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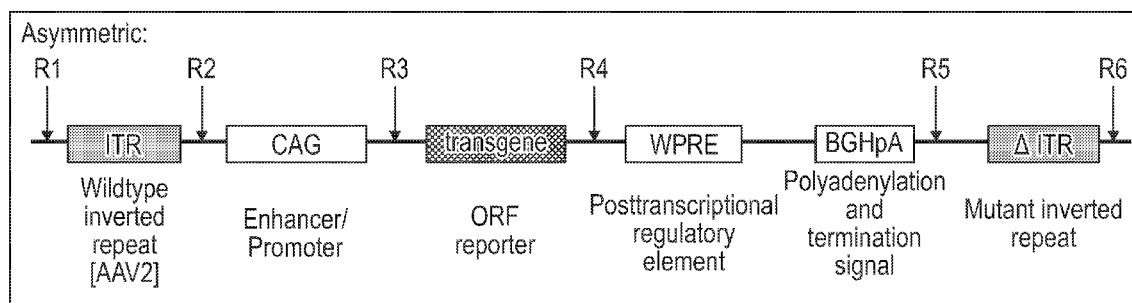
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(54) Title: CLOSED-ENDED DNA (CEDNA) AND IMMUNE MODULATING COMPOUNDS

FIG. 1A



(57) Abstract: Provided herein are methods and constructs related to minimizing immune responses using modified dexamethasone compounds when administering a desired transgene in a cell achieved by delivery of the transgene with one or more doses of a ceDNA construct.

**CLOSED-ENDED DNA (CEDNA) AND IMMUNE MODULATING COMPOUNDS****RELATED APPLICATIONS**

**[0001]** This application claims priority to U.S. Provisional Application No. 62/814,477, filed on March 6, 2019, the contents of which is hereby incorporated by reference in its entirety.

**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 March 5, 2020, is named 131698-05520\_SL.txt and is 116,727 bytes in size.

**TECHNICAL FIELD**

**[0003]** Embodiments of the invention relate to the field of gene therapy, including the delivery of exogenous DNA sequences to a target cell, tissue, organ or organism, and modifications and methods for modulating innate immune responses to the same.

**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.* under-expression 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 (sometimes referred to as 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, functional enzyme, or 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 (rAAV) 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-pathogenic 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. 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.

**[0009]** Mammalian systems have developed a variety of defenses against microbial attack, including systems that detect foreign DNA and engage an immune response that leads to removal

and/or destruction of such DNA. Macrophage lineage cells in particular have multiple DNA sensor pathways, including the TLR9 pathway, the cGAS/STING pathway, and inflammasome pathways.

[0010] Although conceptually elegant, the prospect of using nucleic-acid molecules for gene therapy for treating human diseases remains uncertain. The main cause of this uncertainty is the apparent adverse events relating to host's innate immune response to nucleic acid therapeutics and, thus, the way in which these materials modulate expression of their intended targets in the context of the immune response. The current state of the art surrounding the creation, function, behavior and optimization of nucleic acid molecules that may be adopted for clinical applications has a particular focus on: (1) antisense oligonucleotides and duplex RNAs that directly regulate translation and gene expression; (2) transcriptional gene silencing RNAs that result in long-term epigenetic modifications; (3) antisense oligonucleotides that interact with and alter gene splicing patterns; (4) creation of synthetic or viral vectors that mimic physiological functionalities of naturally occurring AAV or lentiviral genome; and (5) the *in vivo* delivery of therapeutic oligonucleotides. However, despite the advances made in the development of nucleic acid therapeutics that are evident in recent clinical achievements, the field of gene therapy is still severely limited by unwanted adverse events in recipients triggered by the therapeutic nucleic acids, themselves.

[0011] Administration of exogenous DNA for gene therapy may contend with clearance and/or destruction prior to reaching the desired intracellular location or expression of the encoded gene(s) of interest. Modulating such host immune defense against exogenous DNA would be potentially of benefit in facilitating gene therapy efficacy.

[0012] Accordingly, there is a need in the field for a new technology that inhibits (*e.g.*, reduces, ameliorates, mitigates, prevents) the immune response on administration of vectors or nucleic acid to a subject that permits expression of a therapeutic protein in a cell, tissue or subject for the treatment of a wide variety of diseases.

## SUMMARY

[0013] The present disclosure provides methods and pharmaceutical compositions for minimizing or reducing an innate immune response in a subject suffering from a genetic disorder and receiving gene or nucleic acid therapy ("nucleic acid therapeutics" or "therapeutic nucleic acid" (TNA)) Provided herein are non-viral capsid-free DNA vectors with covalently-closed ends (ceDNA vectors) in compositions comprising inhibitors for minimizing and reducing innate immune responses, and methods comprising the same.

[0014] According to some aspects, the disclosure provides methods for inhibiting immune responses when expressing a transgene in a cell, comprising co-administering to a cell a composition comprising a non-viral capsid-free DNA vector with covalently-closed ends (ceDNA vector) and a modified dexamethasone, such that the ceDNA vector comprises a heterologous nucleic acid sequence encoding a transgene operably positioned between two different AAV inverted terminal repeat

sequences (ITRs). According to some embodiments, one of the ITRs comprises a functional AAV terminal resolution site and a Rep binding site. According to some embodiments, one of the ITRs comprises a deletion, insertion, or substitution relative to the other ITR. According to some embodiments, the ceDNA when digested with a restriction enzyme having a single recognition site on the ceDNA vector has the presence of characteristic bands of linear and continuous DNA as compared to linear and non-continuous DNA controls when analyzed on a non-denaturing gel. According to some embodiments, the modified dexamethasone is dexamethasone palmitate.

