaaaca cacagccctc 180

cctgcctgct gaccttggag ctggggcaga ggtcagagac ctctctgggc ccatgccacc 240

tccaacatcc actcgacccc ttggaatttc ggtggagagg agcagaggtt gtcctggcgt 300

ggtttaggta gtgtgagagg gtccgggttc aaaaccactt gctgggtggg gagtcgtcag 360

taagtggcta tgccccgacc ccgaagcctg tttccccatc tgtacaatgg aaatgataaa 420

gacgcccatc tgatagggtt tttgtggcaa ataaacattt ggtttttttg ttttgttttg 480

ttttgttttt tgagatggag gtttgctctg tcgcccaggc tggagtgcag tgacacaatc 540

tcatctcacc acaaccttcc cctgcctcag cctcccaagt agctgggatt acaagcatgt 600

gccaccacac ctggctaatt ttctattttt agtagagacg ggtttctcca tgttggtcag 660

cctcagcctc ccaagtaact gggattacag gcctgtgcca ccacacccgg ctaatttttt 720

ctatttttga cagggacggg gtttcaccat gttggtcagg ctggtctaga 770

<210> 203
<211> 120
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 203
gcgagaactt gtgcctcccc gtgttcctgc tctttgtccc tctgtcctac ttagactaat 60

atttgccttg ggtactgcaa acaggaaatg ggggagggac aggagtaggg cggagggtag 120

<210> 204
<211> 770
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 204
aggctcagag gcacacagga gtttctgggc tcaccctgcc cccttccaac ccctcagttc 60

ccatcctcca gcagctgttt gtgtgctgcc tctgaagtcc acactgaaca aacttcagcc 120

tactcatgtc cctaaaatgg gcaaacattg caagcagcaa acagcaaaca cacagccctc 180

cctgcctgct gaccttggag ctggggcaga ggtcagagac ctctctgggc ccatgccacc 240

tccaacatcc actcgacccc ttggaatttc agtggagagg agcagaggtt gtcctggtgt 300

ggtttaggta gtgtgagagg gtccaggttc aaaaccactt gctgggtggg gagttgtcag 360

taagtggcta tgccccgacc ccgaagcctg tttccccatc tgtacaatgg aaatgataaa 420

gacgcccatc tgatagggtt tttgtggcaa ataaacattt ggtttttttg ttttgttttg 480

ttttgttttt tgagatggag gtttgctctg tcgcccaggc tggagtgcag tgacacaatc 540

tcatctcacc acaaccttcc cctgcctcag cctcccaagt agctgggatt acaagcatgt 600

gccaccacac ctggctaatt ttctattttt agtagagacg ggtttctcca tgttggtcag 660

cctcagcctc ccaagtaact gggattacag gcctgtgcca ccacacccgg ctaatttttt 720

ctatttttga cagggacggg gtttcaccat gttggtcagg ctggtctaga 770

<210> 205
<211> 770
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 205
aggctcagag gcacacagga gtttctgggc tcaccctgcc cccttccaac ccctcagttc 60

ccatcctcca gcagctgttt gtgtgctgcc tctgaagtcc acactgaaca aacttcagcc 120

tactcatgtc cctaaaatgg gcaaacattg caagcagcaa acagcaaaca cacagccctc 180

cctgcctgct gaccttggag ctggggcaga ggtcagagac ctctctgggc ccatgccacc 240

tccaacatcc acttgacccc ttggaatttc ggtggagagg agcagaggtt gtcctggcgt 300

ggtttaggta gtgtgagagg gtccgggttc aaaaccactt gctgggtggg gagtcgtcag 360

taagtggcta tgccccaacc ctgaagcctg tttccccatc tgtacaatgg aaatgataaa 420

gacccccatc tgatagggtt tttgtggcaa ataaacattt ggtttttttg ttttgttttg 480

ttttgttttt tgagatggag gtttgctctg tcgcccaggc tggagtgcag tgacacaatc 540

tcatctcacc acaaccttcc cctgcctcag cctcccaagt agctgggatt acaagcatgt 600

gccaccacac ctggctaatt ttctattttt agtagagacg ggtttctcca tgttggtcag 660

cctcagcctc ccaagtaact gggattacag gcctgtgcca ccacacccgg ctaatttttt 720

ctatttttga cagggacggg gtttcaccat gttggtcagg ctggtctaga 770

<210> 206
<211> 770
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 206
aggctcagag gcacacagga gtttctgggc tcaccctgcc cccttccaac ccctcagttc 60

ccatcctcca gcagctgttt gtgtgctgcc tctgaagtcc acactgaaca aacttcagcc 120

tactcatgtc cctaaaatgg gcaaacattg caagcagcaa acagcaaaca cacagccctc 180

cctgcctgct gaccttggag ctggggcaga ggtcagagac ctctctgggc ccatgccacc 240

tccaacatcc actcgacccc ttggaatttc ggtggagagg agcagaggtt gtcctggcgt 300

ggtttaggta gtgtgagagg gtccgggttc aaaaccactt gctgggtggg gagtcgtcag 360

taagtggcta tgccccgacc ccgaagcctg tttccccatc tgtacaatgg aaatgataaa 420

gacgcccatc tgatagggtt tttgtggcaa ataaacattt ggtttttttg ttttgttttg 480

ttttgttttt tgagatggag gtttgctctg tggcccaggc tggagtgcag tgacacaatc 540

tcatctcacc acaaccttcc cctgcctcag cctcccaagt agctgggatt acaagcatgt 600

gccaccacac ctggctaatt ttctattttt agtagagatg ggtttctcca tgttggtcag 660

cctcagcctc ccaagtaact gggattacag gcctgtgcca ccacacctgg ctaatttttt 720

ctatttttga cagggatggg gtttcaccat gttggtcagg ctggtctaga 770

<210> 207
<211> 770
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 207
aggctcagag gcacacagga gtttctgggc tcaccctgcc cccttccaac ccctcagttc 60

ccatcctcca gcagctgttt gtgtgctgcc tctgaagtcc acactgaaca aacttcagcc 120

tactcatgtc cctaaaatgg gcaaacattg caagcagcaa acagcaaaca cacagccctc 180

cctgcctgct gaccttggag ctggggcaga ggtcagagac ctctctgggc ccatgccacc 240

tccaacatcc acttgacccc ttggaatttc agtggagagg agcagaggtt gtcctggtgt 300

ggtttaggta gtgtgagagg gtccaggttc aaaaccactt gctgggtggg gagttgtcag 360

taagtggcta tgccccaacc ctgaagcctg tttccccatc tgtacaatgg aaatgataaa 420

gacccccatc tgatagggtt tttgtggcaa ataaacattt ggtttttttg ttttgttttg 480

ttttgttttt tgagatggag gtttgctctg tggcccaggc tggagtgcag tgacacaatc 540

tcatctcacc acaaccttcc cctgcctcag cctcccaagt agctgggatt acaagcatgt 600

gccaccacac ctggctaatt ttctattttt agtagagatg ggtttctcca tgttggtcag 660

cctcagcctc ccaagtaact gggattacag gcctgtgcca ccacacctgg ctaatttttt 720

ctatttttga cagggatggg gtttcaccat gttggtcagg ctggtctaga 770

<210> 208
<211> 117
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 208
ccctaaaatg ggcaaacatt gcaagcagca aacagcaaac acacagccct ccctgcctgc 60

tgaccttgga gctggggcag aggtcagaga cctctctggg cccatgccac ctccaac 117

<210> 209
<211> 90
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 209
gttaatcatt aacgcttgta ctttggtaca gttaatcatt aacgcttgta ctttggtaca 60

gttaatcatt aacgcttgta ctttggtaca 90

<210> 210
<211> 348
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 210
gatcttgcta ccagtggaac agccactaag gattctgcag tgagagcaga gggccagcta 60

agtggtactc tcccagagac tgtctgactc acgccacccc ctccaccttg gacacaggac 120

gctgtggttt ctgagccagg tacaatgact cctttcggta agtgcagtgg aagctgtaca 180

ctgcccaggc aaagcgtccg ggcagcgtag gcgggcgact cagatcccag ccagtggact 240

tagcccctgt ttgctcctcc gataactggg gtgaccttgg ttaatattca ccagcagcct 300

cccccgttgc ccctctggat ccactgctta aatacggacg aggacagg 348

<210> 211
<211> 202
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 211
gtctgtctgc acatttcgta gagcgagtgt tccgatactc taatctccct aggcaaggtt 60

catatttgtg taggttactt attctccttt tgttgactaa gtcaataatc agaatcagca 120

ggtttggagt cagcttggca gggatcagca gcctgggttg gaaggagggg gtataaaagc 180

cccttcacca ggagaagccg tc 202

<210> 212
<211> 348
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 212
gatcttgcta ccagtggaac agccactaag gattctgcag tgagagcaga gggccagcta 60

agtggtactc tcccagagac tgtctgactc atgccacccc ctccaccttg gacacaggac 120

actgtggttt ctgagccagg tacaatgact ccttttggta agtgcagtgg aagctgtaca 180

ctgcccaggc aaagtgtccg ggcagcgtag gcgggcgact cagatcccag ccagtggact 240

tagcccctgt ttgctcctcc gataactggg gtgaccttgg ttaatattca ccagcagcct 300

cccccgttgc ccctctggat ccactgctta aatacggacg aggacagg 348

<210> 213
<211> 348
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 213
gatcttgcta ccagtggaac agccactaag gattctgcag tgagagcaga gggccagcta 60

agtggtactc tcccagagac tgtctgactc acgccacccc ctccaccttg gacacaggac 120

gctgtggttt ctgagccagg tacaatgact cctttcggta agtgcagtgg aagctgtaca 180

ctgcccaggc aaagcgtccg ggcagcgtag gcgggcgact cagatcccag ccagtggact 240

tagcccctgt ttgctcctct gataactggg gtgaccttgg ttaatattca ccagcagcct 300

cccctgttgc ccctctggat ccactgctta aatacggaca aggacagg 348

<210> 214
<211> 348
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 214
gatcttgcta ccagtggaac agccactaag gattctgcag tgagagcaga gggccagcta 60

agtggtactc tcccagagac tgtctgactc acgccacccc ctccaccttg gacacaggac 120

gctgtggttt ctgagccagg tacaatgact cctttcggta agtgcagtgg aagctgtaca 180

ctgcccaggc aaagcgtctg ggcagcatag gcaggcgact cagatcccag ccagtggact 240

tagcccctgt ttgctcctcc gataactggg gtgaccttgg ttaatattca ccagcagcct 300

ccccgttgc ccctctggat ccactgctta aatacggacg aggacagg 348

<210> 215
<211> 348
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 215
gatcttgcta ccagtggaac agccactaag gattctgcag tgagagcaga gggccagcta 60

agtggtactc tcccagagac tgtctgactc acgccacccc ctccaccttg gacacaggac 120

gctgtggttt ctgagccagg tacaatgact cctttcggta agtgcagtgg aagctgtaca 180

ctgcccaggc aaagcgtccg ggcagcgtag gcgggcgact cagatcccag ccagtggact 240

tagcccctgt ttgctcctct gataactggg gtgaccttgg ttaatattca ccagcagcct 300

cccccgttgc ccctctggat ccactgctta aatacagacg aggacagg 348

<210> 216
<211> 348
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 216
gatcttgcta ccagtggaac agccactaag gattctgcag tgagagcaga gggccagcta 60

agtggtactc tcccagagac tgtctgactc atgccacccc ctccaccttg gacacaggac 120

actgtggttt ctgagccagg tacaatgact ccttttggta agtgcagtgg aagctgtaca 180

ctgcccaggc aaagtgtctg ggcagcatag gcaggtgact cagatcccag ccagtggact 240

tagcccctgt ttgctcctct gataactggg gtgaccttgg ttaatattca ccagcagcct 300

cccctgttgc ccctctggat ccactgctta aatacagaca aggacagg 348

<210> 217
<211> 318
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 217
gattctgcag tgagagcaga gggccagcta agtggtactc tcccagagac tgtctgactc 60

acgccacccc ctccaccttg gacacaggac gctgtggttt ctgagccagg tacaatgact 120

cctttcggta agtgcagtgg aagctgtaca ctgcccaggc aaagcgtccg ggcagcgtag 180

gcgggcgact cagatcccag ccagtggact tagcccctgt ttgctcctcc gataactggg 240

gtgaccttgg ttaatattca ccagcagcct cccccgttgc ccctctggat ccactgctta 300

aatacggacg aggacagg 318

<210> 218

<400> 218
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<210> 219

<400> 219
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<210> 220

<400> 220
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<210> 221

<400> 221
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<210> 222

<400> 222
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<210> 223

<400> 223
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<210> 224

<400> 224
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<210> 225

<400> 225
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<210> 226

<400> 226
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<210> 227

<400> 227
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<210> 228

<400> 228
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<210> 229

<400> 229
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<210> 230

<400> 230
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<210> 231

<400> 231
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<210> 232

<400> 232
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<210> 233

<400> 233
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<210> 234

<400> 234
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<210> 235
<211> 91
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 235
aagaggtaag ggtttaaggg atggttggtt ggtggggtat taatgtttaa ttacctggag 60

cacctgcctg aaatcacttt ttttcaggtt g 91

<210> 236
<211> 92
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 236
aagaggtaag ggtttaaggg atggttggtt ggtggggtat taatgtttaa ttacctggag 60

cacctgcctg aaatcacttt ttttcaggtt gg 92

<210> 237
<211> 93
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 237
ctctaaggta aatataaaat ttttaagtgt ataatgtgtt aaactactga ttctaattgt 60

ttctctcttt tagattccaa cctttggaac tga 93

<210> 238
<211> 150
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 238
gtaaaggcat gtcctgtagg gtctgatcgg ggccaggatt gtggggatgt ctccttgcta 60

atagtagaaa agagtagtat ggaagcatta cttccagctg ctttttgaag tgtccaccaa 120

aatgaacgac tttttccttt cttcacgcag 150

<210> 239
<211> 250
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 239
gtaaaggcat gtcctgtagg gtctgatcgg ggccaggatt gtggggatgt tatgattctt 60

atcatatggc acctaatgta tcagttgagt tgtgaaaaca gtgtttcggt ttaatggatg 120

ttagggattt atatacaact tcaaatttgc ctccttgcta atagtagaaa agagtagtat 180

ggaagcatta cttccagctg ctttttgaag tgtccaccaa aatgaacgac tttttccttt 240

cttcacgcag 250

<210> 240
<211> 400
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 240
gtaaaggcat gtcctgtagg gtctgatcgg ggccaggatt gtggggatgt aagtctgctt 60

ggaggaaggt gcagacatcg ggttaggatg gttgtgatgc tacctgggcc ccaaagaaac 120

atttctgggt aaggtgtgca cacatctgtg ttattagcag aaatgctaac tgccaattct 180

tttcataggt ctgacctatt tatgattctt atcatatggc acctaatgta tcagttgagt 240

tgtgaaaaca gtgtttcggt ttaatggatg ttagggattt atatacaact tcaaatttgc 300

ctccttgcta atagtagaaa agagtagtat ggaagcatta cttccagctg ctttttgaag 360

tgtccaccaa aatgaacgac tttttccttt cttcacgcag 400

<210> 241
<211> 1000
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 241
gtaaaggcat gtcctgtagg gtctgatcgg ggccaggatt gtggggatgt aagtctgctt 60

ggaggaaggt gcagacatcg ggttaggatg gttgtgatgc tacctgggcc ccaaagaaac 120

atttctgggt aaggtgtgca cacatctgtg ttattagcag aaatgctaac tgccaattct 180

tttcataggt ctgacctatt tgttgatatt ttgttctgtt ttgtccattg cttctcttcg 240

tcatatgctg ctcctccaga atctagagac tggagtagag ggagggtgaa gggacaaaga 300

caaaacttcc ctctgcctgc ccaagcttcc atagagagaa tcaaggcaat gaaatccaat 360

caatatcaca cacaagtttc atgtctggtt ctcttgtgtg tacatgcaat gtgtgttttt 420

ataatatctt ttcctacttt gggtgtaagg ataatatgag ccttgagttc agaagctttt 480

cgtgttttgg gggttctggt tgagtaattg ggagtatctg agtggagaca agaatgaaca 540

ttaatgtagg actgattctg aattacagct tctggcacta gtaggtttca atatacatag 600

tctgctgaaa ggtaattttc tctgtgatat tcctggtatc cctaaggagg gaagtataat 660

tttaggtaca tactgacaaa atttgaacac aaggtactta gatgaaagtt atagtctgtt 720

ttagagatga aattgaagga tgcagtagct attgctttgg caaaggattt ttggctgatg 780

cagtgtatgg ttattgattt tatgattctt atcatatggc acctaatgta tcagttgagt 840

tgtgaaaaca gtgtttcggt ttaatggatg ttagggattt atatacaact tcaaatttgc 900

ctccttgcta atagtagaaa agagtagtat ggaagcatta cttccagctg ctttttgaag 960

tgtccaccaa aatgaacgac tttttccttt cttcacgcag 1000

<210> 242
<211> 130
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 242
gttggtatca aggttacaag acaggtttaa ggagaccaat agaaactggg catgtggaga 60

cagagaagac tcttgggttt ctgataggca ctgactctct ctgcctattg gtctattttc 120

ccacccttag 130

<210> 243
<211> 284
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 243
gtaagcactt tttgactatt ggtactctct cttccccata ctcaaaaagt tcttaccagt 60

tattcaaacc aacaaatcct gaagccatgt ttaaggttag gtacaggtat ggaatcaaat 120

ggcaagggta ttaacatttg gaaactaaca tagctgagca tcagagaagc ttgtattttt 180

ctcactcctt gccttgattg aatcatataa gctgttttag agttggattt gagcctacct 240

agaatttttc ttcccaacct ctcatctttt tttctcttat acag 284

<210> 244
<211> 286
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 244
gtaagcaggt gtctattgtg ctgaccactt tttggtttaa aagaaatggt ctttgtgtac 60

taagaatttt gatatttatc ctacgtgcaa taagaagctt taaataggag agttgatcat 120

tatatttgtg ttttagaaat gtcattctgg aatctactga agaggatgga ttatattttt 180

ggaaggtggg aggcaggttg gactggcata aaaattgatg agaaatccac tctggttcat 240

aggtgagaga gcttatactc ataggattga tgtcttccct ccctag 286

<210> 245
<211> 200
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 245
gtaaaggcat gtcctgtagg gtctgatcgg ggccaggatt gtggggatgt aagtctgctt 60

ggaggaaggt gcagacatcg ggttaggatg gttgtgatgc tacctgggcc ccaaagaaac 120

atttctgggt aaggtgtgca cacatctgtg ttattagcag aaatgctaac tgccaattct 180

tttcataggt ctgacctatt 200

<210> 246
<211> 200
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 246
tatgattctt atcatatggc acctaatgta tcagttgagt tgtgaaaaca gtgtttcggt 60

ttaatggatg ttagggattt atatacaact tcaaatttgc ctccttgcta atagtagaaa 120

agagtagtat ggaagcatta cttccagctg ctttttgaag tgtccaccaa aatgaacgac 180

tttttccttt cttcacgcag 200

<210> 247
<211> 9
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 247
aagaggttg 9

<210> 248
<211> 82
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 248
gtaagggttt aagggatggt tggttggtgg ggtattaatg tttaattacc tggagcacct 60

gcctgaaatc actttttttc ag 82

<210> 249
<211> 48
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 249
atgaagtggg tgaccttcat cagcctgctg ttcagctctg cctacagt 48

<210> 250
<211> 48
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 250
atgaaatggg taacttttat ctctctatta ttctcaagtg cttactct 48

<210> 251
<211> 48
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 251
atgaagtggg tcacctttat atccttactc ttctcctcag catactct 48

<210> 252
<211> 48
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 252
atgcccctgc tgctgctgct gcccctgctg tgggctgggg ccctggca 48

<210> 253
<211> 48
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 253
atgccattac tgctactgtt accactactt tgggctggtg cacttgct 48

<210> 254
<211> 48
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 254
atgccactat tactcctctt gcctctactg tgggctggtg cactggca 48

<210> 255
<211> 54
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 255
atggccttcc tgtggctgct gagctgctgg gccctgctgg gcaccacctt tgga 54

<210> 256
<211> 54
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 256
atggcatttt tatggctttt atcttgctgg gcacttttag gaactacatt tgga 54

<210> 257
<211> 54
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 257
atggcatttc tgtggctgct gtcctgctgg gctctgctgg gcactacctt tggg 54

<210> 258
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 258
atgggggtga aggtgctgtt tgccctgatc tgcattgctg tggctgaggc t 51

<210> 259
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 259
atgggtgtga aagttctttt tgccttgatt tgtattgctg ttgctgaagc a 51

<210> 260
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 260
atgggggtga aagttctgtt tgctctcatc tgtattgctg tggctgaagc t 51

<210> 261
<211> 42

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 261
atgcagctgc tgagctgcat tgccctgatc ctggccctgg tg 42

<210> 262
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 262
atgcagttat tatcttgtat agcattaatc ttagcactag tg 42

<210> 263
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 263
atgcagttgc tgtcttgtat tgccctgatt ttggctctag tg 42

<210> 264
<211> 57
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 264
atgatgaggc ccattgtgct ggtgctgctg tttgccacct ctgccctggc ccaggtg 57

<210> 265
<211> 57
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 265
atgatgagac caattgttct agtactatta tttgccacct ctgctcttgc ccaagtg 57

<210> 266
<211> 57
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 266
atgatgaggc ccattgtcct ggtgcttcta tttgcaacaa gtgccctggc ccaagta 57

<210> 267
<211> 63
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 267
atgtggtgga ggctgtggtg gctgctgctg ctgctgctgc tgctgtggcc catggtgtgg 60

gct 63

<210> 268
<211> 63
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 268
atgtggtgga gattatggtg gttactgtta ttacttttac ttttatggcc aatggtgtgg 60

gct 63

<210> 269
<211> 63

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 269
atgtggtgga ggctgtggtg gttactactg cttctgctcc tgttatggcc aatggtctgg 60

gct 63

<210> 270
<211> 66
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 270
atgtggtgga ggctgtggtg gttactactg cttctgctcc tgttatggcc aatggtctgg 60

gctgct 66

<210> 271
<211> 69
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 271
atggatgcca tgaagagagg cctgtgctgt gtgctgctgc tgtgtggagc tgtgtttgtg 60

agccccagt 69

<210> 272
<211> 69
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 272
atggatgcta tgaaaagagg tttatgttgt gtgttattat tgtgtggtgc tgtttttgtt 60

tccccctct 69

<210> 273
<211> 69
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 273
atggatgcta tgaaaagagg cctctgctgt gtgctgctcc tctgtggagc tgtgtttgtt 60

agccccagt 69

<210> 274
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 274
atgaacctgc tgctgatcct gacctttgtg gctgctgctg tggca 45

<210> 275
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 275
atgaacctgc ttttgatctt gacatttgtt gctgctgctg ttgca 45

<210> 276
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 276
atgaaccttc tcctgattct tacttttgta gctgctgcag tagca 45

<210> 277
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 277
atgatgaggc ccattgtgct ggtgctgctg tttgccacct ctgccctggc t 51

<210> 278
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 278
atgatgagac caattgttct agtactatta tttgccacct ctgctcttgc t 51

<210> 279
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 279
atgatgaggc ccattgtcct ggtgcttcta tttgcaacaa gtgccctggc t 51