**[0015]** Also provided herein, in some aspects, are methods for treating disease in a subject comprising administering to a subject in need thereof, a composition comprising a non-viral capsid-free DNA vector with covalently-closed ends (ceDNA vector), such that the ceDNA vector comprises a heterologous nucleic acid sequence encoding a transgene operably positioned between two different AAV inverted terminal repeat sequences (ITRs). According to some embodiments, one of the ITRs comprises a functional AAV terminal resolution site and a Rep binding site. According to some embodiments, one of the ITRs comprises a deletion, insertion, or substitution relative to the other ITR. According to some embodiments, the ceDNA when digested with a restriction enzyme having a single recognition site on the ceDNA vector has the presence of characteristic bands of linear and continuous DNA as compared to linear and non-continuous DNA controls when analyzed on a non-denaturing gel. According to some embodiments, the method comprises separately administering a modified dexamethasone. According to some embodiments, the modified dexamethasone compound comprises at least one fatty acid. According to some embodiments, the modified dexamethasone compound is dexamethasone palmitate. According to some embodiments, the administration is prior to the administration of the ceDNA vector. According to some embodiments, the administration is simultaneous with the administration of the ceDNA vector. According to some embodiments, the administration is subsequent to the administration of the ceDNA vector. According to some embodiments, the modified dexamethasone compound is co-encapsulated with the ceDNA vector. According to some embodiments, the modified dexamethasone compound is not co-encapsulated with the ceDNA vector. According to some embodiments, the modified dexamethasone compound is co-administered with the ceDNA vector being administered to the cell but is not co-encapsulated with the ceDNA vector. According to some embodiments, increasing the amount of the ceDNA vector in the cell increases expression of the transgene in the cell. According to some embodiments, the heterologous nucleic acid sequence encodes a therapeutic transgene and the desired level of expression of the transgene is a therapeutically effective amount.

**[0016]** According to any of the foregoing embodiments and aspects, at least one additional innate immune pathway inhibitor is administered. According to some embodiments, the at least one additional innate immune inhibitor is an inhibitor of one or more of the cGAS/STING pathway, the TLR9 pathway, or an inflammasome-mediated pathway. According to some embodiments, the at least one additional innate immune inhibitor is an inhibitor of the cGAS/STING pathway. According

to some embodiments, the at least one additional innate immune inhibitor is an inhibitor of the TLR9 pathway. According to some embodiments, the at least one additional innate immune inhibitor is an inhibitor of an inflammasome-mediated pathway.

**[0017]** According to another aspect, the disclosure provides a composition comprising (i) a linear, capsid-free DNA vector with covalently-closed ends (ceDNA vector), wherein the ceDNA vector comprises a heterologous nucleic acid sequence encoding a transgene operably positioned between two AAV inverted terminal repeat sequences (ITRs), and (ii) a modified dexamethasone compound. According to some embodiments, the ceDNA vector when digested with a restriction enzyme having a single recognition site on the ceDNA vector has the presence of characteristic bands of linear and continuous DNA as compared to linear and non-continuous DNA controls when analyzed on a non-denaturing gel. According to some embodiments, the at least one of the ITRs comprises a functional AAV terminal resolution site (TRS) and a Rep binding site. According to some embodiments, both ITRs are naturally occurring AAV ITRs from the same AAV strain. According to some embodiments, one ITR comprises a deletion, insertion, or substitution relative to the other ITR. According to some embodiments, one ITR comprises a deletion, insertion, or substitution relative to the other ITR and neither ITR is a naturally occurring AAV ITR. According to some embodiments, the modified dexamethasone compound comprises at least one fatty acid. According to some embodiments, the modified dexamethasone compound is dexamethasone palmitate. According to some embodiments, the modified dexamethasone compound is co-encapsulated with the ceDNA vector. According to some embodiments, the modified dexamethasone compound is not co-encapsulated with the ceDNA vector. According to some embodiments, the modified dexamethasone compound is co-administered with the ceDNA vector being administered to the cell but is not co-encapsulated with the ceDNA vector.

**[0018]** According to any of the foregoing embodiments and aspects, the composition further comprises at least one additional innate immune pathway inhibitor. According to some embodiments, the at least one additional innate immune inhibitor is an inhibitor of one or more of the cGAS/STING pathway, the TLR9 pathway, or an inflammasome-mediated pathway. According to some embodiments, the at least one additional innate immune inhibitor is an inhibitor of the cGAS/STING pathway. According to some embodiments, the at least one additional innate immune inhibitor is an inhibitor of the TLR9 pathway. According to some embodiments, the at least one additional innate immune inhibitor is an inhibitor of an inflammasome-mediated pathway.

**[0019]** According to some aspects, the disclosure provides a method for inhibiting immune responses when expressing a transgene in a cell, comprising administering to a cell a composition comprising (i) a linear, capsid-free DNA vector with covalently-closed ends (ceDNA vector), wherein the ceDNA vector comprises a heterologous nucleic acid sequence encoding a transgene operably positioned between two AAV inverted terminal repeat sequences (ITRs) and (ii) a modified dexamethasone compound. According to some embodiments, one of the ITRs comprises a functional

AAV terminal resolution site and a Rep binding site. According to some embodiments, one of the ITRs comprises a deletion, insertion, or substitution relative to the other ITR. According to some embodiments, the ceDNA when digested with a restriction enzyme having a single recognition site on the ceDNA vector has the presence of characteristic bands of linear and continuous DNA as compared to linear and non-continuous DNA controls when analyzed on a non-denaturing gel. According to some embodiments, the modified dexamethasone compound comprises at least one fatty acid. According to some embodiments, the modified dexamethasone compound is dexamethasone palmitate. According to some embodiments, the modified dexamethasone compound is co-encapsulated with the ceDNA vector. According to some embodiments, the modified dexamethasone compound is not co-encapsulated with the ceDNA vector. According to some embodiments, both ITRs are naturally occurring AAV ITRs from the same AAV strain. In another aspect, one ITR comprises a deletion, insertion, or substitution relative to the other ITR. In another aspect, one ITR comprises a deletion, insertion or substitution relative to the other ITR and neither ITR is a naturally occurring AAV ITR. In another aspect, the two ITRs are a pair of ITRs selected from the group consisting of (a) SEQ ID NO: 1 (3' WT-ITR) and SEQ ID NO: 4 (5' mod ITR); and (b) SEQ ID NO: 3 (3' mod ITR) and SEQ ID NO: 2 (5' WT-ITR). According to some embodiments, the ceDNA vector is administered in combination with a pharmaceutically acceptable carrier. According to some embodiments, increasing the amount of the ceDNA vector in the cell increases expression of the transgene in the cell. In another aspect, the heterologous nucleic acid sequence encodes a therapeutic transgene and the desired level of expression of the transgene is a therapeutically effective amount.

**[0020]** According to any of the foregoing embodiments and aspects, at least one additional innate immune pathway inhibitor is administered. According to some embodiments, the at least one additional innate immune inhibitor is an inhibitor of one or more of the cGAS/STING pathway, the TLR9 pathway, or an inflammasome-mediated pathway. According to some embodiments, the at least one additional innate immune inhibitor is an inhibitor of the cGAS/STING pathway. According to some embodiments, the at least one additional innate immune inhibitor is an inhibitor of the TLR9 pathway. According to some embodiments, the at least one additional innate immune inhibitor is an inhibitor of an inflammasome-mediated pathway.

**[0021]** In another aspect, the ceDNA vector is obtained from a process comprising the steps of: (a) incubating a population of insect cells harboring a ceDNA vector polynucleotide, which is devoid of viral capsid coding sequences in the presence of Rep protein under conditions effective and for time sufficient to induce production of the closed-ended linear, capsid-free, DNA vector within the insect cells, wherein the insect cells do not comprise production of closed-ended linear, capsid-free, DNA within the insect cells; and (b) harvesting and isolating the closed-ended linear capsid-free, DNA from the insect cells; wherein the presence of the linear, capsid-free, DNA isolated from the insect cells can be confirmed by digesting DNA isolated from the insect cells with a restriction enzyme having a single recognition site on the DNA 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. According to some embodiments, the ceDNA vector is obtained by cell-free synthesis. According to some embodiments, the ceDNA vector is encapsulated. According to some embodiments, the encapsulation is with a liposome. According to some embodiments, the encapsulation is by a lipid nanoparticle.

**[0022]** According to some aspects, the disclosure provides a method for treating a disease in a subject, comprising: administering to a subject in need thereof a composition comprising (i) a linear, capsid-free DNA vector with covalently-closed ends (ceDNA vector), wherein the ceDNA vector comprises a heterologous nucleic acid sequence encoding a transgene operably positioned between two AAV inverted terminal repeat sequences (ITRs) and (ii) a modified dexamethasone compound. According to some embodiments, one of the ITRs comprises a functional AAV terminal resolution site and a Rep binding site. According to some embodiments, one of the ITRs comprises a deletion, insertion, or substitution relative to the other ITR. According to some embodiments, the ceDNA when digested with a restriction enzyme having a single recognition site on the ceDNA vector has the presence of characteristic bands of linear and continuous DNA as compared to linear and non-continuous DNA controls when analyzed on a non-denaturing gel. According to some embodiments, the modified dexamethasone compound comprises at least one fatty acid. According to some embodiments, the modified dexamethasone compound is dexamethasone palmitate. According to some embodiments, the modified dexamethasone compound is co-encapsulated with the ceDNA vector. According to some embodiments, the modified dexamethasone compound is not co-encapsulated with the ceDNA vector. According to some embodiments, both ITRs are naturally occurring AAV ITRs from the same AAV strain. In another aspect, one ITR comprises a deletion, insertion, or substitution relative to the other ITR. In another aspect, one ITR comprises a deletion, insertion or substitution relative to the other ITR and neither ITR is a naturally occurring AAV ITR. In another aspect, the two ITRs are a pair of ITRs selected from the group consisting of (a) SEQ ID NO: 1 (3' WT-ITR) and SEQ ID NO: 4 (5' mod ITR); and (b) SEQ ID NO: 3 (3' mod ITR) and SEQ ID NO: 2 (5' WT-ITR).

**[0023]** In another aspect, the ceDNA vector is administered in combination with a pharmaceutically acceptable carrier. In another aspect, increasing the amount of the ceDNA vector in the cell increases expression of the transgene in the cell. In another aspect, the heterologous nucleic acid sequence encodes a therapeutic transgene and the desired level of expression of the transgene is a therapeutically effective amount.