<210> 280
<211> 72
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 280
atgcccagct ctgtgagctg gggcatcctg ctgctggctg gcctgtgctg cctggtgcct 60

gtgagcctgg ca 72

<210> 281
<211> 72
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 281
atgccaagtt cagttagttg gggaatatta ttacttgctg gcctttgttg ccttgttcct 60

gtatctttag cc 72

<210> 282
<211> 72
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 282
atgccaagtt cagtaagctg ggggatcctt ctgctggctg gtctgtgctg cctggtgcca 60

gtctctcttg ct 72

<210> 283
<211> 225
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 283
tgtgccttct agttgccagc catctgttgt ttgcccctcc cccgtgcctt ccttgaccct 60

ggaaggtgcc actcccactg tcctttccta ataaaatgag gaaattgcat cgcattgtct 120

gagtaggtgt cattctattc tgggggggtgg ggtggggcag gacagcaagg gggaggattg 180

ggaagacaat agcaggcatg ctggggatgc ggtgggctct atggc 225

<210> 284

<211> 581
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 284
gagcatctta ccgccattta ttcccatatt tgttctgttt ttcttgattt gggtatacat 60

ttaaatgtta ataaaacaaa atggtggggc aatcatttac atttttaggg atatgtaatt 120

actagttcag gtgtattgcc acaagacaaa catgttaaga aactttcccg ttatttacgc 180

tctgttcctg ttaatcaacc tctggattac aaaatttgtg aaagattgac tgatattctt 240

aactatgttg ctccttttac gctgtgtgga tatgctgctt tatagcctct gtatctagct 300

attgcttccc gtacggcttt cgttttctcc tccttgtata aatcctggtt gctgtctctt 360

ttagaggagt tgtggcccgt tgtccgtcaa cgtggcgtgg tgtgctctgt gtttgctgac 420

gcaaccccca ctggctgggg cattgccacc acctgtcaac tcctttctgg gactttcgct 480

ttccccctcc cgatcgccac ggcagaactc atcgccgcct gccttgcccg ctgctggaca 540

ggggctaggt tgctgggcac tgataattcc gtggtgttgt c 581

<210> 285
<211> 222
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 285
cagacatgat aagatacatt gatgagtttg gacaaaccac aactagaatg cagtgaaaaa 60

aatgctttat ttgtgaaatt tgtgatgcta ttgctttatt tgtaaccatt ataagctgca 120

ataaacaagt taacaacaac aattgcattc attttatgtt tcaggttcag ggggagatgt 180

gggaggtttt ttaaagcaag taaaacctct acaaatgtgg ta 222

<210> 286
<211> 395
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 286
taaaatacag catagcaaaa ctttaacctc caaatcaagc ctctacttga atccttttct 60

gagggatgaa taaggcatag gcatcagggg ctgttgccaa tgtgcattag ctgtttgcag 120

cctcaccttc tttcatggag tttaagatat agtgtatttt cccaaggttt gaactagctc 180

ttcatttctt tatgttttaa atgcactgac ctcccacatt ccctttttag taaaatattc 240

agaaataatt taaatacatc attgcaatga aaataaatgt tttttattag gcagaatcca 300

gatgctcaag gcccttcata atatccccca gtttagtagt tggacttagg gaacaaagga 360

acctttaata gaaattggac agcaagaaag cgagc 395

<210> 287
<211> 107
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 287
gctcgctttc ttgctgtcca atttctatta aaggttcctt tgttccctaa gtccaactac 60

taaactgggg gatattatga agggccttga gcatctggat tctgcct 107

<210> 288
<211> 395
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 288
gctcgctttc ttgctgtcca atttctatta aaggttcctt tgttccctaa gtccaactac 60

taaactgggg gatattatga agggccttga gcatctggat tctgcctaat aaaaaacatt 120

tattttcatt gcaatgatgt atttaaatta tttctgaata ttttactaaa aagggaatgt 180

gggaggtcag tgcatttaaa acataaagaa atgaagagct agttcaaacc ttgggaaaat 240

acactatatc ttaaactcca tgaaagaagg tgaggctgca aacagctaat gcacattggc 300

aacagcccct gatgcctatg ccttattcat ccctcagaaa aggattcaag tagaggcttg 360

atttggaggt taaagttttg ctatgctgta tttta 395

<210> 289
<211> 107
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 289
gcttgctttc ttgctgtcca atttctatta aaggttcctt tgttccctaa gtccaactac 60

taaactgggg gatattatga agggccttga gcatctggat tctgcct 107

<210> 290
<211> 395
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 290
gcttgctttc ttgctgtcca atttctatta aaggttcctt tgttccctaa gtccaactac 60

taaactgggg gatattatga agggccttga gcatctggat tctgcctaat aaaaaacatt 120

tattttcatt gcaatgatgt atttaaatta tttctgaata ttttactaaa aagggaatgt 180

gggaggtcag tgcatttaaa acataaagaa atgaagagct agttcaaacc ttgggaaaat 240

acactatatc ttaaactcca tgaaagaagg tgaggctgca aacagctaat gcacattggc 300

aacagcccct gatgcctatg ccttattcat ccctcagaaa aggattcaag tagaggcttg 360

atttggaggt taaagttttg ctatgctgta tttta 395

<210> 291
<211> 395
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 291
taaaatacag catagcaaaa ctttaacctc caaatcaagc ctctacttga atccttttct 60

gagggatgaa taaggcatag gcatcagggg ctgttgccaa tgtgcattag ctgtttgcag 120

cctcaccttc tttcatggag tttaagatat agtgtatttt cccaaggttt gaactagctc 180

ttcatttctt tatgttttaa atgcactgac ctcccacatt ccctttttag taaaatattc 240

agaaataatt taaatacatc attgcaatga aaataaatgt tttttattag gcagaatcca 300

gatgctcaag gcccttcata atatccccca gtttagtagt tggacttagg gaacaaagga 360

acctttaata gaaattggac agcaagaaag caagc 395

<210> 292
<211> 674
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 292
gggaggtggt ccctgcagtt acgccaatga taacccccgc cagaaaaatc ttagtagcct 60

tccctttttg ttttccgtgc cccaactcgg cggattgact cggccccttc cggaaacacc 120

cgaatcaact tctagtcaaa ttattgttca cgccgcaatg acccacccct ggcccgcgtc 180

tgtggaactg acccctggtg tacaggagag ttcgctgctg aaagtggtcc caaaggggta 240

ctagttttta agctcccaac tccccctccc ccagcgtctg gaggattcca caccctcgca 300

ccgcaggggc gaggaagtgg gcggagtccg gttttggcgc cagccgctga ggctgccaag 360

cagaaaagcc accgctgagg agactccggt cactgtcctc gccccgcctc ccccttccct 420

cccttgtggg accaccgggc gccacgccgc gaacgttaag tgccgcggtc gtcggcgcct 480

ccgccctccc ccttgggccc caattcccag cgggcgcggc gcgcggcccc tccccccgcc 540

gggcgcgcgc ccgctgcccc gcccttcgtg gccgcccggc gtgggcggtg ccacccctcc 600

ccccggcggc cccgcgcgca gctcccggct ccctcccccc tcggatgtgg cttgagctgt 660

aggcgcggag ggcc 674

<210> 293
<211> 143

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 293
atgcaaattg agttatccac ctgtttcttc ctctgtctcc tgaggttctg cttctctgcc 60

accaggagat actacctggg ggctgtggag ctgtcctggg actacatgca aagtgatctg 120

ggtgagctgc ctgtggatgc aag 143

<210> 294
<211> 4249
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 294
atttcctcct agagtgccaa aatcttttcc attcaacacc tcagtggtgt acaaaaagac 60

cctgtttgtg gagttcacag accacctgtt caacatagcc aagcccaggc ccccctggat 120

gggcctgctg ggccccacca tccaggcaga ggtttatgac acagtggtca tcaccctgaa 180

gaacatggcc tcccaccctg tgtcctcca tgctgtgggg gtgagctact ggaaggccag 240

tgaggggca gaatatgatg accagaccag ccagagggag aaggaggatg acaaggtgtt 300

cccaggggc agccacacct atgtgtggca ggtcctgaag gagaatggcc ccatggcctc 360

tgacccctc tgcctcacct actcctacct gtcccatgtg gacctggtga aggacctcaa 420

ctctggcctg attggagccc tcctggtgtg cagggagggg agcctggcca aggaaaagac 480

ccagaccctg cacaagttta tcctgctctt tgcagtcttt gatgagggga agtcctggca 540

cagtgagacc aagaactccc tgatgcagga cagagatgct gcctcagcca gggcctggcc 600

caagatgcac acagtgaatg ggtatgtcaa caggagcctg ccaggcctga ttgggtgcca 660

cagaaagtca gtgtactggc atgtcattgg catgggcacc accccagagg tgcactccat 720

cttcctggag ggccacacct tcctggtgag gaaccacaga caggccagcc tggagatcag 780

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tctgacagac tctgagatgg atgtggtgag atttgatgat gacaacagcc cctccttcat 1020

ccagatcagg tctgtggcca agaagcaccc taagacctgg gtgcactaca ttgcagctga 1080

ggaggaggac tgggactatg ccccccctggt cctggcccct gatgataggt cctacaagtc 1140

ccagtacctg aacaatggcc cccagaggat agggagaaag tacaagaagg tgaggttcat 1200

ggcctacact gatgagacct tcaagactag ggaagccatc cagcatgagt ctgggatcct 1260

gggccccctc ctctatgggg aggtggggga caccctgctg atcatcttca agaaccaggc 1320

cagcaggccc tacaacattt acccccatgg gatcacagat gtgagacccc tgtacagcag 1380

gagactgcca aaggggggtga agcacctgaa ggacttcccc atcctgccag gggagatctt 1440

caagtacaag tggactgtga cagtggagga tgggcccacc aaatctgacc ctagatgcct 1500

gaccagatac tacagcagct ttgtgaacat ggagagggac ctggcctctg gactgattgg 1560

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gtgcctgcat gaggtggcct actggtacat cctctccatt ggagcccaga ctgacttcct 1860

gtcagtcttc ttcagtggct acaccttcaa gcacaagatg gtctatgagg acactctgac 1920

cctgttcccc ttctctgggg agactgtgtt catgtccatg gagaaccctg gactgtggat 1980

cctgggctgc cacaacagtg acttcaggaa caggggcatg actgccctgc tgaaggtctc 2040

cagctgtgac aagaacactg gggactacta tgaggactcc tatgaggaca tctctgccta 2100

cctgctgtcc aagaacaatg ccattgagcc caggtccttc tcccagaaca gcagacaccc 2160

ctccaccagg cagaagcagt tcaatgcaac caccatccct gaaaatacca ccctgcagag 2220

tgaccaggag gagattgact atgatgacac catctctgtg gagatgaaga aggaggactt 2280

tgacatctat gatgaggatg agaaccagag ccctagaagc ttccagaaga agaccagaca 2340

ctacttcatt gctgcagtgg aaagactgtg ggactatggg atgagctcct ccccccatgt 2400

gctgaggaac agggcccagt ctggctctgt gccccagttc aagaaggtgg tgttccagga 2460

gttcactgat ggctccttca cccagcccct gtacagggga gagctgaatg agcacctggg 2520

cctgctgggc ccctacatca gggctgaggt ggaggacaat atcatggtga ccttcagaaa 2580

ccaggcctcc agaccttact ccttctacag cagcctgatc tcctatgagg aggaccagag 2640

acaggggget gagcccagga agaactttgt gaagcccaat gagaccaaga cctacttctg 2700

gaaggtgcag caccacatgg cccccaccaa ggatgagttt gactgcaagg cctgggccta 2760

cttctctgat gtggacctgg agaaggatgt gcactctggc ctgattggcc ccctgctggt 2820

gtgccacacc aacaccctga accctgccca tggcaggcag gtgactgtgc aggagtttgc 2880

cctgttcttc accatctttg atgagaccaa gagctggtac ttcactgaga acatggagag 2940

gaactgcagg gccccctgca acatccagat ggaggacccc accttcaagg agaactacag 3000

gttccatgcc atcaatggct acatcatgga caccctgcct ggcctggtga tggcccagga 3060

ccagaggatc aggtggtacc tgctgagcat ggggagcaat gagaacatcc acagcatcca 3120

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ggtggagtgc ctgattgggg agcacctgca tgctggcatg agcaccctgt tcctggtgta 3300

cagcaacaag tgccagaccc ccctgggcat ggcctctggc cacatcagag acttccagat 3360

cacagcctca ggccagtatg gccagtgggc ccccaagctg gccaggctgc actactctgg 3420

cagcatcaat gcctggtcca ccaaggagcc cttcagctgg atcaaggtgg acctcctggc 3480

ccccatgatc atccatggca tcaagaccca gggggccagg cagaagttca gctccctgta 3540

catctcccag ttcatcatca tgtactccct ggatggcaag aagtggcaga cctacagagg 3600

caacagcaca ggcaccctga tggtgttctt tggcaatgtg gacagctctg ggatcaagca 3660

caacatcttc aacccccccа tcattgccag gtacatcaga ctgcacccca cccactactc 3720

catcaggtcc accctgagaa tggagctgat gggctgtgac ctgaacagct gcagcatgcc 3780

cctgggcatg gagagcaagg ccatctctga tgcccagatc acagccagca gctacttcac 3840

caacatgttt gccacctggt cccccagcaa ggccaggctg cacctgcagg gcaggagcaa 3900

tgcctggagg ccccaggtga acaaccccaa ggagtggctg caggtggact tccagaagac 3960

catgaaggtg acaggggtga ccacccaggg ggtgaagtcc ctgctgacca gcatgtatgt 4020

gaaggagttc ctgatcagca gctcccagga tggccaccag tggaccctgt tcttccagaa 4080

tggcaaggtg aaggtgttcc agggcaacca ggacagcttc acccctgtgg tgaactccct 4140

ggacccccc ctgctgacca gatacctgag gatccacccc cagtcctggg tgcaccagat 4200

tgccctgaga atggaggtgc tgggctgtga ggcccaggac ctgtactga 4249

<210> 295
<211> 143
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 295
atgcagattg agctgagcac ctgcttcttc ctgtgcctgc tgaggttctg cttctctgcc 60

accaggagat actacctggg ggctgtggag ctgagctggg actacatgca aagtgatctg 120

ggtgagctgc ctgtggatgc aag 143

<210> 296
<211> 4249
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 296
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polynucleotide

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<210> 298
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polynucleotide

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polynucleotide

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polynucleotide

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<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

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<210> 302
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<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

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<210> 303
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<212> DNA
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<223> Description of Artificial Sequence: Synthetic
oligonucleotide

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oligonucleotide

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<213> Artificial Sequence

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<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 305
agcctgcact tagagtgtct agg 23

<210> 306
<211> 70
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 306
tggggacttt ccgctgggga ctttccgctg gggactttcc gctggggact ttccgctggg 60

gactttccgc 70

<210> 307
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 307
acgggtggta cagaatgttc ttttggcgtc gacacgggtg gtacagaatg ttcttttggc 60

<210> 308
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 308
tgacgtcaag atcta 15

<210> 309
<211> 840
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 309
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gcctgctggg gagcctgggg actttccaca cctggttgct gactaattga gatgcatgct 120

ttgcatactt ctgcctgctg gggagcctgg ggactttcca caccttaaga atgtaaattc 180

tgggtggttg ctgactaatt gagatgcatg ctttgcatac ttctgcctgc tggggagcct 240

ggggactttc cacacctggt tgctgactaa ttgagatgca tgctttgcat acttctgcct 300

gctggggagc ctggggactt tccacaccta gttacctaat ttgttagatg gttgctgact 360

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tggttgctga ctaattgaga tgcatgcttt gcatacttct gcctgctggg gagcctgggg 480

actttccaca ccaatggcac tcaaacagta tgtggttgct gactaattga gatgcatgct 540

ttgcatactt ctgcctgctg gggagcctgg ggactttcca cacctggttg ctgactaatt 600

gagatgcatg ctttgcatac ttctgcctgc tggggagcct ggggactttc cacaccactt 660

ttagtcagag gttaattggt tgctgactaa ttgagatgca tgctttgcat acttctgcct 720

gctggggagc ctggggactt tccacacctg gttgctgact aattgagatg catgctttgc 780

atacttctgc ctgctgggga gcctggggac tttccacacc ttggctccta ggtcatgcac 840

<210> 310
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 310
acgggaagaa cattttgtac gtttggcgtc gacacgggaa gaacattttg tacgtttggc 60

<210> 311
<211> 72
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 311
tggttgctga ctaattgaga tgcatgcttt gcatacttct gcctgctggg gagcctgggg 60

actttccaca cc 72

<210> 312
<211> 144
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 312
tggttgctga ctaattgaga tgcatgcttt gcatacttct gcctgctggg gagcctgggg 60

actttccaca cctggttgct gactaattga gatgcatgct ttgcatactt ctgcctgctg 120

gggagcctgg ggactttcca cacc 144

<210> 313
<211> 840
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 313
aacaggttag tttgactagc tggttgctga ctaattgaga tgcatgcttt gcatacttct 60

gcctgctggg gagcctgggg actttccaca cctggttgct gactaattga gatgcatgct 120

ttgcatactt ctgcctgctg gggagcctgg ggactttcca caccttaaga atgtaaattc 180

tgggtggttg ctgactaatt gagatgcatg ctttgcatac ttctgcctgc tggggagcct 240

ggggactttc cacacctggt tgctgactaa ttgagatgca tgctttgcat acttctgcct 300

gctggggagc ctggggactt tccacaccta gttacctaat ttgttagatg gttgctgact 360

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tggttgctga ctaattgaga tgcatgcttt gcatacttct gcctgctggg gagcctgggg 480

actttccaca ccaatggcac tcaaacagta tgtggttgct gactaattga gatgcatgct 540

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gagatgcatg ctttgcatac ttctgcctgc tggggagcct ggggactttc cacaccactt 660

ttagtcagag gttaattggt tgctgactaa ttgagatgca tgctttgcat acttctgcct 720

gctggggagc ctggggactt tccacacctg gttgctgact aattgagatg catgctttgc 780

atacttctgc ctgctgggga gcctggggac tttccacacc ttggctccta gatcatgcac 840

<210> 314
<211> 9
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 314
gccgccacc 9

<210> 315
<211> 7
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 315
agccacc 7

<210> 316
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 316
cgcagccacc 10

<210> 317
<211> 3
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 317
acc 3

<210> 318
<211> 53
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 318
tgtagttaat gattaacccg ccatgctact tatctacgta gccatgctct aga 53

<210> 319
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 319
tgtagttaat gattaacccg ccatgctact tatcgcggcc gc 42

<210> 320
<211> 61
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 320
tctagagcat ggctacgtag ataagtagca tggcgggtta atcattaact acacctgcag 60

g 61

<210> 321
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 321
aacaggttag tttgactagc 20

<210> 322
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 322
ttaagaatgt aaattctggg 20

<210> 323
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 323
tagttaccta atttgttaga 20

<210> 324
<211> 20
<212> DNA
<213> Artificial Sequence

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<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 324
aatggcactc aaacagtatg 20

<210> 325
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 325
actttagtc agaggttaat 20

<210> 326
<211> 20
<212> DNA
<213> Artificial Sequence

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<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 326
ttggctccta ggtcatgcac 20

<210> 327
<211> 20

<212> DNA
<213> Artificial Sequence

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<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 327
ttggctccta gatcatgcac 20

<210> 328
<211> 100
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 328
tcccttgcca atagccggtt taacttgcta taattaattc attggtataa actataaaca 60

ttccaattga gataggggag aaatctagta atcttctaag 100

<210> 329
<211> 800
<212> DNA
<213> Artificial Sequence

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<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 329
ctgtctagct cctcttctgt cattgcaacc tctgtaaggg ttttagttct ccttattttt 60

tatgtcttgc tctggtagtg ctgacagaag cataatagta agagtactgt ggatgtacat 120

aaagcagcag gcagggctac caaccttagc ctggcaactc atgatactgt ctgttcatgg 180

acttttaagt tgcataagag gaagctttat tcctgatagt gatagtagct tcaaatgttg 240

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ctctagtgaa tttaggtcct ctggagcagt atttcaatct actaataggg gctctgcaag 360

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atgtccctgc aatcaaaagt cctagtccac atgattgatt gaccttatac tgaagagtac 540

cctgtggacc aagtcaatct gcagatgcta cattaacaat aaatgttaat tacttcacta 600

gggattagtc tgttatttta caccttttc aaacctctgg cacttgcata ccattgtagc 660

taagatatgc ttctatgaaa tagccaccac aggtgataag tccccctaca cagcaatctt 720

agagcaatat tatccagaga ttgttgcagc agaaactgct gtatgctttt tacctcacac 780

actcctaatg agcttttacc 800

<210> 330
<211> 400
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 330
ttctttgaat gttgttatag agccaccttg atgccttgtt agtgatggtt gctcctatag 60