**[0024]** In any of the foregoing embodiments and aspects, at least one additional innate immune pathway inhibitor is co-administered with the ceDNA vector and the modified dexamethasone compound. According to some embodiments, the at least one additional innate immune inhibitor is an inhibitor of one or more of the cGAS/STING pathway, the TLR9 pathway, or an inflammasome-mediated pathway. According to some embodiments, the at least one additional innate immune

inhibitor is an inhibitor of the cGAS/STING pathway. According to some embodiments, the at least one additional innate immune inhibitor is an inhibitor of the TLR9 pathway. According to some embodiments, the at least one additional innate immune inhibitor is an inhibitor of an inflammasome-mediated pathway.

**[0025]** In another aspect, the ceDNA vector is obtained from a process comprising the steps of: (a) incubating a population of insect cells harboring a ceDNA vector polynucleotide, which is devoid of viral capsid coding sequences in the presence of Rep protein under conditions effective and for time sufficient to induce production of the closed-ended linear, capsid-free, DNA vector within the insect cells, wherein the insect cells do not comprise production of closed-ended linear, capsid-free, DNA within the insect cells; and (b) harvesting and isolating the closed-ended linear capsid-free, DNA from the insect cells. According to some embodiments, the presence of the linear, capsid-free, DNA isolated from the insect cells can be confirmed by digesting DNA isolated from the insect cells with a restriction enzyme having a single recognition site on the DNA 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. According to some embodiments, the ceDNA vector is obtained by cell-free synthesis. According to some embodiments, the ceDNA vector is obtained by cell-free synthesis. According to some embodiments, the ceDNA vector is encapsulated. According to some embodiments, the encapsulation is with a liposome. According to some embodiments, the encapsulation is by a lipid nanoparticle.

### BRIEF DESCRIPTION OF THE DRAWINGS

**[0026]** **FIG. 1A** illustrates an exemplary structure of a ceDNA vector for transgene production as disclosed herein, comprising asymmetric ITRs. In this embodiment, the exemplary ceDNA vector comprises an expression cassette containing CAG promoter, WPRE, and BGHpA. An open reading frame (ORF) encoding a transgene, can be inserted into the cloning site (R3/R4) between the CAG promoter and WPRE. The expression cassette is flanked by two inverted terminal repeats (ITRs) – the wild-type AAV2 ITR on the upstream (5'-end) and the modified ITR on the downstream (3'-end) of the expression cassette, therefore the two ITRs flanking the expression cassette are asymmetric with respect to each other.

**[0027]** **FIG. 1B** illustrates an exemplary structure of a ceDNA vector for transgene production as disclosed herein comprising asymmetric ITRs with an expression cassette containing CAG promoter, WPRE, and BGHpA. An open reading frame (ORF) encoding a transgene, can be inserted into the cloning site between CAG promoter and WPRE. The expression cassette is flanked by two inverted terminal repeats (ITRs) – a modified ITR on the upstream (5'-end) and a wild-type ITR on the downstream (3'-end) of the expression cassette.

**[0028]** **FIG. 1C** illustrates an exemplary structure of a ceDNA vector for transgene production as disclosed herein comprising asymmetric ITRs, with an expression cassette containing an

enhancer/promoter, a transgene, a post transcriptional element (WPRE), and a polyA signal. An open reading frame (ORF) allows insertion of a transgene, into the cloning site between CAG promoter and WPRE. The expression cassette is flanked by two inverted terminal repeats (ITRs) that are asymmetrical with respect to each other; a modified ITR on the upstream (5'-end) and a modified ITR on the downstream (3'-end) of the expression cassette, where the 5' ITR and the 3' ITR are both modified ITRs but have different modifications (*i.e.*, they do not have the same modifications).

**[0029] FIG. 1D** illustrates an exemplary structure of a ceDNA vector for transgene production as disclosed herein, comprising symmetric modified ITRs, or substantially symmetrical modified ITRs as defined herein, with an expression cassette containing CAG promoter, WPRE, and BGHpA. An open reading frame (ORF) encoding a transgene is inserted into the cloning site between CAG promoter and WPRE. The expression cassette is flanked by two modified inverted terminal repeats (ITRs), where the 5' modified ITR and the 3' modified ITR are symmetrical or substantially symmetrical.

**[0030] FIG. 1E** illustrates an exemplary structure of a ceDNA vector for transgene production as disclosed herein comprising symmetric modified ITRs, or substantially symmetrical modified ITRs as defined herein, with an expression cassette containing an enhancer/promoter, a transgene, a post transcriptional element (WPRE), and a polyA signal. An open reading frame (ORF) allows insertion of a transgene, into the cloning site between CAG promoter and WPRE. The expression cassette is flanked by two modified inverted terminal repeats (ITRs), where the 5' modified ITR and the 3' modified ITR are symmetrical or substantially symmetrical.

**[0031] FIG. 1F** illustrates an exemplary structure of a ceDNA vector for transgene production as disclosed herein, comprising symmetric WT-ITRs, or substantially symmetrical WT-ITRs as defined herein, with an expression cassette containing CAG promoter, WPRE, and BGHpA. An open reading frame (ORF) encoding a transgene, is inserted into the cloning site between CAG promoter and WPRE. The expression cassette is flanked by two wild type inverted terminal repeats (WT-ITRs), where the 5' WT-ITR and the 3' WT ITR are symmetrical or substantially symmetrical.

**[0032] FIG. 1G** illustrates an exemplary structure of a ceDNA vector for transgene production as disclosed herein, comprising symmetric modified ITRs, or substantially symmetrical modified ITRs as defined herein, with an expression cassette containing an enhancer/promoter, a transgene, a post transcriptional element (WPRE), and a polyA signal. An open reading frame (ORF) allows insertion of a transgene, into the cloning site between CAG promoter and WPRE. The expression cassette is flanked by two wild type inverted terminal repeats (WT-ITRs), where the 5' WT-ITR and the 3' WT ITR are symmetrical or substantially symmetrical.

**[0033] FIG. 2A** 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. 2B** shows proposed Rep-catalyzed nicking and ligating activities in a wild-type left ITR (SEQ ID NO: 53), 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.

**[0034]** **FIG. 3A** provides the primary structure (polynucleotide sequence) (left) and the secondary structure (right) 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 (SEQ ID NO: 54). **FIG. 3B** shows an exemplary mutated ITR (also referred to as a modified ITR) sequence for the left ITR. Shown is the primary structure (left) and the predicted secondary structure (right) 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) (SEQ ID NO: 113). **FIG. 3C** shows the primary structure (left) and the secondary structure (right) of the RBE-containing portion of the A-A' loop, and the B-B' and C-C' arms of wild type right AAV2 ITR (SEQ ID NO: 55). **FIG. 3D** shows an exemplary right modified ITR. Shown is the primary structure (left) and the predicted secondary structure (right) 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) (SEQ ID NO: 114). 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. 3A-3D** 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. 3A-3D** 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.

**[0035]** **FIG. 4A** 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 antibody or fusion protein production as disclosed herein in the process described in the schematic in **FIG. 4B**. **FIG. 4B** is a schematic of an exemplary method of ceDNA production and **FIG. 4C** illustrates a biochemical method and process to confirm ceDNA vector production. **FIG. 4D** and **FIG. 4E** 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. 4B**. **FIG. 4D** 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 basepairs (*e.g.*, for the double-stranded molecules observed in native conditions). **FIG. 4E** 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. 4E** 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 strands remain connected and produce single strands that migrate as 2kb and 4kb.

**[0036]** **FIG. 5** shows the chemical structures of dexamethasone and dexamethasone palmitate.

**[0037]** **FIGS. 6A, 6B and 6C** provide graphs showing data from the experiments described in Example 6. **FIG. 6A** depicts the body weight of treated mice over time and shows that ceDNA/dexamethasone palmitate treatment results in a lessening of body weight loss relative to ceDNA/polyC-treated control animals. **FIG. 6B** shows that the observed expression of luciferase from ceDNA/polyC-treated animals and ceDNA/dexamethasone palmitate-treated animals was similar, indicating that dexamethasone palmitate does not inhibit the uptake, nuclear translocation or expression of transgene from the administered ceDNA vector. **FIG. 6C** provides the results of the cytokine analyses from serum samples taken after administration, showing that dexamethasone palmitate significantly reduced the levels of certain cytokines (IL-6, TNF-alpha, and RANTES), while not impacting the levels of others.

## DETAILED DESCRIPTION

**[0038]** Nucleic acid transfer vectors and therapeutic agents are promising therapeutics for a variety of applications, such as gene expression and modulation thereof. Viral transfer vectors may comprise transgenes that encode proteins or nucleic acids. Examples of such include AAV vectors, microRNA (miRNA), small interfering RNA (siRNA), as well as antisense oligonucleotides that bind mutation sites in messenger RNA (such as small nuclear RNA (snRNA)). Unfortunately, the promise of these therapeutics has not yet been realized, in large part due to cellular and humoral immune responses directed against the viral transfer vector. These immune responses include antibody, B cell and T cell

responses, and are often specific to viral antigens of the viral transfer vector, such as viral capsid or coat proteins or peptides thereof.

**[0039]** Currently, many potential patients harbor some level of pre-existing immunity against the viruses on which viral transfer vectors are based. In fact, antibodies against viral nucleic acids (both DNA and RNA) or protein are highly prevalent in the human population. In addition, even if the level of pre-existing immunity is low, for example, due to the low immunogenicity of the viral transfer vector, such low levels may still prevent successful transduction (*e.g.*, Jeune *et al.*, Human Gene Therapy Methods, 24:59-67 (2013)). Thus, even low levels of pre-existing immunity may hinder the use of a specific viral transfer vector in a patient, and may require a clinician to choose a viral transfer vector based on a virus of a different serotype that may not be as efficacious, or even opt out for a different type of therapy altogether if another viral transfer vector therapy is not available.

**[0040]** Additionally, viral vectors, such as adeno-associated vectors, can be highly immunogenic and elicit humoral and cell-mediated immunity that can compromise efficacy, particularly with respect to re-administration. In fact, cellular and humoral immune responses against a viral transfer vector can develop after a single administration of the viral transfer vector. After viral transfer vector administration, neutralizing antibody titers can increase and remain high for several years, and can reduce the effectiveness of re-administration of the viral transfer vector. Indeed, repeated administration of a viral transfer vector generally results in enhanced, undesired immune responses. In addition, viral transfer vector-specific CD8<sup>+</sup> T cells may arise and eliminate transduced cells expressing a desired transgene product, for example, on re-exposure to a viral antigen-like viral nucleic acid or capsid protein. For example, it has been shown that AAV nucleic acids or capsid antigens can trigger immune-mediated destruction of hepatocytes transduced with an AAV viral transfer vector. For many therapeutic applications, it is thought that multiple rounds of administration of viral transfer vectors are needed for long-term benefits. The ability to do so, however, would be severely limited, particularly if re-administration is needed, without the methods and compositions provided herein.

**[0041]** Methods and compositions are provided that offer solutions to the aforementioned obstacles to effective use of variety of nucleic acid therapeutics, including viral or non-viral (synthetic) transfer vectors, and other nucleic acid therapeutics for treatment.