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ttgagtcctg ttgagttaac tgtgctacta ctattctgaa gaaaatagtg tctacagctg 180

gaatgagttg gttgctctac ctaggcctaa gatcatactg tgattagtta catataaccc 240

agtttaacag tattgagctt gctaaggaac tttagcttca ggtatactat tttcaagcca 300

tcagcttaat aactataaat ataccactgc tgggtgatga tattagaatt agggtctagt 360

aaaggataga cagtggcatt atgactatat ttcaaaaata 400

<210> 331
<211> 200
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 331
ctaggcctaa gatcatactg tgattagtta catataaccc agtttaacag tattgagctt 60

gctaaggaac tttagcttca ggtatactat tttcaagcca tcagcttaat aactataaat 120

ataccactgc tgggtgatga tattagaatt agggtctagt aaaggataga cagtggcatt 180

atgactatat ttcaaaaata 200

<210> 332
<211> 200
<212> DNA
<213> Artificial Sequence

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<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 332
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tagtattcct gtgatgcaag gtaaatatat tgaagacctg ctgtagacat gaagcagaac 120

ttgagtcctg ttgagttaac tgtgctacta ctattctgaa gaaaatagtg tctacagctg 180

gaatgagttg gttgctctac 200

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<210> 400
<211> 541
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 400

ggggggaggct gctggtgaat attaaccaag gtcaccccag ttatcggagg agcaaacagg 60

ggctaagtcc accgggggag gctgctggtg aatattaacc aaggtcaccc cagttatcgg 120

aggagcaaac aggggctaag tccaccgggg gaggctgctg gtgaatatta accaaggtca 180

ccccagttat cggaggagca aacaggggct aagtccacgg tacccactgg gaggatgttg 240

agtaagatgg aaaactactg atgacccttg cagagacaga gtattaggac atgtttgaac 300

aggggccggg cgatcagcag gtagcactag aggatccccg tctgtctgca catttcgtag 360

agcgagtgtt ccgatactct aatctcccta ggcaaggttc atatttgtgt aggttactta 420

ttctcctttt gttgactaag tcaataatca gaatcagcag gtttggagtc agcttggcag 480

ggatcagcag cctgggttgg aaggaggggg tataaaagcc ccttcaccag gagaagccgt 540

c 541

<210> 401
<211> 1125
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 401
aggctcagag gcacacagga gtttctgggc tcaccctgcc cccttccaac ccctcagttc 60

ccatcctcca gcagctgttt gtgtgctgcc tctgaagtcc acactgaaca aacttcagcc 120

tactcatgtc cctaaaatgg gcaaacattg caagcagcaa acagcaaaca cacagccctc 180

cctgcctgct gaccttggag ctggggcaga ggtcagagac ctctctgggc ccatgccacc 240

tccaacatcc acttgacccc ttggaatttc agtggagagg agcagaggtt gtcctggtgt 300

ggtttaggta gtgtgagagg gtccaggttc aaaaccactt gctgggtggg gagttgtcag 360

taagtggcta tgccccaacc ctgaagcctg tttccccatc tgtacaatgg aaatgataaa 420

gacccccatc tgatagggtt tttgtggcaa ataaacattt ggtttttttg ttttgttttg 480

ttttgttttt tgagatggag gtttgctctg tggcccaggc tggagtgcag tgacacaatc 540

tcatctcacc acaaccttcc cctgcctcag cctcccaagt agctgggatt acaagcatgt 600

gccaccacac ctggctaatt ttctattttt agtagagatg ggtttctcca tgttggtcag 660

cctcagcctc ccaagtaact gggattacag gcctgtgcca ccacacctgg ctaatttttt 720

ctatttttga cagggatggg gtttcaccat gttggtcagg ctggtctaga ggtactggat 780

cttgctacca gtggaacagc cactaaggat tctgcagtga gagcagaggg ccagctaagt 840

ggtactctcc cagagactgt ctgactcatg ccacccccctc caccttggac acaggacact 900

gtggtttctg agccaggtac aatgactcct tttggtaagt gcagtggaag ctgtacactg 960

cccaggcaaa gtgtctgggc agcataggca ggtgactcag atcccagcca gtggacttag 1020

cccctgtttg ctcctctgat aactggggtg accttggtta atattcacca gcagcctccc 1080

ctgttgcccc tctggatcca ctgcttaaat acagacaagg acagg 1125

<210> 402
<211> 1125
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

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ccatcctcca gcagctgttt gtgtgctgcc tctgaagtcc acactgaaca aacttcagcc 120

tactcatgtc cctaaaatgg gcaaacattg caagcagcaa acagcaaaca cacagccctc 180

cctgcctgct gaccttggag ctggggcaga ggtcagagac ctctctgggc ccatgccacc 240

tccaacatcc acttgacccc ttggaatttc agtggagagg agcagaggtt gtcctggtgt 300

ggtttaggta gtgtgagagg gtccaggttc aaaaccactt gctgggtggg gagttgtcag 360

taagtggcta tgccccaacc ctgaagcctg tttccccatc tgtacaatgg aaatgataaa 420

gacccccatc tgatagggtt tttgtggcaa ataaacattt ggtttttttg ttttgttttg 480

ttttgttttt tgagatggag gtttgctctg tggcccaggc tggagtgcag tgacacaatc 540

tcatctcacc acaaccttcc cctgcctcag cctcccaagt agctgggatt acaagcatgt 600

gccaccacac ctggctaatt ttctattttt agtagagatg ggtttctcca tgttggtcag 660

cctcagcctc ccaagtaact gggattacag gcctgtgcca ccacacctgg ctaatttttt 720

ctatttttga cagggatggg gtttcaccat gttggtcagg ctggtctaga ggtactggat 780

cttgctacca gtggaacagc cactaaggat tctgcagtga gagcagaggg ccagctaagt 840

ggtactctcc cagagactgt ctgactcacg ccacccccctc caccttggac acaggacgct 900

gtggtttctg agccaggtac aatgactcct ttcggtaagt gcagtggaag ctgtacactg 960

cccaggcaaa gcgtccgggc agcgtaggcg ggcgactcag atcccagcca gtggacttag 1020

cccctgtttg ctcctccgat aactggggtg accttggtta atattcacca gcagcctccc 1080

ccgttgcccc tctggatcca ctgcttaaat acggacgagg acagg 1125

<210> 403
<211> 1125
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 403
aggctcagag gcacacagga gtttctgggc tcaccctgcc cccttccaac ccctcagttc 60

ccatcctcca gcagctgttt gtgtgctgcc tctgaagtcc acactgaaca aacttcagcc 120

tactcatgtc cctaaaatgg gcaaacattg caagcagcaa acagcaaaca cacagccctc 180

cctgcctgct gaccttggag ctggggcaga ggtcagagac ctctctgggc ccatgccacc 240

tccaacatcc actcgacccc ttggaatttc ggtggagagg agcagaggtt gtcctggcgt 300

ggtttaggta gtgtgagagg gtccgggttc aaaaccactt gctgggtggg gagtcgtcag 360

taagtggcta tgccccgacc ccgaagcctg tttccccatc tgtacaatgg aaatgataaa 420

gacgcccatc tgatagggtt tttgtggcaa ataaacattt ggtttttttg ttttgttttg 480

ttttgttttt tgagatggag gtttgctctg tggcccaggc tggagtgcag tgacacaatc 540

tcatctcacc acaaccttcc cctgcctcag cctcccaagt agctgggatt acaagcatgt 600

gccaccacac ctggctaatt ttctattttt agtagagatg ggtttctcca tgttggtcag 660

cctcagcctc ccaagtaact gggattacag gcctgtgcca ccacacctgg ctaatttttt 720

ctatttttga cagggatggg gtttcaccat gttggtcagg ctggtctaga ggtactggat 780

cttgctacca gtggaacagc cactaaggat tctgcagtga gagcagaggg ccagctaagt 840

ggtactctcc cagagactgt ctgactcacg ccacccccctc caccttggac acaggacgct 900

gtggtttctg agccaggtac aatgactcct ttcggtaagt gcagtggaag ctgtacactg 960

cccaggcaaa gcgtccgggc agcgtaggcg ggcgactcag atcccagcca gtggacttag 1020

cccctgtttg ctcctccgat aactggggtg accttggtta atattcacca gcagcctccc 1080

ccgttgcccc tctggatcca ctgcttaaat acggacgagg acagg 1125

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<211> 1125
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 404
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ccatcctcca gcagctgttt gtgtgctgcc tctgaagtcc acactgaaca aacttcagcc 120

tactcatgtc cctaaaatgg gcaaacattg caagcagcaa acagcaaaca cacagccctc 180

cctgcctgct gaccttggag ctggggcaga ggtcagagac ctctctgggc ccatgccacc 240

tccaacatcc actcgacccc ttggaatttc agtggagagg agcagaggtt gtcctggtgt 300

ggtttaggta gtgtgagagg gtccaggttc aaaaccactt gctgggtggg gagttgtcag 360

taagtggcta tgccccgacc ccgaagcctg tttccccatc tgtacaatgg aaatgataaa 420

gacgcccatc tgatagggtt tttgtggcaa ataaacattt ggtttttttg ttttgttttg 480

ttttgttttt tgagatggag gtttgctctg tcgcccaggc tggagtgcag tgacacaatc 540

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gccaccacac ctggctaatt ttctattttt agtagagacg ggtttctcca tgttggtcag 660

cctcagcctc ccaagtaact gggattacag gcctgtgcca ccacacccgg ctaatttttt 720

ctatttttga cagggacggg gtttcaccat gttggtcagg ctggtctaga ggtactggat 780

cttgctacca gtggaacagc cactaaggat tctgcagtga gagcagaggg ccagctaagt 840

ggtactctcc cagagactgt ctgactcacg ccacccccctc caccttggac acaggacgct 900

gtggtttctg agccaggtac aatgactcct ttcggtaagt gcagtggaag ctgtacactg 960

cccaggcaaa gcgtccgggc agcgtaggcg ggcgactcag atcccagcca gtggacttag 1020

cccctgtttg ctcctccgat aactggggtg accttggtta atattcacca gcagcctccc 1080

ccgttgcccc tctggatcca ctgcttaaat acggacgagg acagg 1125

<210> 405
<211> 1125
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 405
aggctcagag gcacacagga gtttctgggc tcaccctgcc cccttccaac ccctcagttc 60

ccatcctcca gcagctgttt gtgtgctgcc tctgaagtcc acactgaaca aacttcagcc 120

tactcatgtc cctaaaatgg gcaaacattg caagcagcaa acagcaaaca cacagccctc 180

cctgcctgct gaccttggag ctggggcaga ggtcagagac ctctctgggc ccatgccacc 240

tccaacatcc acttgacccc ttggaatttc ggtggagagg agcagaggtt gtcctggcgt 300

ggtttaggta gtgtgagagg gtccgggttc aaaaccactt gctgggtggg gagtcgtcag 360

taagtggcta tgccccaacc ctgaagcctg tttccccatc tgtacaatgg aaatgataaa 420

gacccccatc tgatagggtt tttgtggcaa ataaacattt ggtttttttg ttttgttttg 480

ttttgttttt tgagatggag gtttgctctg tcgcccaggc tggagtgcag tgacacaatc 540

tcatctcacc acaaccttcc cctgcctcag cctcccaagt agctgggatt acaagcatgt 600

gccaccacac ctggctaatt ttctattttt agtagagacg ggtttctcca tgttggtcag 660

cctcagcctc ccaagtaact gggattacag gcctgtgcca ccacacccgg ctaatttttt 720

ctatttttga cagggacggg gtttcaccat gttggtcagg ctggtctaga ggtactggat 780

cttgctacca gtggaacagc cactaaggat tctgcagtga gagcagaggg ccagctaagt 840

ggtactctcc cagagactgt ctgactcacg ccacccccctc caccttggac acaggacgct 900

gtggtttctg agccaggtac aatgactcct ttcggtaagt gcagtggaag ctgtacactg 960

cccaggcaaa gcgtccgggc agcgtaggcg ggcgactcag atcccagcca gtggacttag 1020

cccctgtttg ctcctccgat aactggggtg accttggtta atattcacca gcagcctccc 1080

ccgttgcccc tctggatcca ctgcttaaat acggacgagg acagg 1125

<210> 406
<211> 1125
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 406
aggctcagag gcacacagga gtttctgggc tcaccctgcc cccttccaac ccctcagttc 60

ccatcctcca gcagctgttt gtgtgctgcc tctgaagtcc acactgaaca aacttcagcc 120

tactcatgtc cctaaaatgg gcaaacattg caagcagcaa acagcaaaca cacagccctc 180

cctgcctgct gaccttggag ctggggcaga ggtcagagac ctctctgggc ccatgccacc 240

tccaacatcc actcgacccc ttggaatttc ggtggagagg agcagaggtt gtcctggcgt 300

ggtttaggta gtgtgagagg gtccgggttc aaaaccactt gctgggtggg gagtcgtcag 360

taagtggcta tgccccgacc ccgaagcctg tttccccatc tgtacaatgg aaatgataaa 420

gacgcccatc tgatagggtt tttgtggcaa ataaacattt ggtttttttg ttttgttttg 480

ttttgttttt tgagatggag gtttgctctg tcgcccaggc tggagtgcag tgacacaatc 540

tcatctcacc acaaccttcc cctgcctcag cctcccaagt agctgggatt acaagcatgt 600

gccaccacac ctggctaatt ttctattttt agtagagacg ggtttctcca tgttggtcag 660

cctcagcctc ccaagtaact gggattacag gcctgtgcca ccacacccgg ctaatttttt 720

ctatttttga cagggacggg gtttcaccat gttggtcagg ctggtctaga ggtactggat 780

cttgctacca gtggaacagc cactaaggat tctgcagtga gagcagaggg ccagctaagt 840

ggtactctcc cagagactgt ctgactcatg ccacccccctc caccttggac acaggacact 900

gtggtttctg agccaggtac aatgactcct tttggtaagt gcagtggaag ctgtacactg 960

cccaggcaaa gtgtctgggc agcataggca ggtgactcag atcccagcca gtggacttag 1020

cccctgtttg ctcctctgat aactggggtg accttggtta atattcacca gcagcctccc 1080

ctgttgcccc tctggatcca ctgcttaaat acagacaagg acagg 1125

<210> 407
<211> 1125
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 407
aggctcagag gcacacagga gtttctgggc tcaccctgcc cccttccaac ccctcagttc 60

ccatcctcca gcagctgttt gtgtgctgcc tctgaagtcc acactgaaca aacttcagcc 120

tactcatgtc cctaaaatgg gcaaacattg caagcagcaa acagcaaaca cacagccctc 180

cctgcctgct gaccttggag ctggggcaga ggtcagagac ctctctgggc ccatgccacc 240

tccaacatcc actcgacccc ttggaatttc ggtggagagg agcagaggtt gtcctggcgt 300

ggtttaggta gtgtgagagg gtccgggttc aaaaccactt gctgggtggg gagtcgtcag 360

taagtggcta tgccccgacc ccgaagcctg tttccccatc tgtacaatgg aaatgataaa 420

gacgcccatc tgatagggtt tttgtggcaa ataaacattt ggtttttttg ttttgttttg 480

ttttgttttt tgagatggag gtttgctctg tcgcccaggc tggagtgcag tgacacaatc 540

tcatctcacc acaaccttcc cctgcctcag cctcccaagt agctgggatt acaagcatgt 600

gccaccacac ctggctaatt ttctattttt agtagagacg ggtttctcca tgttggtcag 660

cctcagcctc ccaagtaact gggattacag gcctgtgcca ccacacccgg ctaatttttt 720

ctatttttga cagggacggg gtttcaccat gttggtcagg ctggtctaga ggtactggat 780

cttgctacca gtggaacagc cactaaggat tctgcagtga gagcagaggg ccagctaagt 840

ggtactctcc cagagactgt ctgactcatg ccacccccctc caccttggac acaggacact 900

gtggtttctg agccaggtac aatgactcct tttggtaagt gcagtggaag ctgtacactg 960

cccaggcaaa gtgtccgggc agcgtaggcg ggcgactcag atcccagcca gtggacttag 1020

cccctgtttg ctcctccgat aactggggtg accttggtta atattcacca gcagcctccc 1080

ccgttgcccc tctggatcca ctgcttaaat acggacgagg acagg 1125

<210> 408
<211> 1125
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 408
aggctcagag gcacacagga gtttctgggc tcaccctgcc cccttccaac ccctcagttc 60

ccatcctcca gcagctgttt gtgtgctgcc tctgaagtcc acactgaaca aacttcagcc 120

tactcatgtc cctaaaatgg gcaaacattg caagcagcaa acagcaaaca cacagccctc 180

cctgcctgct gaccttggag ctggggcaga ggtcagagac ctctctgggc ccatgccacc 240

tccaacatcc actcgacccc ttggaatttc ggtggagagg agcagaggtt gtcctggcgt 300

ggtttaggta gtgtgagagg gtccgggttc aaaaccactt gctgggtggg gagtcgtcag 360

taagtggcta tgccccgacc ccgaagcctg tttccccatc tgtacaatgg aaatgataaa 420

gacgcccatc tgatagggtt tttgtggcaa ataaacattt ggtttttttg ttttgttttg 480

ttttgttttt tgagatggag gtttgctctg tcgcccaggc tggagtgcag tgacacaatc 540

tcatctcacc acaaccttcc cctgcctcag cctcccaagt agctgggatt acaagcatgt 600

gccaccacac ctggctaatt ttctattttt agtagagacg ggtttctcca tgttggtcag 660

cctcagcctc ccaagtaact gggattacag gcctgtgcca ccacacccgg ctaatttttt 720

ctatttttga cagggacggg gtttcaccat gttggtcagg ctggtctaga ggtactggat 780

cttgctacca gtggaacagc cactaaggat tctgcagtga gagcagaggg ccagctaagt 840

ggtactctcc cagagactgt ctgactcacg ccacccccctc caccttggac acaggacgct 900

gtggtttctg agccaggtac aatgactcct ttcggtaagt gcagtggaag ctgtacactg 960

cccaggcaaa gcgtccgggc agcgtaggcg ggcgactcag atcccagcca gtggacttag 1020

cccctgtttg ctcctctgat aactggggtg accttggtta atattcacca gcagcctccc 1080

ctgttgcccc tctggatcca ctgcttaaat acggacaagg acagg 1125

<210> 409
<211> 1125
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 409
aggctcagag gcacacagga gtttctgggc tcaccctgcc cccttccaac ccctcagttc 60

ccatcctcca gcagctgttt gtgtgctgcc tctgaagtcc acactgaaca aacttcagcc 120

tactcatgtc cctaaaatgg gcaaacattg caagcagcaa acagcaaaca cacagccctc 180

cctgcctgct gaccttggag ctggggcaga ggtcagagac ctctctgggc ccatgccacc 240

tccaacatcc actcgacccc ttggaatttc ggtggagagg agcagaggtt gtcctggcgt 300

ggtttaggta gtgtgagagg gtccgggttc aaaaccactt gctgggtggg gagtcgtcag 360

taagtggcta tgccccgacc ccgaagcctg tttccccatc tgtacaatgg aaatgataaa 420

gacgcccatc tgatagggtt tttgtggcaa ataaacattt ggtttttttg ttttgttttg 480

ttttgttttt tgagatggag gtttgctctg tcgcccaggc tggagtgcag tgacacaatc 540

tcatctcacc acaaccttcc cctgcctcag cctcccaagt agctgggatt acaagcatgt 600

gccaccacac ctggctaatt ttctattttt agtagagacg ggtttctcca tgttggtcag 660

cctcagcctc ccaagtaact gggattacag gcctgtgcca ccacacccgg ctaatttttt 720

ctatttttga cagggacggg gtttcaccat gttggtcagg ctggtctaga ggtactggat 780

cttgctacca gtggaacagc cactaaggat tctgcagtga gagcagaggg ccagctaagt 840

ggtactctcc cagagactgt ctgactcacg ccacccccctc caccttggac acaggacgct 900

gtggtttctg agccaggtac aatgactcct ttcggtaagt gcagtggaag ctgtacactg 960

cccaggcaaa gcgtctgggc agcataggca ggcgactcag atcccagcca gtggacttag 1020

cccctgtttg ctcctccgat aactggggtg accttggtta atattcacca gcagcctccc 1080

ccgttgcccc tctggatcca ctgcttaaat acggacgagg acagg 1125

<210> 410
<211> 1125
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 410
aggctcagag gcacacagga gtttctgggc tcaccctgcc cccttccaac ccctcagttc 60

ccatcctcca gcagctgttt gtgtgctgcc tctgaagtcc acactgaaca aacttcagcc 120

tactcatgtc cctaaaatgg gcaaacattg caagcagcaa acagcaaaca cacagccctc 180

cctgcctgct gaccttggag ctggggcaga ggtcagagac ctctctgggc ccatgccacc 240

tccaacatcc actcgacccc ttggaatttc ggtggagagg agcagaggtt gtcctggcgt 300

ggtttaggta gtgtgagagg gtccgggttc aaaaccactt gctgggtggg gagtcgtcag 360

taagtggcta tgccccgacc ccgaagcctg tttccccatc tgtacaatgg aaatgataaa 420

gacgcccatc tgatagggtt tttgtggcaa ataaacattt ggtttttttg ttttgttttg 480

ttttgttttt tgagatggag gtttgctctg tggcccaggc tggagtgcag tgacacaatc 540

tcatctcacc acaaccttcc cctgcctcag cctcccaagt agctgggatt acaagcatgt 600

gccaccacac ctggctaatt ttctattttt agtagagatg ggtttctcca tgttggtcag 660

cctcagcctc ccaagtaact gggattacag gcctgtgcca ccacacctgg ctaatttttt 720

ctatttttga cagggatggg gtttcaccat gttggtcagg ctggtctaga ggtactggat 780

cttgctacca gtggaacagc cactaaggat tctgcagtga gagcagaggg ccagctaagt 840

ggtactctcc cagagactgt ctgactcacg ccacccccctc caccttggac acaggacgct 900

gtggtttctg agccaggtac aatgactcct ttcggtaagt gcagtggaag ctgtacactg 960

cccaggcaaa gcgtccgggc agcgtaggcg ggcgactcag atcccagcca gtggacttag 1020

cccctgtttg ctcctctgat aactggggtg accttggtta atattcacca gcagcctccc 1080

ccgttgcccc tctggatcca ctgcttaaat acagacgagg acagg 1125

<210> 411
<211> 129
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 411
acacagatcc acaagctcct gaagaggtaa gggtttaagg gatggttggt tggtggggta 60

ttaatgttta attacctgga gcacctgcct gaaatcactt tttttcaggt tggtttaaac 120

gccgccacc 129

<210> 412
<211> 130
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 412
acacagatcc acaagctcct gaagaggtaa gggtttaagg gatggttggt tggtggggta 60

ttaatgttta attacctgga gcacctgcct gaaatcactt tttttcaggt tgggtttaaa 120

cgccgccacc 130

<210> 413
<211> 129
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 413
acacagatcc acaagctcct gaagaggtaa gggtttaagg gatggttggt tggtggggta 60

ttaatgttta attacctgga gcacctgcct gaaatcactt tttttcaggt tgggttaaac 120

gccgccacc 129

<210> 414
<211> 49
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 414
gccctgtctc ctcagcttca ggcaccacca ctgacctggg acagtgaat 49

<210> 415
<211> 47
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 415
acacagatcc acaagctcct gaagaggttg gtttaaacgc cgccacc 47

<210> 416
<211> 50
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 416
catttcgtgg gctaagttta agctactctc gctccgggat aagagtttca 50

<210> 417
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 417
gggacatcgt agagagtcgt actta 25

<210> 418
<211> 162
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 418
gccctgtctc ctcagcttca ggcaccacca ctgacctggg acagtgaata attactctaa 60

ggtaaatata aaatttttaa gtgtataatg tgttaaacta ctgattctaa ttgtttctct 120

cttttagatt ccaacctttg gaactgagtt taaacagcca cc 162

<210> 419
<211> 165
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 419
gccctgtctc ctcagcttca ggcaccacca ctgacctggg acagtgaata attactctaa 60

ggtaaatata aaatttttaa gtgtataatg tgttaaacta ctgattctaa ttgtttctct 120

cttttagatt ccaacctttg gaactgagtt taaaccgcag ccacc 165

<210> 420
<211> 164
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 420
gccctgtctc ctcagcttca ggcaccacca ctgacctggg acagtgaata attactctaa 60

ggtaaatata aaatttttaa gtgtataatg tgttaaacta ctgattctaa ttgtttctct 120

cttttagatt ccaacctttg gaactgagtt taaacgccgc cacc 164

<210> 421
<211> 230
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 421
gccctgtctc ctcagcttca ggcaccacca ctgacctggg acagtgaata attactctaa 60

ggtaaatata aaatttttaa gtgtataatg tgttaaacta ctgattctaa ttgtttctct 120

cttttagatt ccaacctttg gaactgagtt taaacgggac atcgtagaga gtcgtactta 180

catttcgtgg gctaagttta agctactctc gctccgggat aagagtttca 230

<210> 422
<211> 150
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 422
gccctgtctc ctcagcttca ggcaccacca ctgacctggg acagtgaata attactctaa 60

ggtaaatata aaatttttaa gtgtataatg tgttaaacta ctgattctaa ttgtttctct 120

cttttagatt ccaacctttg gaactgaacc 150

<210> 423

<211> 135
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 423
acacagatcc acaagctcct gtctagaaag aggtaagggt ttaagggatg gttggttggt 60

ggggtattaa tgtttaatta cctggagcac ctgcctgaaa tcacttttt tcaggttggt 120

ttaaacgccg ccacc 135

<210> 424
<211> 136
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 424
acacagatcc acaagctcct gtctagaaag aggtaagggt ttaagggatg gttggttggt 60

ggggtattaa tgtttaatta cctggagcac ctgcctgaaa tcacttttt tcaggttggg 120

tttaaacgcc gccacc 136

<210> 425
<211> 135
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 425
acacagatcc acaagctcct gtctagaaag aggtaagggt ttaagggatg gttggttggt 60

ggggtattaa tgtttaatta cctggagcac ctgcctgaaa tcacttttt tcaggttggg 120

ttaaacgccg ccacc 135

<210> 426
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 426
acacagatcc acaagctcct g 21