**[0042]** The present disclosure relates to the field of gene therapy, including the delivery of exogenous DNA sequences to a target cell, tissue, organ or organism, and compositions and methods for inhibiting innate immune responses to the same. Such compositions and methods for inhibiting innate immune responses can be used to, for example, enhance duration of transgene expression.

**[0043]** It has been unexpectedly discovered that an innate immune response to DNA transfer vector can be attenuated with the methods and related compositions provided herein. Hence, the methods and compositions can potentially increase the efficacy of treatment with viral transfer vectors and other

therapeutic nucleic acid molecules and provide for long-term therapeutic benefits, even if the administration of the viral transfer vector or other nucleic acid therapeutics is repeated.

## **I. Definitions**

**[0044]** 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 invention 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 invention, 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, 6<sup>th</sup> 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, 4<sup>th</sup> 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.

**[0045]** 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.

**[0046]** 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”).

**[0047]** As used herein, an “effective amount” or “therapeutically effective amount” of an active agent or therapeutic agent, such as an immunosuppressant and/or therapeutic nucleic acid, is an amount sufficient to produce the desired effect, *e.g.*, a normalization or reduction of immune response (*e.g.*, innate immune response) and expression or inhibition of expression of a target sequence in comparison to the expression level detected in the absence of a therapeutic nucleic acid and/or immunosuppressant. 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 invention. In prophylactic or preventative applications of the described invention, 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. The terms “dose” and “dosage” are used interchangeably herein.

**[0048]** 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.

**[0049]** 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*, 10<sup>th</sup> Edition, McGraw-Hill (New York) (2001), incorporated herein by reference, are summarized below.

**[0050]** 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.

**[0051]** As used herein, the terms “heterologous nucleotide 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.

**[0052]** 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.

**[0053]** 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<sup>TM</sup>) 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<sup>TM</sup>), 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.

**[0054]** “Nucleotides” contain a sugar deoxyribose (DNA) or ribose (RNA), a base, and a phosphate group. Nucleotides are linked together through the phosphate groups.

**[0055]** “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.

**[0056]** As used herein, the term “interfering RNA” or “RNAi” or “interfering RNA sequence” includes single-stranded RNA (*e.g.*, mature miRNA, ssRNAi oligonucleotides, ssDNAi oligonucleotides), double-stranded RNA (*i.e.*, duplex RNA such as siRNA, Dicer-substrate dsRNA, shRNA, aiRNA, or pre-miRNA), a DNA-RNA hybrid (see, *e.g.*, PCT Publication No. WO

2004/078941), or a DNA-DNA hybrid (see, *e.g.*, PCT Publication No. WO 2004/104199) that is capable of reducing or inhibiting the expression of a target gene or sequence (*e.g.*, by mediating the degradation or inhibiting the translation of mRNAs which are complementary to the interfering RNA sequence) when the interfering RNA is in the same cell as the target gene or sequence. Interfering RNA thus refers to the single-stranded RNA that is complementary to a target mRNA sequence or to the double-stranded RNA formed by two complementary strands or by a single, self-complementary strand. Interfering RNA may have substantial or complete identity to the target gene or sequence, or may comprise a region of mismatch (*i.e.*, a mismatch motif). The sequence of the interfering RNA can correspond to the full-length target gene, or a subsequence thereof. Preferably, the interfering RNA molecules are chemically synthesized. The disclosures of each of the above patent documents are herein incorporated by reference in their entirety for all purposes.

**[0057]** Interfering RNA includes “small-interfering RNA” or “siRNA,” *e.g.*, interfering RNA of about 15-60, 15-50, or 15-40 (duplex) nucleotides in length, more typically about 15-30, 15-25, or 19-25 (duplex) nucleotides in length, and is preferably about 20-24, 21-22, or 21-23 (duplex) nucleotides in length (*e.g.*, each complementary sequence of the double-stranded siRNA is 15-60, 15-50, 15-40, 15-30, 15-25, or 19-25 nucleotides in length, preferably about 20-24, 21-22, or 21-23 nucleotides in length, and the double-stranded siRNA is about 15-60, 15-50, 15-40, 15-30, 15-25, or 19-25 base pairs in length, preferably about 18-22, 19-20, or 19-21 base pairs in length). siRNA duplexes may comprise 3' overhangs of about 1 to about 4 nucleotides or about 2 to about 3 nucleotides and 5' phosphate termini. Examples of siRNA include, without limitation, a double-stranded polynucleotide molecule assembled from two separate stranded molecules, wherein one strand is the sense strand and the other is the complementary antisense strand; a double-stranded polynucleotide molecule assembled from a single stranded molecule, where the sense and antisense regions are linked by a nucleic acid-based or non-nucleic acid-based linker; a double-stranded polynucleotide molecule with a hairpin secondary structure having self-complementary sense and antisense regions; and a circular single-stranded polynucleotide molecule with two or more loop structures and a stem having self-complementary sense and antisense regions, where the circular polynucleotide can be processed *in vivo* or *in vitro* to generate an active double-stranded siRNA molecule. As used herein, the term “siRNA” includes RNA-RNA duplexes as well as DNA-RNA hybrids (see, *e.g.*, PCT Publication No. WO 2004/078941).

**[0058]** 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.

**[0059]** 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.

**[0060]** 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.

**[0061]** A DNA sequence that “encodes” a particular inflammasome antagonist (*e.g.*, any one or more of: an inhibitor of the NLRP3 inflammasome pathway, or an inhibitor of the AIM2 inflammasome pathway, or an inhibitor of caspase 1, or any combination thereof) 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”).