<210> 427
<211> 67
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 427
gccctgtctc ctcagcttca ggcaccacca ctgacctggg acagtgaatg tttaaaccgc 60

agccacc 67

<210> 428
<211> 131
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 428
acacagatcc acaagctcct gaagaggtaa gggtttaagg gatggttggt tggtggggta 60

ttaatgttta attacctgga gcacctgcct gaaatcactt tttttcaggt tgggtttaaa 120

ccgcagccac c 131

<210> 429
<211> 195
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 429
acacagatcc acaagctcct gaagaggtaa gggtttaagg gatggttggt tggtggggta 60

ttaatgttta attacctgga gcacctgcct gaaatcactt tttttcaggt tggtttaaac 120

gggacatcgt agagagtcgt acttacattt cgtgggctaa gtttaagcta ctctcgctcc 180

gggataagag tttca 195

<210> 430
<211> 130
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 430
acacagatcc acaagctcct gaagaggtaa gggtttaagg gatggttggt tggtggggta 60

ttaatgttta attacctgga gcacctgcct gaaatcactt tttttcaggt tgggttaaac 120

cgcagccacc 130

<210> 431
<211> 130
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 431
acacagatcc acaagctcct gaagaggtaa gggtttaagg gatggttggt tggtggggta 60

ttaatgttta attacctgga gcacctgcct gaaatcactt tttttcaggt tggtttaaac 120

cgcagccacc 130

<210> 432
<211> 48
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 432
acacagatcc acaagctcct gaagaggttg gtttaaaccg cagccacc 48

<210> 433

<211> 137
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 433
acacagatcc acaagctcct gtctagaaag aggtaagggt ttaagggatg gttggttggt 60

ggggtattaa tgtttaatta cctggagcac ctgcctgaaa tcacttttt tcaggttggg 120

tttaaaccgc agccacc 137

<210> 434
<211> 136
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 434
acacagatcc acaagctcct gtctagaaag aggtaagggt ttaagggatg gttggttggt 60

ggggtattaa tgtttaatta cctggagcac ctgcctgaaa tcacttttt tcaggttggg 120

ttaaaccgca gccacc 136

<210> 435
<211> 136
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 435
acacagatcc acaagctcct gtctagaaag aggtaagggt ttaagggatg gttggttggt 60

ggggtattaa tgtttaatta cctggagcac ctgcctgaaa tcacttttt tcaggttggt 120

ttaaaccgca gccacc 136

<210> 436
<211> 66
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 436
gccctgtctc ctcagcttca ggcaccacca ctgacctggg acagtgaatg tttaaacgcc 60

gccacc 66

<210> 437
<211> 16
<212> DNA
<213> Adeno-associated virus 2

<400> 437
gcgcgctcgc tcgctc 16

<210> 438
<211> 4
<212> DNA
<213> Unknown

<220>
<223> Description of Unknown: TRS
sequence

<400> 438
agtt 4

<210> 439
<211> 8
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 439
actgaggc 8

<210> 440
<211> 8
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 440
gcctcagt 8

<210> 441
<211> 16
<212> DNA
<213> Adeno-associated virus 2

<400> 441
gagcgagcga gcgcgc 16

<210> 442
<211> 6167
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

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<211> 541
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

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gaaatggggg agggacagga gtagggcgga gggtagcccg gggattctgc agtgagagca 240

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<210> 485
<211> 321
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

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tactcatgtc cctaaaatgg gcaaacattg caagcagcaa acagcaaaca cacagccctc 180

cctgcctgct gaccttggag ctggggcaga ggtcagagac ctctctgggc ccatgccacc 240

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<210> 486
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
peptide

<400> 486
Glu Ile Thr Arg
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<210> 487
<211> 94
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 487
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<210> 488
<211> 31
<212> PRT
<213> Artificial Sequence

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polypeptide

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Phe Gly Ala Thr Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser
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<210> 489
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<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

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ggggagctgc ctgt 134

<210> 490
<211> 45
<212> PRT
<213> Artificial Sequence

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polypeptide

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Cys Phe Ser Ala Thr Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser
            20   25   30

Trp Asp Tyr Met Gln Ser Asp Leu Gly Glu Leu Pro Val
        35   40   45

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<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
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<210> 492
<211> 2351
<212> PRT
<213> Homo sapiens

<400> 492
Met Gln Ile Glu Leu Ser Thr Cys Phe Phe Leu Cys Leu Leu Arg Phe
1   5   10   15

Cys Phe Ser Ala Thr Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser
            20   25   30

Trp Asp Tyr Met Gln Ser Asp Leu Gly Glu Leu Pro Val Asp Ala Arg
        35   40   45

Phe Pro Pro Arg Val Pro Lys Ser Phe Pro Phe Asn Thr Ser Val Val
    50   55   60

Tyr Lys Lys Thr Leu Phe Val Glu Phe Thr Asp His Leu Phe Asn Ile
65   70   75   80

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Ala Lys Pro Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile Gln
                85                  90                  95

Ala Glu Val Tyr Asp Thr Val Val Ile Thr Leu Lys Asn Met Ala Ser
            100                 105                 110

His Pro Val Ser Leu His Ala Val Gly Val Ser Tyr Trp Lys Ala Ser
        115                 120                 125

Glu Gly Ala Glu Tyr Asp Asp Gln Thr Ser Gln Arg Glu Lys Glu Asp
    130                 135                 140

Asp Lys Val Phe Pro Gly Gly Ser His Thr Tyr Val Trp Gln Val Leu
145                 150                 155                 160

Lys Glu Asn Gly Pro Met Ala Ser Asp Pro Leu Cys Leu Thr Tyr Ser
                165                 170                 175

Tyr Leu Ser His Val Asp Leu Val Lys Asp Leu Asn Ser Gly Leu Ile
            180                 185                 190

Gly Ala Leu Leu Val Cys Arg Glu Gly Ser Leu Ala Lys Glu Lys Thr
        195                 200                 205

Gln Thr Leu His Lys Phe Ile Leu Leu Phe Ala Val Phe Asp Glu Gly
    210                 215                 220

Lys Ser Trp His Ser Glu Thr Lys Asn Ser Leu Met Gln Asp Arg Asp
225                 230                 235                 240

Ala Ala Ser Ala Arg Ala Trp Pro Lys Met His Thr Val Asn Gly Tyr
                245                 250                 255

Val Asn Arg Ser Leu Pro Gly Leu Ile Gly Cys His Arg Lys Ser Val
            260                 265                 270

Tyr Trp His Val Ile Gly Met Gly Thr Thr Pro Glu Val His Ser Ile
        275                 280                 285

Phe Leu Glu Gly His Thr Phe Leu Val Arg Asn His Arg Gln Ala Ser
    290                 295                 300
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Leu Glu Ile Ser Pro Ile Thr Phe Leu Thr Ala Gln Thr Leu Leu Met
305                 310                 315                 320

Asp Leu Gly Gln Phe Leu Leu Phe Cys His Ile Ser Ser His Gln His
                325                 330                 335

Asp Gly Met Glu Ala Tyr Val Lys Val Asp Ser Cys Pro Glu Glu Pro
            340                 345                 350

Gln Leu Arg Met Lys Asn Asn Glu Glu Ala Glu Asp Tyr Asp Asp Asp
        355                 360                 365

Leu Thr Asp Ser Glu Met Asp Val Val Arg Phe Asp Asp Asp Asn Ser
    370                 375                 380

Pro Ser Phe Ile Gln Ile Arg Ser Val Ala Lys Lys His Pro Lys Thr
385                 390                 395                 400

Trp Val His Tyr Ile Ala Ala Glu Glu Glu Asp Trp Asp Tyr Ala Pro
                405                 410                 415

Leu Val Leu Ala Pro Asp Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn
            420                 425                 430

Asn Gly Pro Gln Arg Ile Gly Arg Lys Tyr Lys Lys Val Arg Phe Met
        435                 440                 445

Ala Tyr Thr Asp Glu Thr Phe Lys Thr Arg Glu Ala Ile Gln His Glu
    450                 455                 460

Ser Gly Ile Leu Gly Pro Leu Leu Tyr Gly Glu Val Gly Asp Thr Leu
465                 470                 475                 480

Leu Ile Ile Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn Ile Tyr Pro
                485                 490                 495

His Gly Ile Thr Asp Val Arg Pro Leu Tyr Ser Arg Arg Leu Pro Lys
            500                 505                 510
```

```
Gly Val Lys His Leu Lys Asp Phe Pro Ile Leu Pro Gly Glu Ile Phe
        515                 520                 525

Lys Tyr Lys Trp Thr Val Thr Val Glu Asp Gly Pro Thr Lys Ser Asp
    530                 535                 540

Pro Arg Cys Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg
545                 550                 555                 560

Asp Leu Ala Ser Gly Leu Ile Gly Pro Leu Leu Ile Cys Tyr Lys Glu
                565                 570                 575

Ser Val Asp Gln Arg Gly Asn Gln Ile Met Ser Asp Lys Arg Asn Val
            580                 585                 590

Ile Leu Phe Ser Val Phe Asp Glu Asn Arg Ser Trp Tyr Leu Thr Glu
        595                 600                 605

Asn Ile Gln Arg Phe Leu Pro Asn Pro Ala Gly Val Gln Leu Glu Asp
    610                 615                 620

Pro Glu Phe Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val
625                 630                 635                 640

Phe Asp Ser Leu Gln Leu Ser Val Cys Leu His Glu Val Ala Tyr Trp
                645                 650                 655

Tyr Ile Leu Ser Ile Gly Ala Gln Thr Asp Phe Leu Ser Val Phe Phe
            660                 665                 670

Ser Gly Tyr Thr Phe Lys His Lys Met Val Tyr Glu Asp Thr Leu Thr
        675                 680                 685

Leu Phe Pro Phe Ser Gly Glu Thr Val Phe Met Ser Met Glu Asn Pro
    690                 695                 700

Gly Leu Trp Ile Leu Gly Cys His Asn Ser Asp Phe Arg Asn Arg Gly
705                 710                 715                 720

Met Thr Ala Leu Leu Lys Val Ser Ser Cys Asp Lys Asn Thr Gly Asp
                725                 730                 735
```

```
Tyr Tyr Glu Asp Ser Tyr Glu Asp Ile Ser Ala Tyr Leu Leu Ser Lys
            740                 745                 750

Asn Asn Ala Ile Glu Pro Arg Ser Phe Ser Gln Asn Ser Arg His Pro
        755                 760                 765

Ser Thr Arg Gln Lys Gln Phe Asn Ala Thr Thr Ile Pro Glu Asn Asp
    770                 775                 780

Ile Glu Lys Thr Asp Pro Trp Phe Ala His Arg Thr Pro Met Pro Lys
785                 790                 795                 800

Ile Gln Asn Val Ser Ser Ser Asp Leu Leu Met Leu Leu Arg Gln Ser
                805                 810                 815

Pro Thr Pro His Gly Leu Ser Leu Ser Asp Leu Gln Glu Ala Lys Tyr
            820                 825                 830

Glu Thr Phe Ser Asp Asp Pro Ser Pro Gly Ala Ile Asp Ser Asn Asn
        835                 840                 845

Ser Leu Ser Glu Met Thr His Phe Arg Pro Gln Leu His His Ser Gly
    850                 855                 860

Asp Met Val Phe Thr Pro Glu Ser Gly Leu Gln Leu Arg Leu Asn Glu
865                 870                 875                 880

Lys Leu Gly Thr Thr Ala Ala Thr Glu Leu Lys Lys Leu Asp Phe Lys
                885                 890                 895

Val Ser Ser Thr Ser Asn Asn Leu Ile Ser Thr Ile Pro Ser Asp Asn
            900                 905                 910

Leu Ala Ala Gly Thr Asp Asn Thr Ser Ser Leu Gly Pro Pro Ser Met
        915                 920                 925

Pro Val His Tyr Asp Ser Gln Leu Asp Thr Thr Leu Phe Gly Lys Lys
    930                 935                 940
```

```
Ser Ser Pro Leu Thr Glu Ser Gly Gly Pro Leu Ser Leu Ser Glu Glu
945                 950                 955                 960


Asn Asn Asp Ser Lys Leu Leu Glu Ser Gly Leu Met Asn Ser Gln Glu
                965                 970                 975


Ser Ser Trp Gly Lys Asn Val Ser Ser Thr Glu Ser Gly Arg Leu Phe
            980                 985                 990


Lys Gly Lys Arg Ala His Gly Pro  Ala Leu Leu Thr Lys  Asp Asn Ala
        995                 1000                1005


Leu Phe  Lys Val Ser Ile Ser  Leu Leu Lys Thr Asn  Lys Thr Ser
    1010                1015                1020


Asn Asn  Ser Ala Thr Asn Arg  Lys Thr His Ile Asp  Gly Pro Ser
    1025                1030                1035


Leu Leu  Ile Glu Asn Ser Pro  Ser Val Trp Gln Asn  Ile Leu Glu
    1040                1045                1050


Ser Asp  Thr Glu Phe Lys Lys  Val Thr Pro Leu Ile  His Asp Arg
    1055                1060                1065


Met Leu  Met Asp Lys Asn Ala  Thr Ala Leu Arg Leu  Asn His Met
    1070                1075                1080


Ser Asn  Lys Thr Thr Ser Ser  Lys Asn Met Glu Met  Val Gln Gln
    1085                1090                1095


Lys Lys  Glu Gly Pro Ile Pro  Pro Asp Ala Gln Asn  Pro Asp Met
    1100                1105                1110


Ser Phe  Phe Lys Met Leu Phe  Leu Pro Glu Ser Ala  Arg Trp Ile
    1115                1120                1125


Gln Arg  Thr His Gly Lys Asn  Ser Leu Asn Ser Gly  Gln Gly Pro
    1130                1135                1140


Ser Pro  Lys Gln Leu Val Ser  Leu Gly Pro Glu Lys  Ser Val Glu
    1145                1150                1155
```

```
Gly Gln  Asn Phe Leu Ser Glu  Lys Asn Lys Val Val  Val Gly Lys
    1160                1165                1170

Gly Glu  Phe Thr Lys Asp Val  Gly Leu Lys Glu Met  Val Phe Pro
    1175                1180                1185

Ser Ser  Arg Asn Leu Phe Leu  Thr Asn Leu Asp Asn  Leu His Glu
    1190                1195                1200

Asn Asn  Thr His Asn Gln Glu  Lys Lys Ile Gln Glu  Glu Ile Glu
    1205                1210                1215

Lys Lys  Glu Thr Leu Ile Gln  Glu Asn Val Val Leu  Pro Gln Ile
    1220                1225                1230

His Thr  Val Thr Gly Thr Lys  Asn Phe Met Lys Asn  Leu Phe Leu
    1235                1240                1245

Leu Ser  Thr Arg Gln Asn Val  Glu Gly Ser Tyr Asp  Gly Ala Tyr
    1250                1255                1260

Ala Pro  Val Leu Gln Asp Phe  Arg Ser Leu Asn Asp  Ser Thr Asn
    1265                1270                1275

Arg Thr  Lys Lys His Thr Ala  His Phe Ser Lys Lys  Gly Glu Glu
    1280                1285                1290

Glu Asn  Leu Glu Gly Leu Gly  Asn Gln Thr Lys Gln  Ile Val Glu
    1295                1300                1305

Lys Tyr  Ala Cys Thr Thr Arg  Ile Ser Pro Asn Thr  Ser Gln Gln
    1310                1315                1320

Asn Phe  Val Thr Gln Arg Ser  Lys Arg Ala Leu Lys  Gln Phe Arg
    1325                1330                1335

Leu Pro  Leu Glu Glu Thr Glu  Leu Glu Lys Arg Ile  Ile Val Asp
    1340                1345                1350
```

```
Asp Thr  Ser Thr Gln Trp Ser  Lys Asn Met Lys His  Leu Thr Pro
    1355                1360                1365

Ser Thr  Leu Thr Gln Ile Asp  Tyr Asn Glu Lys Glu  Lys Gly Ala
    1370                1375                1380

Ile Thr  Gln Ser Pro Leu Ser  Asp Cys Leu Thr Arg  Ser His Ser
    1385                1390                1395

Ile Pro  Gln Ala Asn Arg Ser  Pro Leu Pro Ile Ala  Lys Val Ser
    1400                1405                1410

Ser Phe  Pro Ser Ile Arg Pro  Ile Tyr Leu Thr Arg  Val Leu Phe
    1415                1420                1425

Gln Asp  Asn Ser Ser His Leu  Pro Ala Ala Ser Tyr  Arg Lys Lys
    1430                1435                1440

Asp Ser  Gly Val Gln Glu Ser  Ser His Phe Leu Gln  Gly Ala Lys
    1445                1450                1455

Lys Asn  Asn Leu Ser Leu Ala  Ile Leu Thr Leu Glu  Met Thr Gly
    1460                1465                1470

Asp Gln  Arg Glu Val Gly Ser  Leu Gly Thr Ser Ala  Thr Asn Ser
    1475                1480                1485

Val Thr  Tyr Lys Lys Val Glu  Asn Thr Val Leu Pro  Lys Pro Asp
    1490                1495                1500

Leu Pro  Lys Thr Ser Gly Lys  Val Glu Leu Leu Pro  Lys Val His
    1505                1510                1515

Ile Tyr  Gln Lys Asp Leu Phe  Pro Thr Glu Thr Ser  Asn Gly Ser
    1520                1525                1530

Pro Gly  His Leu Asp Leu Val  Glu Gly Ser Leu Leu  Gln Gly Thr
    1535                1540                1545

Glu Gly  Ala Ile Lys Trp Asn  Glu Ala Asn Arg Pro  Gly Lys Val
    1550                1555                1560
```

```
Pro Phe  Leu Arg Val Ala Thr  Glu Ser Ser Ala Lys  Thr Pro Ser
    1565                1570                1575

Lys Leu  Leu Asp Pro Leu Ala  Trp Asp Asn His Tyr  Gly Thr Gln
    1580                1585                1590

Ile Pro  Lys Glu Glu Trp Lys  Ser Gln Glu Lys Ser  Pro Glu Lys
    1595                1600                1605

Thr Ala  Phe Lys Lys Lys Asp  Thr Ile Leu Ser Leu  Asn Ala Cys
    1610                1615                1620

Glu Ser  Asn His Ala Ile Ala  Ala Ile Asn Glu Gly  Gln Asn Lys
    1625                1630                1635

Pro Glu  Ile Glu Val Thr Trp  Ala Lys Gln Gly Arg  Thr Glu Arg
    1640                1645                1650

Leu Cys  Ser Gln Asn Pro Pro  Val Leu Lys Arg His  Gln Arg Glu
    1655                1660                1665

Ile Thr  Arg Thr Thr Leu Gln  Ser Asp Gln Glu Glu  Ile Asp Tyr
    1670                1675                1680

Asp Asp  Thr Ile Ser Val Glu  Met Lys Lys Glu Asp  Phe Asp Ile
    1685                1690                1695

Tyr Asp  Glu Asp Glu Asn Gln  Ser Pro Arg Ser Phe  Gln Lys Lys
    1700                1705                1710

Thr Arg  His Tyr Phe Ile Ala  Ala Val Glu Arg Leu  Trp Asp Tyr
    1715                1720                1725

Gly Met  Ser Ser Ser Pro His  Val Leu Arg Asn Arg  Ala Gln Ser
    1730                1735                1740

Gly Ser  Val Pro Gln Phe Lys  Lys Val Val Phe Gln  Glu Phe Thr
    1745                1750                1755
```

```
Asp Gly  Ser Phe Thr Gln Pro  Leu Tyr Arg Gly Glu  Leu Asn Glu
    1760                1765                1770

His Leu  Gly Leu Leu Gly Pro  Tyr Ile Arg Ala Glu  Val Glu Asp
    1775                1780                1785

Asn Ile  Met Val Thr Phe Arg  Asn Gln Ala Ser Arg  Pro Tyr Ser
    1790                1795                1800

Phe Tyr  Ser Ser Leu Ile Ser  Tyr Glu Glu Asp Gln  Arg Gln Gly
    1805                1810                1815

Ala Glu  Pro Arg Lys Asn Phe  Val Lys Pro Asn Glu  Thr Lys Thr
    1820                1825                1830

Tyr Phe  Trp Lys Val Gln His  His Met Ala Pro Thr  Lys Asp Glu
    1835                1840                1845

Phe Asp  Cys Lys Ala Trp Ala  Tyr Phe Ser Asp Val  Asp Leu Glu
    1850                1855                1860

Lys Asp  Val His Ser Gly Leu  Ile Gly Pro Leu Leu  Val Cys His
    1865                1870                1875

Thr Asn  Thr Leu Asn Pro Ala  His Gly Arg Gln Val  Thr Val Gln
    1880                1885                1890

Glu Phe  Ala Leu Phe Phe Thr  Ile Phe Asp Glu Thr  Lys Ser Trp
    1895                1900                1905

Tyr Phe  Thr Glu Asn Met Glu  Arg Asn Cys Arg Ala  Pro Cys Asn
    1910                1915                1920

Ile Gln  Met Glu Asp Pro Thr  Phe Lys Glu Asn Tyr  Arg Phe His
    1925                1930                1935

Ala Ile  Asn Gly Tyr Ile Met  Asp Thr Leu Pro Gly  Leu Val Met
    1940                1945                1950

Ala Gln  Asp Gln Arg Ile Arg  Trp Tyr Leu Leu Ser  Met Gly Ser
    1955                1960                1965
```

```
Asn Glu  Asn Ile His Ser Ile  His Phe Ser Gly His  Val Phe Thr
    1970                1975                1980

Val Arg  Lys Lys Glu Glu Tyr  Lys Met Ala Leu Tyr  Asn Leu Tyr
    1985                1990                1995

Pro Gly  Val Phe Glu Thr Val  Glu Met Leu Pro Ser  Lys Ala Gly
    2000                2005                2010

Ile Trp  Arg Val Glu Cys Leu  Ile Gly Glu His Leu  His Ala Gly
    2015                2020                2025

Met Ser  Thr Leu Phe Leu Val  Tyr Ser Asn Lys Cys  Gln Thr Pro
    2030                2035                2040

Leu Gly  Met Ala Ser Gly His  Ile Arg Asp Phe Gln  Ile Thr Ala
    2045                2050                2055

Ser Gly  Gln Tyr Gly Gln Trp  Ala Pro Lys Leu Ala  Arg Leu His
    2060                2065                2070

Tyr Ser  Gly Ser Ile Asn Ala  Trp Ser Thr Lys Glu  Pro Phe Ser
    2075                2080                2085

Trp Ile  Lys Val Asp Leu Leu  Ala Pro Met Ile Ile  His Gly Ile
    2090                2095                2100

Lys Thr  Gln Gly Ala Arg Gln  Lys Phe Ser Ser Leu  Tyr Ile Ser
    2105                2110                2115

Gln Phe  Ile Ile Met Tyr Ser  Leu Asp Gly Lys Lys  Trp Gln Thr
    2120                2125                2130

Tyr Arg  Gly Asn Ser Thr Gly  Thr Leu Met Val Phe  Phe Gly Asn
    2135                2140                2145

Val Asp  Ser Ser Gly Ile Lys  His Asn Ile Phe Asn  Pro Pro Ile
    2150                2155                2160
```

```
Ile Ala  Arg Tyr Ile Arg Leu  His Pro Thr His Tyr  Ser Ile Arg
    2165                2170                2175

Ser Thr  Leu Arg Met Glu Leu  Met Gly Cys Asp Leu  Asn Ser Cys
    2180                2185                2190

Ser Met  Pro Leu Gly Met Glu  Ser Lys Ala Ile Ser  Asp Ala Gln
    2195                2200                2205

Ile Thr  Ala Ser Ser Tyr Phe  Thr Asn Met Phe Ala  Thr Trp Ser
    2210                2215                2220

Pro Ser  Lys Ala Arg Leu His  Leu Gln Gly Arg Ser  Asn Ala Trp
    2225                2230                2235