**[0062]** 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) one an inflammasome antagonist (*e.g.*, any one or more of: an inhibitor of the NLRP3 inflammasome pathway, or an inhibitor of the AIM2 inflammasome pathway, or an inhibitor of caspase 1, or any combination thereof) or fragment thereof and (ii) at least one non-Gene of interest (GOI) protein or alternatively, a different inflammasome antagonist protein. Fusion proteins encompassed herein include, but are not limited to, an antibody, or Fc or antigen-binding fragment of an antibody fused to an inflammasome antagonist (*e.g.*, any one or more of: an inhibitor of the NLRP3 inflammasome pathway, or an inhibitor of the AIM2 inflammasome pathway, or an inhibitor of caspase 1, or any combination thereof), *e.g.*, an extracellular domain of a receptor, ligand, enzyme or peptide. An inflammasome antagonist (*e.g.*, any one or more of: an inhibitor of the NLRP3

inflammasome pathway, or an inhibitor of the AIM2 inflammasome pathway, or an inhibitor of caspase 1, or any combination thereof) or fragment thereof that is part of a fusion protein can be a monospecific antibody or a bispecific or multispecific antibody.

**[0063]** 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.

**[0064]** As used herein, the term “gene delivery” means a process by which foreign DNA is transferred to host cells for applications of gene therapy.

**[0065]** 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 invention 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”.

**[0066]** 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 nucleotide 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).

**[0067]** 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.

**[0068]** 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.

**[0069]** 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.

**[0070]** 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”.

**[0071]** 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 a modified ITR can be considered substantially symmetrical, even if it has some nucleotide 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 nucleotide 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 nucleotide 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.

**[0072]** 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 cdNA vector.

**[0073]** 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. 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.

**[0074]** 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.

**[0075]** 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. By “decrease,” “decreasing,” “reduce,” or “reducing” of an immune response (*e.g.*, an immune response (*e.g.*, innate immune response)) by an immunosuppressant is intended to mean a detectable decrease of an immune response to a given immunosuppressant. The amount of decrease of an immune response by the immunosuppressant may be determined relative to the level of an immune response in the presence of an immunosuppressant. A detectable decrease can be about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, or more lower than the immune response detected in the presence of the immunosuppressant.

**[0076]** 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.

**[0077]** As used herein, the term “ceDNA-plasmid” refers to a plasmid that comprises a ceDNA genome as an intermolecular duplex.

**[0078]** 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.

**[0079]** As used herein, the term “ceDNA-baculovirus” refers to a baculovirus that comprises a ceDNA genome as an intermolecular duplex within the baculovirus genome.

**[0080]** 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.

**[0081]** 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.

**[0082]** 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.

**[0083]** 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.

**[0084]** 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).

**[0085]** As used herein, the terms “gap” and “nick” are used interchangeably and refer to a discontinued portion of synthetic DNA vector of the present invention, 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.

**[0086]** 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: 60), an RBS sequence identified in AAV2. Any known RBS sequence may be used in the embodiments of the invention, including other known AAV RBS sequences and other naturally known or synthetic RBS sequences. Without being bound by theory it is thought that the nuclease domain of a Rep protein binds to the duplex nucleotide 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: 60). 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.

**[0087]** 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' (SEQ ID NO: 61), the hexanucleotide sequence identified in AAV2. Any known TRS sequence may be used in the embodiments of the invention, including other known AAV TRS sequences and other naturally known or synthetic TRS sequences such as AGTT (SEQ ID NO: 62), GGTTGG (SEQ ID NO: 63), AGTTGG (SEQ ID NO: 64), AGTTGA (SEQ ID NO: 65), and other motifs such as RRTTRR (SEQ ID NO: 66).

**[0088]** 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.

**[0089]** 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.

**[0090]** 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  $\beta$ -galactosidase convert a substrate to a colored product. Exemplary reporter polypeptides useful for experimental or diagnostic purposes include, but are not limited to  $\beta$ -lactamase,  $\beta$ -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.

**[0091]** 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.

**[0092]** Transcriptional regulators refer to transcriptional activators and repressors that either activate or repress transcription of a gene of interest, such as an inflammasome antagonist (*e.g.*, inhibitor of one or more of NLRP3 and/or AIM2 inflammasome pathway, or a caspase 1 inhibitor). 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.

**[0093]** 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.

**[0094]** 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.

**[0095]** 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.

**[0096]** 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.

**[0097]** 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 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.

**[0098]** 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 pairs 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.

**[0099]** 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.

**[00100]** 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.

**[00101]** 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.

**[00102]** 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 of 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.

**[00103]** 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.

**[00104]** The phrase “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 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. Promoters can also be of AAV origin.

**[00105]** 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 invention, 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, cynomolgous 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.

**[00106]** 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<sup>+</sup> 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.

**[00107]** 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.

**[00108]** The term “sequence identity” refers to the relatedness between two nucleotide 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.

**[00109]** 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.

**[00110]** 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.

**[00111]** 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 cDNA 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.

**[00112]** 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).

**[00113]** By “recombinant vector” is meant a vector that includes a 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.

[00114] 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, thalassaemias, xeroderma pigmentosum, Fanconi's anemia, retinitis pigmentosa, ataxia telangiectasia, Bloom's syndrome, retinoblastoma, and Tay-Sachs disease.