Arg Pro  Gln Val Asn Asn Pro  Lys Glu Trp Leu Gln  Val Asp Phe
    2240                2245                2250

Gln Lys  Thr Met Lys Val Thr  Gly Val Thr Thr Gln  Gly Val Lys
    2255                2260                2265

Ser Leu  Leu Thr Ser Met Tyr  Val Lys Glu Phe Leu  Ile Ser Ser
    2270                2275                2280

Ser Gln  Asp Gly His Gln Trp  Thr Leu Phe Phe Gln  Asn Gly Lys
    2285                2290                2295

Val Lys  Val Phe Gln Gly Asn  Gln Asp Ser Phe Thr  Pro Val Val
    2300                2305                2310

Asn Ser  Leu Asp Pro Pro Leu  Leu Thr Arg Tyr Leu  Arg Ile His
    2315                2320                2325

Pro Gln  Ser Trp Val His Gln  Ile Ala Leu Arg Met  Glu Val Leu
    2330                2335                2340

Gly Cys  Glu Ala Gln Asp Leu  Tyr
    2345                2350
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<211> 143

<212> DNA
<213> Adeno-associated virus 1

<400> 493
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agacggcaga ggtctcctct gccggcccca ccgagcgagc gacgcgcgca gagagggagt 120

gggcaactcc atcactaggg taa 143

<210> 494
<211> 143
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ggcagaggag acctctgccg tctgcggacc tttggtccgc aggccccacc gagcgagcga 120

gcgcgcagag agggagtggg caa 143

<210> 495
<211> 141
<212> DNA
<213> Adeno-associated virus 2

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cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccgggcaaag cccgggcgtc 60

gggcgacctt tggtcgcccg gcctcagtga gcgagcgagc gcgcagagag ggagtggcca 120

actccatcac taggggttcc t 141

<210> 496
<211> 141
<212> DNA
<213> Adeno-associated virus 2

<400> 496
aggaacccct agtgatggag ttggccactc cctctctgcg cgctcgctcg ctcactgagg 60

ccgggcgacc aaaggtcgcc cgacgcccgg gctttgcccg ggcggcctca gtgagcgagc 120

gagcgcgcag ctgcctgcag g 141

<210> 497
<211> 144
<212> DNA
<213> Adeno-associated virus 3

<400> 497
ttggccactc cctctatgcg cactcgctcg ctcggtgggg cctggcgacc aaaggtcgcc 60

agacggacgt gggtttccac gtccggcccc accgagcgag cgagtgcgca tagagggagt 120

ggccaactcc atcactagag gtat 144

<210> 498
<211> 144
<212> DNA
<213> Adeno-associated virus 3

<400> 498
atacctctag tgatggagtt ggccactccc tctatgcgca ctcgctcgct cggtggggcc 60

ggacgtggaa acccacgtcc gtctggcgac ctttggtcgc caggccccac cgagcgagcg 120

agtgcgcata gagggagtgg ccaa 144

<210> 499
<211> 127
<212> DNA
<213> Adeno-associated virus 4

<400> 499
ttggccactc cctctatgcg cgctcgctca ctcactcggc cctggagacc aaaggtctcc 60

agactgccgg cctctggccg gcagggccga gtgagtgagc gagcgcgcat agagggagtg 120

gccaact 127

<210> 500
<211> 127
<212> DNA
<213> Adeno-associated virus 4

<400> 500
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ccagactgcc ggcctctggc cggcagggcc gagtgagtga gcgagcgcgc atagagggag 120

tggccaa 127

<210> 501
<211> 166
<212> DNA
<213> Adeno-associated virus 5

<400> 501

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gtcgtctggc agctctttga gctgccaccc ccccaaacga gccagcgagc gagcgaacgc 120

gacaggggggg agagtgccac actctcaagc aaggggggttt tgtaag 166

<210> 502
<211> 166
<212> DNA
<213> Adeno-associated virus 5

<400> 502
cttacaaaac ccccttgctt gagagtgtgg cactctcccc cctgtcgcgt tcgctcgctc 60

gctggctcgt ttgggggggt ggcagctcaa agagctgcca gacgacggcc ctctggccgt 120

cgccccccca aacgagccag cgagcgagcg aacgcgacag gggggga 166

<210> 503
<211> 144
<212> DNA
<213> Adeno-associated virus 6

<400> 503
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cagacggcag aggtctcctc tgccggcccc accgagcgag cgagcgcgca tagagggagt 120

gggcaactcc atcactaggg gtat 144

<210> 504
<211> 144
<212> DNA
<213> Adeno-associated virus 6

<400> 504
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ggcagaggag acctctgccg tctgcggacc tttggtccgc aggccccacc gagcgagcga 120

gcgcgcatta gagggagtgg gcaa 144

<210> 505
<211> 120
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

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<211> 122
<212> DNA
<213> Artificial Sequence

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gcctgcagg 129

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ccgggcgaca aagtcgcccg acgcccgggc tttgcccggg cggcctcagt gagcgagcga 120

gcgcgcagct gcctgcagg 139

<210> 510
<211> 137
<212> DNA
<213> Artificial Sequence

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gcgcagctgc ctgcagg 137

<210> 511
<211> 135
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

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ccgggcgaaa cgcccgacgc ccgggctttg cccgggcggc ctcagtgagc gagcgagcgc 120

gcagctgcct gcagg 135

<210> 512

<211> 133
<212> DNA
<213> Artificial Sequence

<220>
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ccgggcaaag cccgacgccc gggctttgcc cgggcggcct cagtgagcga gcgagcgcgc 120

agctgcctgc agg 133

<210> 513
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<212> DNA
<213> Artificial Sequence

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gcgcgcagct gcctgcagg 139

<210> 514
<211> 137
<212> DNA
<213> Artificial Sequence

<220>
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polynucleotide

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gcgcagctgc ctgcagg 137

<210> 515
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polynucleotide

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gcagctgcct gcagg 135

<210> 516
<211> 133
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

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ccgggcgacc aaaggtcgcc cgacgccctt tgggcggcct cagtgagcga gcgagcgcgc 120

agctgcctgc agg 133

<210> 517
<211> 131
<212> DNA
<213> Artificial Sequence

<220>
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polynucleotide

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ccgggcgacc aaaggtcgcc cgacgccttt ggcggcctca gtgagcgagc gagcgcgcag 120

ctgcctgcag g 131

<210> 518
<211> 129
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic

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ccgggcgacc aaaggtcgcc cgacgctttg cggcctcagt gagcgagcga gcgcgcagct 120

gcctgcagg 129

<210> 519
<211> 127
<212> DNA
<213> Artificial Sequence

<220>
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ccgggcgacc aaaggtcgcc cgacgtttcg gcctcagtga gcgagcgagc gcgcagctgc 120

ctgcagg 127

<210> 520
<211> 122
<212> DNA
<213> Artificial Sequence

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gg 122

<210> 521
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polynucleotide

<400> 522
cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccgggaaacc cgggcgtgcg 60

cctcagtgag cgagcgagcg cgcagagagg gagtggccaa ctccatcact aggggttcct 120

<210> 523
<211> 122
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 523
cctgcaggca gctgcgcgct cgctcgctca ctgaggccgt cgggcgacct ttggtcgccc 60

ggcctcagtg agcgagcgag cgcgcagaga gggagtggcc aactccatca ctaggggttc 120

ct 122

<210> 524
<211> 122
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 524
cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccgggcaaag cccgggcgtc 60

ggcctcagtg agcgagcgag cgcgcagaga gggagtggcc aactccatca ctaggggttc 120

ct 122

<210> 525
<211> 129
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 525
cctgcaggca gctgcgcgct cgctcgctca ctgaggcgcc cgggcgtcgg gcgacctttg 60

gtcgcccggc ctcagtgagc gagcgagcgc gcagagaggg agtggccaac tccatcacta 120

ggggttcct 129

<210> 526
<211> 101
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 526
cctgcaggca gctgcgcgct cgctcgctca ctgaggcaaa gcctcagtga gcgagcgagc 60

gcgcagagag ggagtggcca actccatcac taggggttcc t 101

<210> 527
<211> 139
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 527
cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccgggcaaag cccgggcgtc 60

gggcgacttt gtcgcccggc ctcagtgagc gagcgagcgc gcagagaggg agtggccaac 120

tccatcacta ggggttcct 139

<210> 528
<211> 137
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 528
cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccgggcaaag cccgggcgtc 60

gggcgatttt cgcccggcct cagtgagcga gcgagcgcgc agagagggag tggccaactc 120

catcactagg ggttcct 137

<210> 529
<211> 135
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 529
cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccgggcaaag cccgggcgtc 60

gggcgtttcg cccggcctca gtgagcgagc gagcgcgcag agagggagtg gccaactcca 120

tcactagggg ttcct 135

<210> 530
<211> 133
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 530
cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccgggcaaag cccgggcgtc 60

gggctttgcc cggcctcagt gagcgagcga gcgcgcagag agggagtggc caactccatc 120

actaggggtt cct 133

<210> 531
<211> 139
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 531
cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccgggaaacc cgggcgtcgg 60

gcgacctttg gtcgcccggc ctcagtgagc gagcgagcgc gcagagaggg agtggccaac 120

tccatcacta ggggttcct 139

<210> 532
<211> 137
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 532
cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccggaaaccg ggcgtcgggc 60

gacctttggt cgcccggcct cagtgagcga gcgagcgcgc agagagggag tggccaactc 120

catcactagg ggttcct 137

<210> 533
<211> 135
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 533
cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccgaaacggg cgtcgggcga 60

cctttggtcg cccggcctca gtgagcgagc gagcgcgcag agagggagtg gccaactcca 120

tcactagggg ttcct 135

<210> 534
<211> 133
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 534
cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccaaagggcg tcgggcgacc 60

tttggtcgcc cggcctcagt gagcgagcga gcgcgcagag agggagtggc caactccatc 120

actaggggtt cct 133

<210> 535
<211> 131
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 535
cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc caaaggcgtc gggcgacctt 60

tggtcgcccg gcctcagtga gcgagcgagc gcgcagagag ggagtggcca actccatcac 120

taggggttcc t 131

<210> 536
<211> 129
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 536
cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc aaagcgtcgg gcgacctttg 60

gtcgcccggc ctcagtgagc gagcgagcgc gcagagaggg agtggccaac tccatcacta 120

ggggttcct 129

<210> 537
<211> 127
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 537
cctgcaggca gctgcgcgct cgctcgctca ctgaggccga aacgtcgggc gacctttggt 60

cgcccggcct cagtgagcga gcgagcgcgc agagagggag tggccaactc catcactagg 120

ggttcct 127

<210> 538
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 538
atcgaacgat cg 12

<210> 539
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 539
cgatcgttcg at 12

<210> 540
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 540
atcgaaccat cg 12

<210> 541
<211> 225
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 541

ccgtctgtct gcacatttcg tagagcgagt gttccgatac tctaatctcc ctaggcaagg 60

ttcatatttg tgtaggttac ttattctcct tttgttgact aagtcaataa tcagaatcag 120

caggtttgga gtcagcttgg cagggatcag cagcctgggt tggaaggagg gggtataaaa 180

gccccttcac caggagaagc cgtcacacag atccacaagc tcctg 225

<210> 542
<211> 103
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 542
taacaaacac cattgtcaca ctccatcacc aaacaccatt gtcacactcc acgatcaaac 60

accattgtca cactccatca ccaaacacca ttgtcacact cca 103

<210> 543
<211> 77
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 543
tccataaagt aggaaacact acacgattcc ataaagtagg aaacactaca tcactccata 60

aagtaggaaa cactaca 77

<210> 544
<211> 165
<212> DNA
<213> Adeno-associated virus 2

<400> 544
aggaacccct agtgatggag ttggccactc cctctctgcg cgctcgctcg ctcactgagg 60

ccgcccgggc aaagcccggg cgtcgggcga cctttggtcg cccggcctca gtgagcgagc 120

gagcgcgcag agagggagtg gccaactcca tcactagggg ttcct 165

<210> 545
<211> 140

<212> DNA
<213> Adeno-associated virus 2

<400> 545
cccctagtga tggagttggc cactccctct ctgcgcgctc gctcgctcac tgaggccgcc 60

cgggcaaagc ccgggcgtcg ggcgaccttt ggtcgcccgg cctcagtgag cgagcgagcg 120

cgcagagaga tcactagggg 140

<210> 546
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 546
gggactttcc 10

<210> 547
<211> 91
<212> DNA
<213> Adeno-associated virus 2

<400> 547
gcgcgctcgc tcgctcactg aggccgcccg ggcaaagccc gggcgtcggg cgacctttgg 60

tcgcccggcc tcagtgagcg agcgagcgcg c 91

<210> 548
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 548
ggggactttc c 11

<210> 549
<211> 80
<212> DNA
<213> Adeno-associated virus 2

<400> 549

gcgcgctcgc tcgctcactg aggccgcccg ggcgtcgggc gacctttggt cgcccggcct 60

cagtgagcga gcgagcgcgc 80

<210> 550
<211> 91
<212> DNA
<213> Adeno-associated virus 2

<400> 550
gcgcgctcgc tcgctcactg aggccgggcg accaaaggtc gcccgacgcc cgggctttgc 60

ccgggcggcc tcagtgagcg agcgagcgcg c 91

<210> 551
<211> 80
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 551
gcgcgctcgc tcgctcactg aggccgggcg accaaaggtc gcccgacgcc cgggcggcct 60

cagtgagcga gcgagcgcgc 80

<210> 552
<211> 1463
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 552
Met Gln Ile Glu Leu Ser Thr Cys Phe Phe Leu Cys Leu Leu Arg Phe
1 5 10 15

Cys Phe Ser Ala Thr Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser
20 25 30

Trp Asp Tyr Met Gln Ser Asp Leu Gly Glu Leu Pro Val Asp Ala Arg
35 40 45

Phe Pro Pro Arg Val Pro Lys Ser Phe Pro Phe Asn Thr Ser Val Val

```
    50                  55                  60

Tyr Lys Lys Thr Leu Phe Val Glu Phe Thr Asp His Leu Phe Asn Ile
65                  70                  75                  80

Ala Lys Pro Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile Gln
                85                  90                  95

Ala Glu Val Tyr Asp Thr Val Val Ile Thr Leu Lys Asn Met Ala Ser
            100                 105                 110

His Pro Val Ser Leu His Ala Val Gly Val Ser Tyr Trp Lys Ala Ser
        115                 120                 125

Glu Gly Ala Glu Tyr Asp Asp Gln Thr Ser Gln Arg Glu Lys Glu Asp
    130                 135                 140

Asp Lys Val Phe Pro Gly Gly Ser His Thr Tyr Val Trp Gln Val Leu
145                 150                 155                 160

Lys Glu Asn Gly Pro Met Ala Ser Asp Pro Leu Cys Leu Thr Tyr Ser
                165                 170                 175

Tyr Leu Ser His Val Asp Leu Val Lys Asp Leu Asn Ser Gly Leu Ile
            180                 185                 190

Gly Ala Leu Leu Val Cys Arg Glu Gly Ser Leu Ala Lys Glu Lys Thr
        195                 200                 205

Gln Thr Leu His Lys Phe Ile Leu Leu Phe Ala Val Phe Asp Glu Gly
    210                 215                 220

Lys Ser Trp His Ser Glu Thr Lys Asn Ser Leu Met Gln Asp Arg Asp
225                 230                 235                 240

Ala Ala Ser Ala Arg Ala Trp Pro Lys Met His Thr Val Asn Gly Tyr
                245                 250                 255

Val Asn Arg Ser Leu Pro Gly Leu Ile Gly Cys His Arg Lys Ser Val
            260                 265                 270
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Tyr Trp His Val Ile Gly Met Gly Thr Thr Pro Glu Val His Ser Ile
        275                 280                 285

Phe Leu Glu Gly His Thr Phe Leu Val Arg Asn His Arg Gln Ala Ser
    290                 295                 300

Leu Glu Ile Ser Pro Ile Thr Phe Leu Thr Ala Gln Thr Leu Leu Met
305                 310                 315                 320

Asp Leu Gly Gln Phe Leu Leu Phe Cys His Ile Ser Ser His Gln His
                325                 330                 335

Asp Gly Met Glu Ala Tyr Val Lys Val Asp Ser Cys Pro Glu Glu Pro
            340                 345                 350

Gln Leu Arg Met Lys Asn Asn Glu Glu Ala Glu Asp Tyr Asp Asp Asp
        355                 360                 365

Leu Thr Asp Ser Glu Met Asp Val Val Arg Phe Asp Asp Asp Asn Ser
    370                 375                 380

Pro Ser Phe Ile Gln Ile Arg Ser Val Ala Lys Lys His Pro Lys Thr
385                 390                 395                 400

Trp Val His Tyr Ile Ala Ala Glu Glu Glu Asp Trp Asp Tyr Ala Pro
                405                 410                 415

Leu Val Leu Ala Pro Asp Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn
            420                 425                 430

Asn Gly Pro Gln Arg Ile Gly Arg Lys Tyr Lys Lys Val Arg Phe Met
        435                 440                 445

Ala Tyr Thr Asp Glu Thr Phe Lys Thr Arg Glu Ala Ile Gln His Glu
    450                 455                 460

Ser Gly Ile Leu Gly Pro Leu Leu Tyr Gly Glu Val Gly Asp Thr Leu
465                 470                 475                 480

Leu Ile Ile Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn Ile Tyr Pro
```

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                485                 490                 495

His Gly Ile Thr Asp Val Arg Pro Leu Tyr Ser Arg Arg Leu Pro Lys
            500                 505                 510

Gly Val Lys His Leu Lys Asp Phe Pro Ile Leu Pro Gly Glu Ile Phe
        515                 520                 525

Lys Tyr Lys Trp Thr Val Thr Val Glu Asp Gly Pro Thr Lys Ser Asp
    530                 535                 540

Pro Arg Cys Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg
545                 550                 555                 560

Asp Leu Ala Ser Gly Leu Ile Gly Pro Leu Leu Ile Cys Tyr Lys Glu
                565                 570                 575

Ser Val Asp Gln Arg Gly Asn Gln Ile Met Ser Asp Lys Arg Asn Val
            580                 585                 590

Ile Leu Phe Ser Val Phe Asp Glu Asn Arg Ser Trp Tyr Leu Thr Glu
        595                 600                 605

Asn Ile Gln Arg Phe Leu Pro Asn Pro Ala Gly Val Gln Leu Glu Asp
    610                 615                 620

Pro Glu Phe Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val
625                 630                 635                 640

Phe Asp Ser Leu Gln Leu Ser Val Cys Leu His Glu Val Ala Tyr Trp
                645                 650                 655

Tyr Ile Leu Ser Ile Gly Ala Gln Thr Asp Phe Leu Ser Val Phe Phe
            660                 665                 670

Ser Gly Tyr Thr Phe Lys His Lys Met Val Tyr Glu Asp Thr Leu Thr
        675                 680                 685

Leu Phe Pro Phe Ser Gly Glu Thr Val Phe Met Ser Met Glu Asn Pro
    690                 695                 700
```

```
Gly Leu Trp Ile Leu Gly Cys His Asn Ser Asp Phe Arg Asn Arg Gly
705                 710                 715                 720


Met Thr Ala Leu Leu Lys Val Ser Ser Cys Asp Lys Asn Thr Gly Asp
                725                 730                 735


Tyr Tyr Glu Asp Ser Tyr Glu Asp Ile Ser Ala Tyr Leu Leu Ser Lys
            740                 745                 750


Asn Asn Ala Ile Glu Pro Arg Ser Phe Ser Gln Asn Ser Arg His Pro
        755                 760                 765


Ser Thr Arg Gln Lys Gln Phe Asn Ala Thr Thr Ile Pro Glu Asn Thr
    770                 775                 780


Thr Leu Gln Ser Asp Gln Glu Glu Ile Asp Tyr Asp Asp Thr Ile Ser
785                 790                 795                 800


Val Glu Met Lys Lys Glu Asp Phe Asp Ile Tyr Asp Glu Asp Glu Asn
                805                 810                 815


Gln Ser Pro Arg Ser Phe Gln Lys Lys Thr Arg His Tyr Phe Ile Ala
            820                 825                 830


Ala Val Glu Arg Leu Trp Asp Tyr Gly Met Ser Ser Ser Pro His Val
        835                 840                 845


Leu Arg Asn Arg Ala Gln Ser Gly Ser Val Pro Gln Phe Lys Lys Val
    850                 855                 860


Val Phe Gln Glu Phe Thr Asp Gly Ser Phe Thr Gln Pro Leu Tyr Arg
865                 870                 875                 880


Gly Glu Leu Asn Glu His Leu Gly Leu Leu Gly Pro Tyr Ile Arg Ala
                885                 890                 895


Glu Val Glu Asp Asn Ile Met Val Thr Phe Arg Asn Gln Ala Ser Arg
            900                 905                 910


Pro Tyr Ser Phe Tyr Ser Ser Leu Ile Ser Tyr Glu Glu Asp Gln Arg
```

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        915                 920                 925

Gln Gly Ala Glu Pro Arg Lys Asn Phe Val Lys Pro Asn Glu Thr Lys
    930                 935                 940

Thr Tyr Phe Trp Lys Val Gln His His Met Ala Pro Thr Lys Asp Glu
945                 950                 955                 960

Phe Asp Cys Lys Ala Trp Ala Tyr Phe Ser Asp Val Asp Leu Glu Lys
                965                 970                 975

Asp Val His Ser Gly Leu Ile Gly Pro Leu Leu Val Cys His Thr Asn
            980                 985                 990

Thr Leu Asn Pro Ala His Gly Arg  Gln Val Thr Val Gln  Glu Phe Ala
        995                 1000                1005

Leu Phe  Phe Thr Ile Phe Asp  Glu Thr Lys Ser Trp  Tyr Phe Thr
    1010                1015                1020

Glu Asn  Met Glu Arg Asn Cys  Arg Ala Pro Cys Asn  Ile Gln Met
    1025                1030                1035

Glu Asp  Pro Thr Phe Lys Glu  Asn Tyr Arg Phe His  Ala Ile Asn
    1040                1045                1050

Gly Tyr  Ile Met Asp Thr Leu  Pro Gly Leu Val Met  Ala Gln Asp
    1055                1060                1065

Gln Arg  Ile Arg Trp Tyr Leu  Leu Ser Met Gly Ser  Asn Glu Asn
    1070                1075                1080

Ile His  Ser Ile His Phe Ser  Gly His Val Phe Thr  Val Arg Lys
    1085                1090                1095

Lys Glu  Glu Tyr Lys Met Ala  Leu Tyr Asn Leu Tyr  Pro Gly Val
    1100                1105                1110

Phe Glu  Thr Val Glu Met Leu  Pro Ser Lys Ala Gly  Ile Trp Arg
    1115                1120                1125
```

```
Val Glu  Cys Leu Ile Gly Glu  His Leu His Ala Gly  Met Ser Thr
    1130                1135                1140

Leu Phe  Leu Val Tyr Ser Asn  Lys Cys Gln Thr Pro  Leu Gly Met
    1145                1150                1155

Ala Ser  Gly His Ile Arg Asp  Phe Gln Ile Thr Ala  Ser Gly Gln
    1160                1165                1170

Tyr Gly  Gln Trp Ala Pro Lys  Leu Ala Arg Leu His  Tyr Ser Gly
    1175                1180                1185

Ser Ile  Asn Ala Trp Ser Thr  Lys Glu Pro Phe Ser  Trp Ile Lys
    1190                1195                1200

Val Asp  Leu Leu Ala Pro Met  Ile Ile His Gly Ile  Lys Thr Gln
    1205                1210                1215

Gly Ala  Arg Gln Lys Phe Ser  Ser Leu Tyr Ile Ser  Gln Phe Ile
    1220                1225                1230

Ile Met  Tyr Ser Leu Asp Gly  Lys Lys Trp Gln Thr  Tyr Arg Gly
    1235                1240                1245

Asn Ser  Thr Gly Thr Leu Met  Val Phe Phe Gly Asn  Val Asp Ser
    1250                1255                1260

Ser Gly  Ile Lys His Asn Ile  Phe Asn Pro Pro Ile  Ile Ala Arg
    1265                1270                1275

Tyr Ile  Arg Leu His Pro Thr  His Tyr Ser Ile Arg  Ser Thr Leu
    1280                1285                1290

Arg Met  Glu Leu Met Gly Cys  Asp Leu Asn Ser Cys  Ser Met Pro
    1295                1300                1305

Leu Gly  Met Glu Ser Lys Ala  Ile Ser Asp Ala Gln  Ile Thr Ala
    1310                1315                1320

Ser Ser  Tyr Phe Thr Asn Met  Phe Ala Thr Trp Ser  Pro Ser Lys
```

1325 1330 1335

Ala Arg Leu His Leu Gln Gly Arg Ser Asn Ala Trp Arg Pro Gln
1340 1345 1350

Val Asn Asn Pro Lys Glu Trp Leu Gln Val Asp Phe Gln Lys Thr
1355 1360 1365

Met Lys Val Thr Gly Val Thr Thr Gln Gly Val Lys Ser Leu Leu
1370 1375 1380

Thr Ser Met Tyr Val Lys Glu Phe Leu Ile Ser Ser Ser Gln Asp
1385 1390 1395

Gly His Gln Trp Thr Leu Phe Phe Gln Asn Gly Lys Val Lys Val
1400 1405 1410

Phe Gln Gly Asn Gln Asp Ser Phe Thr Pro Val Val Asn Ser Leu
1415 1420 1425

Asp Pro Pro Leu Leu Thr Arg Tyr Leu Arg Ile His Pro Gln Ser
1430 1435 1440

Trp Val His Gln Ile Ala Leu Arg Met Glu Val Leu Gly Cys Glu
1445 1450 1455

Ala Gln Asp Leu Tyr
1460

<210> 553
<211> 520
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 553
atgcaaattg agttatccac ctgtttcttc ctctgtctcc tgaggttctg cttctctgcc 60

accaggagat actacctggg ggctgtggag ctgtcctggg actacatgca aagtgatctg 120

ggtgagctgc ctgtggatgc aaggtaaagg catgtcctgt agggtctgat cggggccagg 180

attgtgggga tgtctccttg ctaatagtag aaaagagtag tatggaagca ttacttccag 240

ctgctttttg aagtgtccac caaaatgaac gacttttttcc tttcttcacg cagatttcct 300

cctagagtgc caaaatcttt tccattcaac acctcagtgg tgtacaaaaa gaccctgttt 360

gtggagttca cagaccacct gttcaacata gccaagccca ggccccccctg gatgggcctg 420

ctgggcccca ccatccaggc agaggtttat gacacagtgg tcatcaccct gaagaacatg 480

gcctcccacc ctgtgtccct ccatgctgtg ggggtgagct 520

<210> 554
<211> 666
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 554
atgcagattg agctgagcac ctgcttcttc ctgtgcctgc tgaggttctg cttctctgcc 60

accaggagat actacctggg ggctgtggag ctgagctggg actacatgca gtctgacctg 120

ggggagctgc ctgtggatgc caggtaaagg catgtcctgt agggtctgat cggggccagg 180

attgtgggga tgtaagtctg cttggaggaa ggtgcagaca tcgggttagg atggttgtga 240

tgctacctgg gccccaaaga aacatttctg ggtaaggtgt gcacacatct gtgttattag 300

cagaaatgct aactgccaat tcttttcata ggtctgacct attgttaatc attaacgctt 360

gtactttggt acagttaatc attaacgctt gtactttggt acagttaatc attaacgctt 420

gtactttggt acatatgatt cttatcatat ggcacctaat gtatcagttg agttgtgaaa 480

acagtgtttc ggtttaatgg atgttaggga tttatataca acttcaaatt tgcctccttg 540

ctaatagtag aaaagagtag tatggaagca ttacttccag ctgctttttg aagtgtccac 600

caaaatgaac gacttttttcc tttcttcacg caggttcccc cccagagtgc ccaagagctt 660

cccctt 666

<210> 555
<211> 1444
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polypeptide

<400> 555
Ala Thr Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser Trp Asp Tyr
1 5 10 15

Met Gln Ser Asp Leu Gly Glu Leu Pro Val Asp Ala Arg Phe Pro Pro
20 25 30

Arg Val Pro Lys Ser Phe Pro Phe Asn Thr Ser Val Val Tyr Lys Lys
35 40 45

Thr Leu Phe Val Glu Phe Thr Asp His Leu Phe Asn Ile Ala Lys Pro
50 55 60

Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile Gln Ala Glu Val
65 70 75 80

Tyr Asp Thr Val Val Ile Thr Leu Lys Asn Met Ala Ser His Pro Val
85 90 95

Ser Leu His Ala Val Gly Val Ser Tyr Trp Lys Ala Ser Glu Gly Ala
100 105 110

Glu Tyr Asp Asp Gln Thr Ser Gln Arg Glu Lys Glu Asp Asp Lys Val
115 120 125

Phe Pro Gly Gly Ser His Thr Tyr Val Trp Gln Val Leu Lys Glu Asn
130 135 140

Gly Pro Met Ala Ser Asp Pro Leu Cys Leu Thr Tyr Ser Tyr Leu Ser
145 150 155 160

His Val Asp Leu Val Lys Asp Leu Asn Ser Gly Leu Ile Gly Ala Leu
165 170 175

Leu Val Cys Arg Glu Gly Ser Leu Ala Lys Glu Lys Thr Gln Thr Leu
180 185 190

His Lys Phe Ile Leu Leu Phe Ala Val Phe Asp Glu Gly Lys Ser Trp

```
        195                 200                 205

His Ser Glu Thr Lys Asn Ser Leu Met Gln Asp Arg Asp Ala Ala Ser
    210                 215                 220

Ala Arg Ala Trp Pro Lys Met His Thr Val Asn Gly Tyr Val Asn Arg
225                 230                 235                 240

Ser Leu Pro Gly Leu Ile Gly Cys His Arg Lys Ser Val Tyr Trp His
                245                 250                 255

Val Ile Gly Met Gly Thr Thr Pro Glu Val His Ser Ile Phe Leu Glu
            260                 265                 270

Gly His Thr Phe Leu Val Arg Asn His Arg Gln Ala Ser Leu Glu Ile
        275                 280                 285

Ser Pro Ile Thr Phe Leu Thr Ala Gln Thr Leu Leu Met Asp Leu Gly
    290                 295                 300

Gln Phe Leu Leu Phe Cys His Ile Ser Ser His Gln His Asp Gly Met
305                 310                 315                 320

Glu Ala Tyr Val Lys Val Asp Ser Cys Pro Glu Glu Pro Gln Leu Arg
                325                 330                 335

Met Lys Asn Asn Glu Glu Ala Glu Asp Tyr Asp Asp Asp Leu Thr Asp
            340                 345                 350

Ser Glu Met Asp Val Val Arg Phe Asp Asp Asp Asn Ser Pro Ser Phe
        355                 360                 365

Ile Gln Ile Arg Ser Val Ala Lys Lys His Pro Lys Thr Trp Val His
    370                 375                 380

Tyr Ile Ala Ala Glu Glu Glu Asp Trp Asp Tyr Ala Pro Leu Val Leu
385                 390                 395                 400

Ala Pro Asp Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn Asn Gly Pro
                405                 410                 415
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```
Gln Arg Ile Gly Arg Lys Tyr Lys Lys Val Arg Phe Met Ala Tyr Thr
            420                 425                 430

Asp Glu Thr Phe Lys Thr Arg Glu Ala Ile Gln His Glu Ser Gly Ile
        435                 440                 445

Leu Gly Pro Leu Leu Tyr Gly Glu Val Gly Asp Thr Leu Leu Ile Ile
    450                 455                 460

Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn Ile Tyr Pro His Gly Ile
465                 470                 475                 480

Thr Asp Val Arg Pro Leu Tyr Ser Arg Arg Leu Pro Lys Gly Val Lys
                485                 490                 495

His Leu Lys Asp Phe Pro Ile Leu Pro Gly Glu Ile Phe Lys Tyr Lys
            500                 505                 510

Trp Thr Val Thr Val Glu Asp Gly Pro Thr Lys Ser Asp Pro Arg Cys
        515                 520                 525

Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg Asp Leu Ala
    530                 535                 540

Ser Gly Leu Ile Gly Pro Leu Leu Ile Cys Tyr Lys Glu Ser Val Asp
545                 550                 555                 560

Gln Arg Gly Asn Gln Ile Met Ser Asp Lys Arg Asn Val Ile Leu Phe
                565                 570                 575

Ser Val Phe Asp Glu Asn Arg Ser Trp Tyr Leu Thr Glu Asn Ile Gln
            580                 585                 590

Arg Phe Leu Pro Asn Pro Ala Gly Val Gln Leu Glu Asp Pro Glu Phe
        595                 600                 605

Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val Phe Asp Ser
    610                 615                 620

Leu Gln Leu Ser Val Cys Leu His Glu Val Ala Tyr Trp Tyr Ile Leu
```

625 630 635 640

Ser Ile Gly Ala Gln Thr Asp Phe Leu Ser Val Phe Phe Ser Gly Tyr
645 650 655

Thr Phe Lys His Lys Met Val Tyr Glu Asp Thr Leu Thr Leu Phe Pro
660 665 670

Phe Ser Gly Glu Thr Val Phe Met Ser Met Glu Asn Pro Gly Leu Trp
675 680 685

Ile Leu Gly Cys His Asn Ser Asp Phe Arg Asn Arg Gly Met Thr Ala
690 695 700

Leu Leu Lys Val Ser Ser Cys Asp Lys Asn Thr Gly Asp Tyr Tyr Glu
705 710 715 720

Asp Ser Tyr Glu Asp Ile Ser Ala Tyr Leu Leu Ser Lys Asn Asn Ala
725 730 735

Ile Glu Pro Arg Ser Phe Ser Gln Asn Ser Arg His Pro Ser Thr Arg
740 745 750

Gln Lys Gln Phe Asn Ala Thr Thr Ile Pro Glu Asn Thr Thr Leu Gln
755 760 765

Ser Asp Gln Glu Glu Ile Asp Tyr Asp Asp Thr Ile Ser Val Glu Met
770 775 780

Lys Lys Glu Asp Phe Asp Ile Tyr Asp Glu Asp Glu Asn Gln Ser Pro
785 790 795 800

Arg Ser Phe Gln Lys Lys Thr Arg His Tyr Phe Ile Ala Ala Val Glu
805 810 815

Arg Leu Trp Asp Tyr Gly Met Ser Ser Ser Pro His Val Leu Arg Asn
820 825 830

Arg Ala Gln Ser Gly Ser Val Pro Gln Phe Lys Lys Val Val Phe Gln
835 840 845

```
Glu Phe Thr Asp Gly Ser Phe Thr Gln Pro Leu Tyr Arg Gly Glu Leu
    850                 855                 860

Asn Glu His Leu Gly Leu Leu Gly Pro Tyr Ile Arg Ala Glu Val Glu
865                 870                 875                 880

Asp Asn Ile Met Val Thr Phe Arg Asn Gln Ala Ser Arg Pro Tyr Ser
                885                 890                 895

Phe Tyr Ser Ser Leu Ile Ser Tyr Glu Glu Asp Gln Arg Gln Gly Ala
            900                 905                 910

Glu Pro Arg Lys Asn Phe Val Lys Pro Asn Glu Thr Lys Thr Tyr Phe
        915                 920                 925

Trp Lys Val Gln His His Met Ala Pro Thr Lys Asp Glu Phe Asp Cys
    930                 935                 940

Lys Ala Trp Ala Tyr Phe Ser Asp Val Asp Leu Glu Lys Asp Val His
945                 950                 955                 960

Ser Gly Leu Ile Gly Pro Leu Leu Val Cys His Thr Asn Thr Leu Asn
                965                 970                 975

Pro Ala His Gly Arg Gln Val Thr Val Gln Glu Phe Ala Leu Phe Phe
            980                 985                 990

Thr Ile Phe Asp Glu Thr Lys Ser  Trp Tyr Phe Thr Glu  Asn Met Glu
        995                 1000                1005

Arg Asn  Cys Arg Ala Pro Cys  Asn Ile Gln Met Glu  Asp Pro Thr
    1010                1015                1020

Phe Lys  Glu Asn Tyr Arg Phe  His Ala Ile Asn Gly  Tyr Ile Met
    1025                1030                1035

Asp Thr  Leu Pro Gly Leu Val  Met Ala Gln Asp Gln  Arg Ile Arg
    1040                1045                1050

Trp Tyr  Leu Leu Ser Met Gly  Ser Asn Glu Asn Ile  His Ser Ile
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    1055                1060                1065

His Phe  Ser Gly His Val Phe  Thr Val Arg Lys Lys  Glu Glu Tyr
    1070                1075                1080

Lys Met  Ala Leu Tyr Asn Leu  Tyr Pro Gly Val Phe  Glu Thr Val
    1085                1090                1095

Glu Met  Leu Pro Ser Lys Ala  Gly Ile Trp Arg Val  Glu Cys Leu
    1100                1105                1110

Ile Gly  Glu His Leu His Ala  Gly Met Ser Thr Leu  Phe Leu Val
    1115                1120                1125

Tyr Ser  Asn Lys Cys Gln Thr  Pro Leu Gly Met Ala  Ser Gly His
    1130                1135                1140

Ile Arg  Asp Phe Gln Ile Thr  Ala Ser Gly Gln Tyr  Gly Gln Trp
    1145                1150                1155

Ala Pro  Lys Leu Ala Arg Leu  His Tyr Ser Gly Ser  Ile Asn Ala
    1160                1165                1170

Trp Ser  Thr Lys Glu Pro Phe  Ser Trp Ile Lys Val  Asp Leu Leu
    1175                1180                1185

Ala Pro  Met Ile Ile His Gly  Ile Lys Thr Gln Gly  Ala Arg Gln
    1190                1195                1200

Lys Phe  Ser Ser Leu Tyr Ile  Ser Gln Phe Ile Ile  Met Tyr Ser
    1205                1210                1215

Leu Asp  Gly Lys Lys Trp Gln  Thr Tyr Arg Gly Asn  Ser Thr Gly
    1220                1225                1230

Thr Leu  Met Val Phe Phe Gly  Asn Val Asp Ser Ser  Gly Ile Lys
    1235                1240                1245

His Asn  Ile Phe Asn Pro Pro  Ile Ile Ala Arg Tyr  Ile Arg Leu
    1250                1255                1260
```

```
His Pro  Thr His Tyr Ser Ile  Arg Ser Thr Leu Arg  Met Glu Leu
    1265                1270                1275

Met Gly  Cys Asp Leu Asn Ser  Cys Ser Met Pro Leu  Gly Met Glu
    1280                1285                1290

Ser Lys  Ala Ile Ser Asp Ala  Gln Ile Thr Ala Ser  Ser Tyr Phe
    1295                1300                1305

Thr Asn  Met Phe Ala Thr Trp  Ser Pro Ser Lys Ala  Arg Leu His
    1310                1315                1320

Leu Gln  Gly Arg Ser Asn Ala  Trp Arg Pro Gln Val  Asn Asn Pro
    1325                1330                1335

Lys Glu  Trp Leu Gln Val Asp  Phe Gln Lys Thr Met  Lys Val Thr
    1340                1345                1350

Gly Val  Thr Thr Gln Gly Val  Lys Ser Leu Leu Thr  Ser Met Tyr
    1355                1360                1365

Val Lys  Glu Phe Leu Ile Ser  Ser Ser Gln Asp Gly  His Gln Trp
    1370                1375                1380

Thr Leu  Phe Phe Gln Asn Gly  Lys Val Lys Val Phe  Gln Gly Asn
    1385                1390                1395

Gln Asp  Ser Phe Thr Pro Val  Val Asn Ser Leu Asp  Pro Pro Leu
    1400                1405                1410

Leu Thr  Arg Tyr Leu Arg Ile  His Pro Gln Ser Trp  Val His Gln
    1415                1420                1425

Ile Ala  Leu Arg Met Glu Val  Leu Gly Cys Glu Ala  Gln Asp Leu
    1430                1435                1440

Tyr
```

<210> 556

<211> 4392
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 556
atgcagattg agctgagcac ctgcttcttc ctgtgcctgc tgaggttctg cttctctgcc 60

accaggagat actacctggg ggctgtggag ctgagctggg actacatgca gtctgacctg 120

ggggagctgc ctgtggatgc caggttcccc cccagagtgc ccaagagctt ccccttcaac 180

acctctgtgg tgtacaagaa gaccctgttt gtggagttca ctgaccacct gttcaacatt 240

gccaagccca ggcccccctg gatgggcctg ctgggcccca ccatccaggc tgaggtgtat 300

gacactgtgg tgatcaccct gaagaacatg gccagccacc ctgtgagcct gcatgctgtg 360

ggggtgagct actggaaggc ctctgagggg gctgagtatg atgaccagac cagccagagg 420

gagaaggagg atgacaaggt gttccctggg ggcagccaca cctatgtgtg gcaggtgctg 480

aaggagaatg gccccatggc ctctgacccc ctgtgcctga cctacagcta cctgagccat 540

gtggacctgg tgaaggacct gaactctggc ctgattgggg ccctgctggt gtgcagggag 600

ggcagcctgg ccaaggagaa gacccagacc ctgcacaagt tcatcctgct gtttgctgtg 660

tttgatgagg gcaagagctg gcactctgaa accaagaaca gcctgatgca ggacagggat 720

gctgcctctg ccagggcctg gcccaagatg cacactgtga atggctatgt gaacaggagc 780

ctgcctggcc tgattggctg ccacaggaag tctgtgtact ggcatgtgat tggcatgggc 840

accacccctg aggtgcacag catcttcctg gagggccaca ccttcctggt caggaaccac 900

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gaggctgagg actatgatga tgacctgact gactctgaga tggatgtggt gaggtttgat 1140

gatgacaaca gccccagctt catccagatc aggtctgtgg ccaagaagca ccccaagacc 1200

tgggtgcact acattgctgc tgaggaggag gactgggact atgcccccct ggtgctggcc 1260

cctgatgaca ggagctacaa gagccagtac ctgaacaatg gcccccagag gattggcagg 1320

aagtacaaga aggtcaggtt catggcctac actgatgaaa ccttcaagac cagggaggcc 1380

atccagcatg agtctggcat cctgggcccc ctgctgtatg gggaggtggg ggacaccctg 1440

ctgatcatct tcaagaacca ggccagcagg ccctacaaca tctaccccca tggcatcact 1500

gatgtgaggc ccctgtacag caggaggctg cccaaggggg tgaagcacct gaaggacttc 1560

cccatcctgc ctggggagat cttcaagtac aagtggactg tgactgtgga ggatggcccc 1620

accaagtctg accccaggtg cctgaccaga tactacagca gctttgtgaa catggagagg 1680

gacctggcct ctggcctgat tggccccctg ctgatctgct acaaggagtc tgtggaccag 1740

aggggcaacc agatcatgtc tgacaagagg aatgtgatcc tgttctctgt gtttgatgag 1800

aacaggagct ggtacctgac tgagaacatc cagaggttcc tgcccaaccc tgctggggtg 1860

cagctggagg accctgagtt ccaggccagc aacatcatgc acagcatcaa tggctatgtg 1920

tttgacagcc tgcagctgtc tgtgtgcctg catgaggtgg cctactggta catcctgagc 1980

attggggccc agactgactt cctgtctgtg ttcttctctg gctacacctt caagcacaag 2040

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agctatgagg acatctctgc ctacctgctg agcaagaaca atgccattga gcccaggagc 2280

ttcagccaga atagcaggca ccccagcacc aggcagaagc agttcaatgc caccaccatc 2340

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ggcatgagca gcagccccca tgtgctgagg aacagggccc agtctggctc tgtgccccag 2580

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cccagcaagg ctggcatctg gagggtggag tgcctgattg gggagcacct gcatgctggc 3420

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ggccacatca gggacttcca gatcactgcc tctggccagt atggccagtg ggcccccaag 3540

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ttcacccctg tggtgaacag cctggacccc cccctgctga ccagatacct gaggattcac 4320

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gacctgtact ga 4392

<210> 557
<211> 70
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 557
gggggaagct actggtgaat attaaccaag gtcacccagt tatcagggag caaacaggag 60

ctaagtccat 70

<210> 558
<211> 218
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 558
gggggaggct gctggtaaac attaaccaag gtcaccccag ttatcagagg agcaaacagg 60

ggcaaagtcc accgggggag gctgctggta aacattaacc aaggtcaccc cagttatcag 120

aggagcaaac aggggcaaag tccaccgggg gaggctgctg gtaaacatta accaaggtca 180

ccccagttat cagaggagca aacaggggca aagtccac 218

<210> 559
<211> 218
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 559
aggggaggct gctggtaaac attaaccaag gtcaccccag ttatcagagg agcaaacagg 60

ggcaaagtcc acaggggggag gctgctggta aacattaacc aaggtcaccc cagttatcag 120

aggagcaaac aggggcaaag tccacagggg gaggctgctg gtaaacatta accaaggtca 180

ccccagttat cagaggagca aacaggggca aagtccat 218

<210> 560
<211> 215
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 560
gagggaggct gctggtaaac attaaccaag gtcacccagt tatcagagga gcaaacaggg 60

gcaaagtcca ccgagggagg ctgctggtaa acattaacca aggtcaccca gttatcagag 120

gagcaaacag gggcaaagtc caccgaggga ggctgctggt aaacattaac caaggtcacc 180

cagttatcag aggagcaaac aggggcaaag tccac 215

<210> 561
<211> 214
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 561
agggaggctg ctggtaaaca ttaaccaagg tcacccagtt atcagaggag caaacagggg 60

caaagtccac agagggaggc tgctggtaaa cattaaccaa ggtcacccag ttatcagagg 120

agcaaacagg ggcaaagtcc acagagggag gctgctggta aacattaacc aaggtcaccc 180

agttatcaga ggagcaaaca ggggcaaagt ccat 214

<210> 562
<211> 218
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 562
gggggaggct gctggtaaac attaaccaag gtcacctcag ttatcagagg agcaaacagg 60

gacaaagtcc accgggggag gctgctggta aacattaacc aaggtcacct cagttatcag 120

aggagcaaac agggacaaag tccaccgggg gaggctgctg gtaaacatta accaaggtca 180

cctcagttat cagaggagca aacagggaca aagtccac 218

<210> 563
<211> 219

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 563
aggggaggct gctggtaaac attaaccaag gtcacctcag ttatcagagg agcaaacagg 60

gacaaagtcc acaggggggag gctgctggta aacattaacc aaggtcacct cagttatcag 120

aggagcaaac agggacaaag tccacagggg gaggctgctg gtaaacatta accaaggtca 180

cctcagttat cagaggagca aacagggaca aagtccaca 219

<210> 564
<211> 212
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 564
gggaggctgc tggtaaacat taaccaaggt caccccagtt atcagaggag caaacaaggg 60

caaagtccac cgggaggctg ctggtaaaca ttaaccaagg tcaccccagt tatcagagga 120

gcaaacaagg gcaaagtcca ccgggaggct gctggtaaac attaaccaag gtcaccccag 180

ttatcagagg agcaaacaag ggcaaagtcc ac 212

<210> 565
<211> 214
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 565
agggaggctg ctggtaaaca ttaaccaagg tcaccccagt tatcagagga gcaaacaagg 60

gcaaagtcca cagggaggct gctggtaaac attaaccaag gtcaccccag ttatcagagg 120

agcaaacaag ggcaaagtcc acagggaggc tgctggtaaa cattaaccaa ggtcacccca 180

gttatcagag gagcaaacaa gggcaaagtc caca 214

<210> 566
<211> 220
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 566
aggaggaggc tgctggtaaa cattaaccaa ggtcacctca gttatcagag gagcaaacag 60

gggcaaagtc cacaggagga ggctgctggt aaacattaac caaggtcacc tcagttatca 120

gaggagcaaa caggggcaaa gtccacagga ggaggctgct ggtaaacatt aaccaaggtc 180

acctcagtta tcagaggagc aaacaggggc aaagtccaca 220

<210> 567
<211> 220
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 567
agggggaggc tgctggtaaa cattaaccaa ggtcacctca gttatcagag gagcaaacag 60

gtgcaaagtc cacaggggga ggctgctggt aaacattaac caaggtcacc tcagttatca 120

gaggagcaaa caggtgcaaa gtccacaggg ggaggctgct ggtaaacatt aaccaaggtc 180

acctcagtta tcagaggagc aaacaggtgc aaagtccaca 220

<210> 568
<211> 220
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 568
aggaggaggc tgctggtaaa cattaaccaa ggtcacccca gttatcagag gagcaaacag 60

gtgcaaagtc cacaggagga ggctgctggt aaacattaac caaggtcacc ccagttatca 120

gaggagcaaa caggtgcaaa gtccacagga ggaggctgct ggtaaacatt aaccaaggtc 180

accccagtta tcagaggagc aaacaggtgc aaagtccaca 220

<210> 569
<211> 209
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 569
ggaggctgtt ggtgaatatt aaccaaggtc acctcagtta tcggaggagc aaacaagggc 60

taagtccacc ggaggctgtt ggtgaatatt aaccaaggtc acctcagtta tcggaggagc 120

aaacaagggc taagtccacc ggaggctgtt ggtgaatatt aaccaaggtc acctcagtta 180

tcggaggagc aaacaagggc taagtccac 209

<210> 570
<211> 211
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 570
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ctaagtccac aggaggctgt tggtgaatat taaccaaggt cacctcagtt atcagaggag 120

caaacaaggg ctaagtccac aggaggctgt tggtgaatat taaccaaggt cacctcagtt 180

atcagaggag caaacaaggg ctaagtccac a 211

<210> 571
<211> 218
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 571
gggggaggct gctggtgaat attaaccaag gtcaccccag ttatcggagg agcaaacagg 60

ggctaagtcc acagggggag gctgctggtg aatattaacc aaggtcaccc cagttatcgg 120

aggagcaaac aggggctaag tccacagggg gaggctgctg gtgaatatta accaaggtca 180

ccccagttat cggaggagca aacaggggct aagtccac 218

<210> 572
<211> 212
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 572
aggggaagct actggtgaat attaaccaag gtcacccagt tatcagggag caaacaggag 60

ctaagtccat agggggaagc tactggtgaa tattaaccaa ggtcacccag ttatcaggga 120

gcaaacagga gctaagtcca taggggggaag ctactggtga atattaacca aggtcaccca 180

gttatcaggg agcaaacagg agctaagtcc at 212

<210> 573
<211> 364
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 573
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ggcaaagtcc accgggggag gctgctggta aacattaacc aaggtcaccc cagttatcag 120

aggagcaaac aggggcaaag tccaccgggg gaggctgctg gtaaacatta accaaggtca 180

ccccagttat cagaggagca aacaggggca aagtccaccg ggggaggctg ctggtaaaca 240

ttaaccaagg tcaccccagt atcagagga gcaaacaggg gcaaagtcca ccgggggagg 300

ctgctggtaa acattaacca aggtcacccc agttatcaga ggagcaaaca ggggcaaagt 360

ccac 364

<210> 574
<211> 359
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 574
gagggaggct gctggtaaac attaaccaag gtcacccagt tatcagagga gcaaacaggg 60

gcaaagtcca ccgagggagg ctgctggtaa acattaacca aggtcaccca gttatcagag 120

gagcaaacag gggcaaagtc caccgaggga ggctgctggt aaacattaac caaggtcacc 180

cagttatcag aggagcaaac aggggcaaag tccaccgagg gaggctgctg gtaaacatta 240

accaaggtca cccagttatc agaggagcaa acaggggcaa agtccaccga gggaggctgc 300

tggtaaacat taaccaaggt cacccagtta tcagaggagc aaacaggggc aaagtccac 359

<210> 575
<211> 358
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 575
agggaggctg ctggtaaaca ttaaccaagg tcacccagtt atcagaggag caaacagggg 60

caaagtccac agagggaggc tgctggtaaa cattaaccaa ggtcacccag ttatcagagg 120

agcaaacagg ggcaaagtcc acagagggag gctgctggta aacattaacc aaggtcaccc 180

agttatcaga ggagcaaaca ggggcaaagt ccacagaggg aggctgctgg taaacattaa 240

ccaaggtcac ccagttatca gaggagcaaa caggggcaaa gtccacagag ggaggctgct 300

ggtaaacatt aaccaaggtc acccagttat cagaggagca aacaggggca aagtccat 358

<210> 576
<211> 364
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 576
gggggaggct gctggtaaac attaaccaag gtcacctcag ttatcagagg agcaaacagg 60

gacaaagtcc accgggggag gctgctggta aacattaacc aaggtcacct cagttatcag 120

aggagcaaac agggacaaag tccaccgggg gaggctgctg gtaaacatta accaaggtca 180

cctcagttat cagaggagca aacagggaca aagtccaccg ggggaggctg ctggtaaaca 240

ttaaccaagg tcacctcagt tatcagagga gcaaacaggg acaaagtcca ccgggggagg 300

ctgctggtaa acattaacca aggtcacctc agttatcaga ggagcaaaca gggacaaagt 360

ccac 364

<210> 577
<211> 365
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 577
aggggaggct gctggtaaac attaaccaag gtcacctcag ttatcagagg agcaaacagg 60

gacaaagtcc acagggggag gctgctggta aacattaacc aaggtcacct cagttatcag 120

aggagcaaac agggacaaag tccacagggg gaggctgctg gtaaacatta accaaggtca 180

cctcagttat cagaggagca aacagggaca aagtccacag ggggaggctg ctggtaaaca 240

ttaaccaagg tcacctcagt tatcagagga gcaaacaggg acaaagtcca cagggggagg 300

ctgctggtaa acattaacca aggtcacctc agttatcaga ggagcaaaca gggacaaagt 360

ccaca 365

<210> 578
<211> 354
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 578
gggaggctgc tggtaaacat taaccaaggt caccccagtt atcagaggag caaacaaggg 60

caaagtccac cgggaggctg ctggtaaaca ttaaccaagg tcaccccagt tatcagagga 120

gcaaacaagg gcaaagtcca ccgggaggct gctggtaaac attaaccaag gtcaccccag 180

ttatcagagg agcaaacaag ggcaaagtcc accgggaggc tgctggtaaa cattaaccaa 240

ggtcacccca gttatcagag gagcaaacaa gggcaaagtc caccgggagg ctgctggtaa 300

acattaacca aggtcacccc agttatcaga ggagcaaaca agggcaaagt ccac 354

<210> 579
<211> 356
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 579
agggaggctg ctggtaaaca ttaaccaagg tcaccccagt tatcagagga gcaaacaagg 60

gcaaagtcca cagggaggct gctggtaaac attaaccaag gtcaccccag ttatcagagg 120

agcaaacaag ggcaaagtcc acagggaggc tgctggtaaa cattaaccaa ggtcacccca 180

gttatcagag gagcaaacaa gggcaaagtc cacagggagg ctgctggtaa acattaacca 240

aggtcacccc agttatcaga ggagcaaaca agggcaaagt ccacagggag gctgctggta 300

aacattaacc aaggtcaccc cagttatcag aggagcaaac aagggcaaag tccaca 356

<210> 580
<211> 366
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 580
aggaggaggc tgctggtaaa cattaaccaa ggtcacctca gttatcagag gagcaaacag 60

gggcaaagtc cacaggagga ggctgctggt aaacattaac caaggtcacc tcagttatca 120

gaggagcaaa caggggcaaa gtccacagga ggaggctgct ggtaaacatt aaccaaggtc 180

acctcagtta tcagaggagc aaacaggggc aaagtccaca ggaggaggct gctggtaaac 240

attaaccaag gtcacctcag ttatcagagg agcaaacagg ggcaaagtcc acaggaggag 300

gctgctggta aacattaacc aaggtcacct cagttatcag aggagcaaac aggggcaaag 360

tccaca 366

<210> 581
<211> 366
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 581
agggggaggc tgctggtaaa cattaaccaa ggtcacctca gttatcagag gagcaaacag 60

gtgcaaagtc cacaggggga ggctgctggt aaacattaac caaggtcacc tcagttatca 120

gaggagcaaa caggtgcaaa gtccacaggg ggaggctgct ggtaaacatt aaccaaggtc 180

acctcagtta tcagaggagc aaacaggtgc aaagtccaca gggggaggct gctggtaaac 240

attaaccaag gtcacctcag ttatcagagg agcaaacagg tgcaaagtcc acaggggggag 300

gctgctggta aacattaacc aaggtcacct cagttatcag aggagcaaac aggtgcaaag 360

tccaca 366

<210> 582
<211> 366
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 582
aggaggaggc tgctggtaaa cattaaccaa ggtcacccca gttatcagag gagcaaacag 60

gtgcaaagtc cacaggagga ggctgctggt aaacattaac caaggtcacc ccagttatca 120

gaggagcaaa caggtgcaaa gtccacagga ggaggctgct ggtaaacatt aaccaaggtc 180

accccagtta tcagaggagc aaacaggtgc aaagtccaca ggaggaggct gctggtaaac 240

attaaccaag gtcaccccag ttatcagagg agcaaacagg tgcaaagtcc acaggaggag 300

gctgctggta aacattaacc aaggtcaccc cagttatcag aggagcaaac aggtgcaaag 360

tccaca 366

<210> 583
<211> 349

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 583
ggaggctgtt ggtgaatatt aaccaaggtc acctcagtta tcggaggagc aaacaagggc 60

taagtccacc ggaggctgtt ggtgaatatt aaccaaggtc acctcagtta tcggaggagc 120

aaacaagggc taagtccacc ggaggctgtt ggtgaatatt aaccaaggtc acctcagtta 180

tcggaggagc aaacaagggc taagtccacc ggaggctgtt ggtgaatatt aaccaaggtc 240

acctcagtta tcggaggagc aaacaagggc taagtccacc ggaggctgtt ggtgaatatt 300

aaccaaggtc acctcagtta tcggaggagc aaacaagggc taagtccac 349

<210> 584
<211> 351
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 584
aggaggctgt tggtgaatat taaccaaggt cacctcagtt atcagaggag caaacaaggg 60

ctaagtccac aggaggctgt tggtgaatat taaccaaggt cacctcagtt atcagaggag 120

caaacaaggg ctaagtccac aggaggctgt tggtgaatat taaccaaggt cacctcagtt 180

atcagaggag caaacaaggg ctaagtccac aggaggctgt tggtgaatat taaccaaggt 240

cacctcagtt atcagaggag caaacaaggg ctaagtccac aggaggctgt tggtgaatat 300

taaccaaggt cacctcagtt atcagaggag caaacaaggg ctaagtccac a 351

<210> 585
<211> 354
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 585

aggggaagct actggtgaat attaaccaag gtcacccagt tatcagggag caaacaggag 60

ctaagtccat agggggaagc tactggtgaa tattaaccaa ggtcacccag ttatcaggga 120

gcaaacagga gctaagtcca taggggggaag ctactggtga atattaacca aggtcaccca 180

gttatcaggg agcaaacagg agctaagtcc atagggggaa gctactggtg aatattaacc 240

aaggtcaccc agttatcagg gagcaaacag gagctaagtc catagggggga agctactggt 300

gaatattaac caaggtcacc cagttatcag ggagcaaaca ggagctaagt ccat 354

<210> 586
<211> 729
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 586
gggggaggct gctggtaaac attaaccaag gtcaccccag ttatcagagg agcaaacagg 60

ggcaaagtcc accgggggag gctgctggta aacattaacc aaggtcaccc cagttatcag 120

aggagcaaac aggggcaaag tccaccgggg gaggctgctg gtaaacatta accaaggtca 180

ccccagttat cagaggagca aacaggggca aagtccaccg ggggaggctg ctggtaaaca 240

ttaaccaagg tcaccccagt tatcagagga gcaaacaggg gcaaagtcca ccgggggagg 300

ctgctggtaa acattaacca aggtcacccc agttatcaga ggagcaaaca ggggcaaagt 360

ccaccggggg aggctgctgg taaacattaa ccaaggtcac cccagttatc agaggagcaa 420

acaggggcaa agtccaccgg gggaggctgc tggtaaacat taaccaaggt caccccagtt 480

atcagaggag caaacagggg caaagtccac cgggggaggc tgctggtaaa cattaaccaa 540

ggtcacccca gttatcagag gagcaaacag gggcaaagtc caccgggggga ggctgctggt 600

aaacattaac caaggtcacc ccagttatca gaggagcaaa caggggcaaa gtccaccggg 660

ggaggctgct ggtaaacatt aaccaaggtc accccagtta tcagaggagc aaacaggggc 720

aaagtccac 729

<210> 587
<211> 719
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 587
gagggaggct gctggtaaac attaaccaag gtcacccagt tatcagagga gcaaacaggg 60

gcaaagtcca ccgagggagg ctgctggtaa acattaacca aggtcaccca gttatcagag 120

gagcaaacag gggcaaagtc caccgaggga ggctgctggt aaacattaac caaggtcacc 180

cagttatcag aggagcaaac aggggcaaag tccaccgagg gaggctgctg gtaaacatta 240

accaaggtca cccagttatc agaggagcaa acaggggcaa agtccaccga gggaggctgc 300

tggtaaacat taaccaaggt cacccagtta tcagaggagc aaacaggggc aaagtccacc 360

gagggaggct gctggtaaac attaaccaag gtcacccagt tatcagagga gcaaacaggg 420

gcaaagtcca ccgagggagg ctgctggtaa acattaacca aggtcaccca gttatcagag 480

gagcaaacag gggcaaagtc caccgaggga ggctgctggt aaacattaac caaggtcacc 540

cagttatcag aggagcaaac aggggcaaag tccaccgagg gaggctgctg gtaaacatta 600

accaaggtca cccagttatc agaggagcaa acaggggcaa agtccaccga gggaggctgc 660

tggtaaacat taaccaaggt cacccagtta tcagaggagc aaacaggggc aaagtccac 719

<210> 588
<211> 718
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 588
agggaggctg ctggtaaaca ttaaccaagg tcacccagtt atcagaggag caaacagggg 60

caaagtccac agagggaggc tgctggtaaa cattaaccaa ggtcacccag ttatcagagg 120

agcaaacagg ggcaaagtcc acagagggag gctgctggta aacattaacc aaggtcaccc 180

agttatcaga ggagcaaaca ggggcaaagt ccacagaggg aggctgctgg taaacattaa 240

ccaaggtcac ccagttatca gaggagcaaa caggggcaaa gtccacagag ggaggctgct 300

ggtaaacatt aaccaaggtc acccagttat cagaggagca aacaggggca aagtccacag 360

agggaggctg ctggtaaaca ttaaccaagg tcacccagtt atcagaggag caaacagggg 420

caaagtccac agagggaggc tgctggtaaa cattaaccaa ggtcacccag ttatcagagg 480

agcaaacagg ggcaaagtcc acagagggag gctgctggta aacattaacc aaggtcaccc 540

agttatcaga ggagcaaaca ggggcaaagt ccacagaggg aggctgctgg taaacattaa 600

ccaaggtcac ccagttatca gaggagcaaa caggggcaaa gtccacagag ggaggctgct 660

ggtaaacatt aaccaaggtc acccagttat cagaggagca aacaggggca aagtccat 718

<210> 589
<211> 729
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 589
gggggaggct gctggtaaac attaaccaag gtcacctcag ttatcagagg agcaaacagg 60

gacaaagtcc accgggggag gctgctggta aacattaacc aaggtcacct cagttatcag 120

aggagcaaac agggacaaag tccaccgggg gaggctgctg gtaaacatta accaaggtca 180

cctcagttat cagaggagca aacagggaca aagtccaccg ggggaggctg ctggtaaaca 240

ttaaccaagg tcacctcagt tatcagagga gcaaacaggg acaaagtcca ccgggggagg 300

ctgctggtaa acattaacca aggtcacctc agttatcaga ggagcaaaca gggacaaagt 360

ccaccggggg aggctgctgg taaacattaa ccaaggtcac ctcagttatc agaggagcaa 420

acagggacaa agtccaccgg gggaggctgc tggtaaacat taaccaaggt cacctcagtt 480

atcagaggag caaacaggga caaagtccac cgggggaggc tgctggtaaa cattaaccaa 540

ggtcacctca gttatcagag gagcaaacag ggacaaagtc caccgggggа ggctgctggt 600

aaacattaac caaggtcacc tcagttatca gaggagcaaa cagggacaaa gtccaccggg 660

ggaggctgct ggtaaacatt aaccaaggtc acctcagtta tcagaggagc aaacagggac 720

aaagtccac 729

<210> 590
<211> 730
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 590
aggggaggct gctggtaaac attaaccaag gtcacctcag ttatcagagg agcaaacagg 60

gacaaagtcc acaggggggag gctgctggta aacattaacc aaggtcacct cagttatcag 120

aggagcaaac agggacaaag tccacagggg gaggctgctg gtaaacatta accaaggtca 180

cctcagttat cagaggagca aacagggaca aagtccacag ggggaggctg ctggtaaaca 240

ttaaccaagg tcacctcagt tatcagagga gcaaacaggg acaaagtcca cagggggagg 300

ctgctggtaa acattaacca aggtcacctc agttatcaga ggagcaaaca gggacaaagt 360

ccacaggggg aggctgctgg taaacattaa ccaaggtcac ctcagttatc agaggagcaa 420

acagggacaa agtccacagg gggaggctgc tggtaaacat taaccaaggt cacctcagtt 480

atcagaggag caaacaggga caaagtccac agggggaggc tgctggtaaa cattaaccaa 540

ggtcacctca gttatcagag gagcaaacag ggacaaagtc cacaggggga ggctgctggt 600

aaacattaac caaggtcacc tcagttatca gaggagcaaa cagggacaaa gtccacaggg 660

ggaggctgct ggtaaacatt aaccaaggtc acctcagtta tcagaggagc aaacagggac 720

aaagtccaca 730

<210> 591
<211> 709
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 591
gggaggctgc tggtaaacat taaccaaggt caccccagtt atcagaggag caaacaaggg 60

caaagtccac cgggaggctg ctggtaaaca ttaaccaagg tcaccccagt tatcagagga 120

gcaaacaagg gcaaagtcca ccgggaggct gctggtaaac attaaccaag gtcaccccag 180

ttatcagagg agcaaacaag ggcaaagtcc accgggaggc tgctggtaaa cattaaccaa 240

ggtcacccca gttatcagag gagcaaacaa gggcaaagtc caccgggagg ctgctggtaa 300

acattaacca aggtcacccc agttatcaga ggagcaaaca agggcaaagt ccaccgggag 360

gctgctggta aacattaacc aaggtcaccc cagttatcag aggagcaaac aagggcaaag 420

tccaccggga ggctgctggt aaacattaac caaggtcacc ccagttatca gaggagcaaa 480

caagggcaaa gtccaccggg aggctgctgg taaacattaa ccaaggtcac cccagttatc 540

agaggagcaa acaagggcaa agtccaccgg gaggctgctg gtaaacatta accaaggtca 600

ccccagttat cagaggagca aacaagggca aagtccaccg ggaggctgct ggtaaacatt 660

aaccaaggtc accccagtta tcagaggagc aaacaagggc aaagtccac 709

<210> 592
<211> 711
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 592
agggaggctg ctggtaaaca ttaaccaagg tcaccccagt tatcagagga gcaaacaagg 60

gcaaagtcca cagggaggct gctggtaaac attaaccaag gtcaccccag ttatcagagg 120

agcaaacaag ggcaaagtcc acagggaggc tgctggtaaa cattaaccaa ggtcacccca 180

gttatcagag gagcaaacaa gggcaaagtc cacagggagg ctgctggtaa acattaacca 240

aggtcacccc agttatcaga ggagcaaaca agggcaaagt ccacagggag gctgctggta 300

aacattaacc aaggtcaccc cagttatcag aggagcaaac aagggcaaag tccacaggga 360

ggctgctggt aaacattaac caaggtcacc ccagttatca gaggagcaaa caagggcaaa 420

gtccacaggg aggctgctgg taaacattaa ccaaggtcac cccagttatc agaggagcaa 480

acaagggcaa agtccacagg gaggctgctg gtaaacatta accaaggtca ccccagttat 540

cagaggagca aacaagggca aagtccacag ggaggctgct ggtaaacatt aaccaaggtc 600

accccagtta tcagaggagc aaacaagggc aaagtccaca gggaggctgc tggtaaacat 660

taaccaaggt caccccagtt atcagaggag caaacaaggg caaagtccac a 711

<210> 593
<211> 729
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 593
gggggaggct gctggtgaat attaaccaag gtcaccccag ttatcggagg agcaaacagg 60

ggctaagtcc accgggggag gctgctggtg aatattaacc aaggtcaccc cagttatcgg 120

aggagcaaac aggggctaag tccaccgggg gaggctgctg gtgaatatta accaaggtca 180

ccccagttat cggaggagca aacaggggct aagtccaccg ggggaggctg ctggtgaata 240

ttaaccaagg tcaccccagt tatcggagga gcaaacaggg gctaagtcca ccgggggagg 300

ctgctggtga atattaacca aggtcacccc agttatcgga ggagcaaaca ggggctaagt 360

ccaccggggg aggctgctgg tgaatattaa ccaaggtcac cccagttatc ggaggagcaa 420

acaggggcta agtccaccgg gggaggctgc tggtgaatat taaccaaggt caccccagtt 480

atcggaggag caaacagggg ctaagtccac cgggggaggc tgctggtgaa tattaaccaa 540

ggtcacccca gttatcggag gagcaaacag gggctaagtc caccgggggа ggctgctggt 600

gaatattaac caaggtcacc ccagttatcg gaggagcaaa caggggctaa gtccaccggg 660

ggaggctgct ggtgaatatt aaccaaggtc accccagtta tcggaggagc aaacaggggc 720

taagtccac 729

<210> 594
<211> 709
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 594
aggggaagct actggtgaat attaaccaag gtcacccagt tatcagggag caaacaggag 60

ctaagtccat agggggaagc tactggtgaa tattaaccaa ggtcacccag ttatcaggga 120

gcaaacagga gctaagtcca taggggggaag ctactggtga atattaacca aggtcaccca 180

gttatcaggg agcaaacagg agctaagtcc atagggggaa gctactggtg aatattaacc 240

aaggtcaccc agttatcagg gagcaaacag gagctaagtc catagggggа agctactggt 300

gaatattaac caaggtcacc cagttatcag ggagcaaaca ggagctaagt ccatagggggg 360

aagctactgg tgaatattaa ccaaggtcac ccagttatca gggagcaaac aggagctaag 420

tccatagggg gaagctactg gtgaatatta accaaggtca cccagttatc agggagcaaa 480

caggagctaa gtccataggg ggaagctact ggtgaatatt aaccaaggtc acccagttat 540

cagggagcaa acaggagcta agtccatagg gggaagctac tggtgaatat taaccaaggt 600

cacccagtta tcagggagca aacaggagct aagtccatag ggggaagcta ctggtgaata 660

ttaaccaagg tcacccagtt atcagggagc aaacaggagc taagtccat 709

<210> 595
<211> 216
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 595
gggggaagct actggtgaat attaaccaag gtcacccagt tatcagggag caaacaggag 60

ctaagtccat agggggaggc tgctggtaaa cattaaccaa ggtcacccca gttatcagag 120

gagcaaacag gggcaaagtc cacagaggga ggctgctggt gaatattaac caaggtcacc 180

tcagttatca gaggagcaaa caggggcaaa gtccat 216

<210> 596
<211> 217
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 596
agagggaggc tgctggtgaa tattaaccaa ggtcacctca gttatcagag gagcaaacag 60

gggcaaagtc catagaggga agctactggt gaatattaac caaggtcacc cagttatcag 120

ggagcaaaca ggagctaagt ccatagggggg aggctgctgg taaacattaa ccaaggtcac 180

cccagttatc agaggagcaa acaggggcaa agtccac 217

<210> 597
<211> 220
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 597
gggggaggct gctggtgaat attaaccaag gtcaccccag ttatcggagg agcaaacagg 60

ggctaagtcc acaaggggga ggctgctggt gaatattaac caaggtcacc ccagttatcg 120

gaggagcaaa caggggctaa gtccaccagg gggaggctgc tggtgaatat taaccaaggt 180

caccccagtt atcggaggag caaacagggg ctaagtccac 220

<210> 598
<211> 220
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 598
gggggaggct gctggtgaat attaaccaag gtcaccccag ttatcggagg agcaaacagg 60

ggctaagtcc acaaggggga ggctgctggt gaatattaac caaggtcacc ccagttatcg 120

gaggagcaaa caggggctaa gtccacttgg gggaggctgc tggtgaatat taaccaaggt 180

caccccagtt atcggaggag caaacagggg ctaagtccac 220

<210> 599
<211> 220
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 599
gggggaggct gctggtgaat attaaccaag gtcaccccag ttatcggagg agcaaacagg 60

ggctaagtcc accaggggga ggctgctggt gaatattaac caaggtcacc ccagttatcg 120

gaggagcaaa caggggctaa gtccacttgg gggaggctgc tggtgaatat taaccaaggt 180

caccccagtt atcggaggag caaacagggg ctaagtccac 220

<210> 600

<211> 220
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 600
gggggaggct gctggtgaat attaaccaag gtcaccccag ttatcggagg agcaaacagg 60

ggctaagtcc acctgggggа ggctgctggt gaatattaac caaggtcacc ccagttatcg 120

gaggagcaaa caggggctaa gtccacaagg gggaggctgc tggtgaatat taaccaaggt 180

caccccagtt atcggaggag caaacagggg ctaagtccac 220

<210> 601
<211> 220
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 601
gggggaggct gctggtgaat attaaccaag gtcaccccag ttatcggagg agcaaacagg 60

ggctaagtcc acctgggggа ggctgctggt gaatattaac caaggtcacc ccagttatcg 120

gaggagcaaa caggggctaa gtccaccagg gggaggctgc tggtgaatat taaccaaggt 180

caccccagtt atcggaggag caaacagggg ctaagtccac 220

<210> 602
<211> 220
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 602
gggggaggct gctggtgaat attaaccaag gtcaccccag ttatcggagg agcaaacagg 60

ggctaagtcc acctgggggа ggctgctggt gaatattaac caaggtcacc ccagttatcg 120

gaggagcaaa caggggctaa gtccacttgg gggaggctgc tggtgaatat taaccaaggt 180

caccccagtt atcggaggag caaacagggg ctaagtccac 220

<210> 603
<211> 220
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 603
gggggaggct gctggtgaat attaaccaag gtcaccccag ttatcggagg agcaaacagg 60

ggctaagtcc acttggggga ggctgctggt gaatattaac caaggtcacc ccagttatcg 120

gaggagcaaa caggggctaa gtccacaagg gggaggctgc tggtgaatat taaccaaggt 180

caccccagtt atcggaggag caaacagggg ctaagtccac 220

<210> 604
<211> 222
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 604
gggggaggct gctggtgaat attaaccaag gtcaccccag ttatcggagg agcaaacagg 60

ggctaagtcc acttaggggg aggctgctgg tgaatattaa ccaaggtcac cccagttatc 120

ggaggagcaa acaggggcta agtccactgt gggggaggct gctggtgaat attaaccaag 180

gtcaccccag ttatcggagg agcaaacagg ggctaagtcc ac 222

<210> 605
<211> 222
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 605
gggggaggct gctggtgaat attaaccaag gtcaccccag ttatcggagg agcaaacagg 60

ggctaagtcc acagaggggg aggctgctgg tgaatattaa ccaaggtcac cccagttatc 120

ggaggagcaa acaggggcta agtccactga gggggaggct gctggtgaat attaaccaag 180

gtcaccccag ttatcggagg agcaaacagg ggctaagtcc ac 222

<210> 606
<211> 226
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 606
gggggaggct gctggtgaat attaaccaag gtcaccccag ttatcggagg agcaaacagg 60

ggctaagtcc acacataggg ggaggctgct ggtgaatatt aaccaaggtc accccagtta 120

tcggaggagc aaacaggggc taagtccacc tgtaggggga ggctgctggt gaatattaac 180

caaggtcacc ccagttatcg gaggagcaaa caggggctaa gtccac 226

<210> 607
<211> 226
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 607
gggggaggct gctggtgaat attaaccaag gtcaccccag ttatcggagg agcaaacagg 60

ggctaagtcc acaacaaggg ggaggctgct ggtgaatatt aaccaaggtc accccagtta 120

tcggaggagc aaacaggggc taagtccacc atcaggggga ggctgctggt gaatattaac 180

caaggtcacc ccagttatcg gaggagcaaa caggggctaa gtccac 226

<210> 608
<211> 226
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 608
gggggaggct gctggtgaat attaaccaag gtcaccccag ttatcggagg agcaaacagg 60

ggctaagtcc accaattggg ggaggctgct ggtgaatatt aaccaaggtc accccagtta 120

tcggaggagc aaacaggggc taagtccact tgctgggga ggctgctggt gaatattaac 180

caaggtcacc ccagttatcg gaggagcaaa caggggctaa gtccac 226

<210> 609
<211> 238
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 609
gggggaggct gctggtgaat attaaccaag gtcaccccag ttatcggagg agcaaacagg 60

ggctaagtcc acccttggga ccaggggggag gctgctggtg aatattaacc aaggtcaccc 120

cagttatcgg aggagcaaac aggggctaag tccacaagct gttccagggg gaggctgctg 180

gtgaatatta accaaggtca ccccagttat cggaggagca aacaggggct aagtccac 238

<210> 610
<211> 238
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 610
gggggaggct gctggtgaat attaaccaag gtcaccccag ttatcggagg agcaaacagg 60

ggctaagtcc acaggctggt tgaggggggag gctgctggtg aatattaacc aaggtcaccc 120

cagttatcgg aggagcaaac aggggctaag tccactgata atagctgggg gaggctgctg 180

gtgaatatta accaaggtca ccccagttat cggaggagca aacaggggct aagtccac 238

<210> 611
<211> 238
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 611
gggggaggct gctggtgaat attaaccaag gtcaccccag ttatcggagg agcaaacagg 60

ggctaagtcc accattctgc tttgggggag gctgctggtg aatattaacc aaggtcaccc 120

cagttatcgg aggagcaaac aggggctaag tccacttgat taagaagggg gaggctgctg 180

gtgaatatta accaaggtca ccccagttat cggaggagca aacaggggct aagtccac 238

<210> 612
<211> 238
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 612
gggggaggct gctggtgaat attaaccaag gtcaccccag ttatcggagg agcaaacagg 60

ggctaagtcc acaacaaagt ccaggggggag gctgctggtg aatattaacc aaggtcaccc 120

cagttatcgg aggagcaaac aggggctaag tccaccttgt aaacaagggg gaggctgctg 180

gtgaatatta accaaggtca ccccagttat cggaggagca aacaggggct aagtccac 238

<210> 613
<211> 238
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 613
gggggaggct gctggtgaat attaaccaag gtcaccccag ttatcggagg agcaaacagg 60

ggctaagtcc actgcaaagt cctgggggag gctgctggtg aatattaacc aaggtcaccc 120

cagttatcgg aggagcaaac aggggctaag tccacagtgt ttacaagggg gaggctgctg 180

gtgaatatta accaaggtca ccccagttat cggaggagca aacaggggct aagtccac 238

<210> 614
<211> 238
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 614
gggggaggct gctggtgaat attaaccaag gtcaccccag ttatcggagg agcaaacagg 60

ggctaagtcc acaggacttt gaaggggggag gctgctggtg aatattaacc aaggtcaccc 120

cagttatcgg aggagcaaac aggggctaag tccacagtgt aaacaagggg gaggctgctg 180

gtgaatatta accaaggtca ccccagttat cggaggagca aacaggggct aagtccac 238

<210> 615
<211> 238
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 615
gggggaggct gctggtgaat attaaccaag gtcaccccag ttatcggagg agcaaacagg 60

ggctaagtcc actggacttt ggtgggggag gctgctggtg aatattaacc aaggtcaccc 120

cagttatcgg aggagcaaac aggggctaag tccactctgt ttacaagggg gaggctgctg 180

gtgaatatta accaaggtca ccccagttat cggaggagca aacaggggct aagtccac 238

<210> 616
<211> 276
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 616
gggggaggct gctggtgaat attaaccaag gtcaccccag ttatcggagg agcaaacagg 60

ggctaagtcc acctgcttga catctgcagt aatctttgat tagggggagg ctgctggtga 120

atattaacca aggtcacccc agttatcgga ggagcaaaca ggggctaagt ccacctctga 180

tactttgata tctagtctac tgctgggggga ggctgctggt gaatattaac caaggtcacc 240

ccagttatcg gaggagcaaa caggggctaa gtccac 276

<210> 617
<211> 69
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 617
ggaggctgtt ggtgaatatt aaccaaggtc acctcagtta tcggaggagc aaacaagggc 60

taagtccac 69

<210> 618
<211> 541
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 618
gggggaggct gctggtaaac attaaccaag gtcacctcag ttatcagagg agcaaacagg 60

gacaaagtcc accgggggag gctgctggta aacattaacc aaggtcacct cagttatcag 120

aggagcaaac agggacaaag tccaccgggg gaggctgctg gtaaacatta accaaggtca 180

cctcagttat cagaggagca aacagggaca aagtccacgg tacccactgg gaggatgttg 240

agtaagatgg aaaactactg atgacccttg cagagacaga gtattaggac atgtttgaac 300

aggggccggg cgatcagcag gtagctctag aggatccccg tctgtctgca catttcgtag 360

agcgagtgtt ccgatactct aatctcccta ggcaaggttc atatttgtgt aggttactta 420

ttctccttt gttgactaag tcaataatca gaatcagcag gtttggagtc agcttggcag 480

ggatcagcag cctgggttgg aaggaggggg tataaaagcc ccttcaccag gagaagccgt 540

c 541

<210> 619
<211> 542
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 619
aggggaggct gctggtaaac attaaccaag gtcacctcag ttatcagagg agcaaacagg 60

gacaaagtcc acaggggggag gctgctggta aacattaacc aaggtcacct cagttatcag 120

aggagcaaac agggacaaag tccacagggg gaggctgctg gtaaacatta accaaggtca 180

cctcagttat cagaggagca aacagggaca aagtccacag gtacccactg ggaggatgtt 240

gagtaagatg gaaaactact gatgaccctt gcagagacag agtattagga catgtttgaa 300

caggggccgg gcgatcagca ggtagctcta gaggatcccc gtctgtctgc acatttcgta 360

gagcgagtgt tccgatactc taatctccct aggcaaggtt catatttgtg taggttactt 420

attctccttt tgttgactaa gtcaataatc agaatcagca ggtttggagt cagcttggca 480

gggatcagca gcctgggttg gaaggagggg gtataaaagc cccttcacca ggagaagccg 540

tc 542

<210> 620
<211> 535
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 620
aggggaagct actggtgaat attaaccaag gtcacccagt tatcagggag caaacaggag 60

ctaagtccat agggggaagc tactggtgaa tattaaccaa ggtcacccag ttatcaggga 120

gcaaacagga gctaagtcca taggggggaag ctactggtga atattaacca aggtcaccca 180

gttatcaggg agcaaacagg agctaagtcc atggtaccca ctgggaggat gttgagtaag 240

atggaaaact actgatgacc cttgcagaga cagagtatta ggacatgttt gaacaggggc 300

cgggcgatca gcaggtagct ctagaggatc cccgtctgtc tgcacatttc gtagagcgag 360

tgttccgata ctctaatctc cctaggcaag gttcatattt gtgtaggtta cttattctcc 420

ttttgttgac taagtcaata atcagaatca gcaggtttgg agtcagcttg gcagggatca 480

gcagcctggg ttggaaggag ggggtataaa agccccttca ccaggagaag ccgtc 535

<210> 621
<211> 539

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 621
gggggaagct actggtgaat attaaccaag gtcacccagt tatcagggag caaacaggag 60

ctaagtccat agggggaggc tgctggtaaa cattaaccaa ggtcacccca gttatcagag 120

gagcaaacag gggcaaagtc cacagaggga ggctgctggt gaatattaac caaggtcacc 180

tcagttatca gaggagcaaa caggggcaaa gtccatggta cccactggga ggatgttgag 240

taagatggaa aactactgat gacccttgca gagacagagt attaggacat gtttgaacag 300

gggccgggcg atcagcaggt agctctagag gatccccgtc tgtctgcaca tttcgtagag 360

cgagtgttcc gatactctaa tctccctagg caaggttcat atttgtgtag gttacttatt 420

ctccttttgt tgactaagtc aataatcaga atcagcaggt ttggagtcag cttggcaggg 480

atcagcagcc tgggttggaa ggaggggggta taaaagcccc ttcaccagga gaagccgtc 539

<210> 622
<211> 540
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 622
agagggaggc tgctggtgaa tattaaccaa ggtcacctca gttatcagag gagcaaacag 60

gggcaaagtc catagaggga agctactggt gaatattaac caaggtcacc cagttatcag 120

ggagcaaaca ggagctaagt ccatagggggg aggctgctgg taaacattaa ccaaggtcac 180

cccagttatc agaggagcaa acaggggcaa agtccacggt acccactggg aggatgttga 240

gtaagatgga aaactactga tgacccttgc agagacagag tattaggaca tgtttgaaca 300

ggggccgggc gatcagcagg tagctctaga ggatccccgt ctgtctgcac atttcgtaga 360

gcgagtgttc cgatactcta atctccctag gcaaggttca tatttgtgta ggttacttat 420

tctccttttg ttgactaagt caataatcag aatcagcagg tttggagtca gcttggcagg 480

gatcagcagc ctgggttgga aggaggggggt ataaaagccc cttcaccagg agaagccgtc 540

<210> 623
<211> 532
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 623
ggaggctgtt ggtgaatatt aaccaaggtc acctcagtta tcggaggagc aaacaagggc 60

taagtccacc ggaggctgtt ggtgaatatt aaccaaggtc acctcagtta tcggaggagc 120

aaacaagggc taagtccacc ggaggctgtt ggtgaatatt aaccaaggtc acctcagtta 180

tcggaggagc aaacaagggc taagtccacg gtacccactg ggaggatgtt gagtaagatg 240

gaaaactact gatgaccctt gcagagacag agtattagga catgtttgaa caggggccgg 300

gcgatcagca ggtagctcta gaggatcccc gtctgtctgc acatttcgta gagcgagtgt 360

tccgatactc taatctccct aggcaaggtt catatttgtg taggttactt attctccttt 420

tgttgactaa gtcaataatc agaatcagca ggtttggagt cagcttggca gggatcagca 480

gcctgggttg gaaggagggg gtataaaagc cccttcacca ggagaagccg tc 532

<210> 624
<211> 534
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 624
aggaggctgt tggtgaatat taaccaaggt cacctcagtt atcagaggag caaacaaggg 60

ctaagtccac aggaggctgt tggtgaatat taaccaaggt cacctcagtt atcagaggag 120

caaacaaggg ctaagtccac aggaggctgt tggtgaatat taaccaaggt cacctcagtt 180

atcagaggag caaacaaggg ctaagtccac aggtacccac tgggaggatg ttgagtaaga 240

tggaaaacta ctgatgaccc ttgcagagac agagtattag gacatgtttg aacaggggcc 300

gggcgatcag caggtagctc tagaggatcc ccgtctgtct gcacatttcg tagagcgagt 360

gttccgatac tctaatctcc ctaggcaagg ttcatatttg tgtaggttac ttattctcct 420

tttgttgact aagtcaataa tcagaatcag caggtttgga gtcagcttgg cagggatcag 480

cagcctgggt tggaaggagg gggtataaaa gccccttcac caggagaagc cgtc 534

<210> 625
<211> 72
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 625
gggggaggct gctggtaaac attaaccaag gtcaccccag ttatcagagg agcaaacagg 60

ggcaaagtcc ac 72

<210> 626
<211> 4392
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 626
atgcagattg agctgagcac ctgcttcttc ctgtgcctgc tgaggttctg cttctctgcc 60

accaggagat actacctggg ggctgtggag ctgagctggg actacatgca gtctgacctg 120

ggggagctgc ctgtggacgc caggttcccc cccagagtgc ccaagagctt ccccttcaac 180

acctctgtgg tgtacaagaa gaccctgttt gtggagttca ctgaccacct gttcaacatt 240

gccaagccca ggcccccctg gatgggcctg ctgggcccca ccatccaggc tgaggtgtat 300

gacactgtgg tgatcaccct gaagaacatg gccagccacc ctgtgagcct gcatgctgtg 360

ggggtgagct actggaaggc ctctgagggg gctgagtatg atgaccagac cagccagagg 420

gagaaggagg atgacaaggt gttccctggg ggcagccaca cctatgtgtg gcaggtgctg 480

aaggagaatg gccccatggc ctctgacccc ctgtgcctga cctacagcta cctgagccat 540

gtggacctgg tgaaggacct gaactctggc ctgattgggg ccctgctggt gtgcagggag 600

ggcagcctgg ccaaggagaa gacccagacc ctgcacaagt tcatcctgct gtttgctgtg 660

tttgatgagg gcaagagctg gcactctgaa accaagaaca gcctgatgca ggacagggat 720

gctgcctctg ccagggcctg gcccaagatg cacactgtga atggctatgt gaacaggagc 780

ctgcctggcc tgattggctg ccacaggaag tctgtgtact ggcatgtgat tggcatgggc 840

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aggcaggcca gcctggagat cagccccatc accttcctga ctgcccagac cctgctgatg 960

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gaggctgagg actatgatga tgacctgact gactctgaga tggatgtggt gaggtttgat 1140

gatgacaaca gccccagctt catccagatc aggtctgtgg ccaagaagca ccccaagacc 1200

tgggtgcact acattgctgc tgaggaggag gactgggact atgcccccct ggtgctggcc 1260

cctgatgaca ggagctacaa gagccagtac ctgaacaatg gcccccagag gattggcagg 1320

aagtacaaga aggtcaggtt catggcctac actgatgaaa ccttcaagac cagggaggcc 1380

atccagcatg agtctggcat cctgggcccc ctgctgtatg gggaggtggg ggacaccctg 1440

ctgatcatct tcaagaacca ggccagcagg ccctacaaca tctaccccca tggcatcact 1500

gatgtgaggc ccctgtacag caggaggctg cccaaggggg tgaagcacct gaaggacttc 1560

cccatcctgc ctggggagat cttcaagtac aagtggactg tgactgtgga ggatggcccc 1620

accaagtctg accccaggtg cctgaccaga tactacagca gctttgtgaa catggagagg 1680

gacctggcct ctggcctgat tggccccctg ctgatctgct acaaggagtc tgtggaccag 1740

aggggcaacc agatcatgtc tgacaagagg aatgtgatcc tgttctctgt gtttgatgag 1800

aacaggagct ggtacctgac tgagaacatc cagaggttcc tgcccaaccc tgctggggtg 1860

cagctggagg accctgagtt ccaggccagc aacatcatgc acagcatcaa tggctatgtg 1920

tttgacagcc tgcagctgtc tgtgtgcctg catgaggtgg cctactggta catcctgagc 1980

attggggccc agactgactt cctgtctgtg ttcttctctg gctacacctt caagcacaag 2040

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atggagaacc ctggcctgtg gattctgggc tgccacaact ctgacttcag gaacaggggc 2160

atgactgccc tgctgaaagt ctccagctgt gacaagaaca ctggggacta ctatgaggac 2220

agctatgagg acatctctgc ctacctgctg agcaagaaca atgccattga gcccaggagc 2280

ttcagccaga atagcaggca ccccagcacc aggcagaagc agttcaatgc caccaccatc 2340

ccagagaata ccaccctgca gtctgaccag gaggagattg actatgatga caccatctct 2400

gtggagatga agaaggagga ctttgacatc tacgacgagg acgagaacca gagccccagg 2460

agcttccaga agaagaccag gcactacttc attgctgctg tggagaggct gtgggactat 2520

ggcatgagca gcagccccca tgtgctgagg aacagggccc agtctggctc tgtgccccag 2580

ttcaagaagg tggtgttcca ggagttcact gatggcagct tcacccagcc cctgtacaga 2640

ggggagctga atgagcacct gggcctgctg ggcccctaca tcagggctga ggtggaggac 2700

aacatcatgg tgaccttcag gaaccaggcc agcaggccct acagcttcta cagcagcctg 2760

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aatgaaacca agacctactt ctggaaggtg cagcaccaca tggcccccac caaggatgag 2880

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caggtgactg tgcaggagtt tgccctgttc ttcaccatct ttgatgaaac caagagctgg 3060

tacttcactg agaacatgga gaggaactgc agggcccccc gcaacatcca gatggaggac 3120

cccaccttca aggagaacta caggttccat gccatcaatg gctacatcat ggacaccctg 3180

cctggcctgg tgatggccca ggaccagagg atcaggtggt acctgctgag catgggcagc 3240

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<210> 633
<211> 4392
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

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gccaagccca ggcccccctg gatgggcctg ctgggcccca ccatccaggc tgaggtgtat 300

gacactgtgg tgatcaccct gaagaacatg gccagccacc ctgtgagcct gcatgctgtg 360

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gacctgtact ga 4392

<210> 634
<211> 43
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 634
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<210> 635
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<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 635
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<210> 636
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 636
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<210> 637
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 637
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<210> 638
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<212> DNA
<213> Artificial Sequence

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<223> Description of Artificial Sequence: Synthetic
oligonucleotide

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gattaaccca ccatgctact tatcaattg 89

<210> 639
<211> 54
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 639
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<210> 640
<211> 49
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
oligonucleotide

<400> 640
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<210> 641
<211> 537
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

<400> 641
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agcaaacagg ggcaaagtcc acagagggag gctgctggta aacattaacc aaggtcaccc 180

agttatcaga ggagcaaaca ggggcaaagt ccatggtacc cactgggagg atgttgagta 240

agatggaaaa ctactgatga cccttgcaga gacagagtat taggacatgt ttgaacaggg 300

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ccttttgttg actaagtcaa taatcagaat cagcaggttt ggagtcagct tggcagggat 480

cagcagcctg ggttggaagg agggggtata aaagcccctt caccaggaga agccgtc 537