[00115] An “inhibitory polynucleotide” as used herein refers to a DNA or RNA molecule that reduces or prevents expression (transcription or translation) of a second (target) polynucleotide. Inhibitory polynucleotides include antisense polynucleotides, ribozymes, and external guide sequences. The term “inhibitory polynucleotide” further includes DNA and RNA molecules, *e.g.*, RNAi that encode the actual inhibitory species, such as DNA molecules that encode ribozymes.

[00116] As used herein, “gene silencing” or “gene silenced” in reference to an activity of an RNAi molecule, for example a siRNA or miRNA refers to a decrease in the mRNA level in a cell for a target gene (*e.g.* NLRP3, AIM2 or caspase-1 mRNA) by at least about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 99%, about 100% of the mRNA level found in the cell without the presence of the miRNA or RNA interference molecule. In one preferred embodiment, the mRNA levels are decreased by at least about 70%, about 80%, about 90%, about 95%, about 99%, about 100%.

[00117] As used herein, the term “RNAi” refers to any type of interfering RNA, including but not limited to, siRNAi, shRNAi, endogenous microRNA and artificial microRNA. For instance, it includes sequences previously identified as siRNA, regardless of the mechanism of down-stream processing of the RNA (*i.e.* although siRNAs are believed to have a specific method of *in vivo* processing resulting in the cleavage of mRNA, such sequences can be incorporated into the vectors in the context of the flanking sequences described herein). The term “RNAi” can include both gene silencing RNAi molecules, and also RNAi effector molecules which activate the expression of a gene. By way of an example only, in some embodiments RNAi agents which serve to inhibit or gene silence are useful in the methods, kits and compositions disclosed herein, *e.g.*, to inhibit the immune response (*e.g.*, the innate immune response).

[00118] 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.

[00119] 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.

**[00120]** 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.

**[00121]** 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 invention.

**[00122]** 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.”

**[00123]** Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein should be understood as modified in all instances by the term “about.” The term “about” when used in connection with percentages can mean  $\pm 1\%$ . The present invention is further explained in detail by the following examples, but the scope of the invention should not be limited thereto.

**[00124]** Groupings of alternative elements or embodiments of the invention 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.

**[00125]** 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.

**[00126]** Other terms are defined herein within the description of the various aspects of the invention.

## II. Nucleic Acids

[00127] Nucleic acids are large, highly charged, rapidly degraded and cleared from the body, and offer generally poor pharmacological properties because they are recognized as a foreign matter to the body and become a target of an immune response (*e.g.*, innate immune response). Hence, certain nucleic acids, such as therapeutic nucleic acids or nucleic acids used for research purposes (*e.g.*, antisense oligonucleotide or viral vectors) can often trigger immune responses *in vivo*. The present disclosure provides pharmaceutical compositions and methods that may ameliorate, reduce or eliminate such immune responses and enhance efficacy of the nucleic acids by increasing expression levels through maximizing the durability of the nucleic acid in a reduced immune-responsive state in a subject recipient. This may also minimize any potential adverse events that may lead to an organ damage or other toxicity in the course of gene therapy. Many of the compositions and methods provided herein relate to the administration of a specific inhibitor of the immune response (*e.g.*, innate immune response) in conjunction with a nucleic acid (*e.g.*, a therapeutic nucleic acid or a nucleic acid used for research purposes), thereby reducing the immune response (*e.g.*, innate immune response) triggered by the presence of the nucleic acid.

[00128] The characterization and development of nucleic acid molecules for potential therapeutic use in conjunction with antagonists of the immune response (*e.g.*, innate immune response) are provided herein. In some embodiments, chemical modification of oligonucleotides for the purpose of altered and improved *in vivo* properties (delivery, stability, life-time, folding, target specificity), as well as their biological function and mechanism that directly correlate with therapeutic application, are described where appropriate.

[00129] Illustrative therapeutic nucleic acids of the present disclosure that can be immunostimulatory and require use of immunosuppressants disclosed herein can include, but are not limited to, minigenes, plasmids, minicircles, small interfering RNA (siRNA), microRNA (miRNA), antisense oligonucleotides (ASO), ribozymes, closed ended double stranded DNA (*e.g.*, ceDNA, CELiD, linear covalently closed DNA (“ministring”), doggybone (dbDNA<sup>TM</sup>), protelomere closed ended DNA, or dumbbell linear DNA), dicer-substrate dsRNA, small hairpin RNA (shRNA), asymmetrical interfering RNA (aiRNA), microRNAs (miRNA), mRNA, tRNA, rRNA, and DNA viral vectors, viral RNA vector, and any combination thereof.

[00130] siRNA or miRNA that can downregulate the intracellular levels of specific proteins through a process called RNA interference (RNAi) are also contemplated by the present invention to be nucleic acid therapeutics. After siRNA or miRNA is introduced into the cytoplasm of a host cell, these double-stranded RNA constructs can bind to a protein called RISC. The sense strand of the siRNA or miRNA is removed by the RISC complex. The RISC complex, when combined with the complementary mRNA, cleaves the mRNA and release the cut strands. RNAi is by inducing specific destruction of mRNA that results in downregulation of a corresponding protein.

**[00131]** Antisense oligonucleotides (ASO) and ribozymes that inhibit mRNA translation into protein can be nucleic acid therapeutics. For antisense constructs, these single stranded deoxy nucleic acids have a complementary sequence to the sequence of the target protein mRNA, and Watson - capable of binding to the mRNA by Crick base pairing. This binding prevents translation of a target mRNA, and / or triggers RNaseH degradation of the mRNA transcript. As a result, the antisense oligonucleotide has increased specificity of action (*i.e.*, down-regulation of a specific disease-related protein).

**[00132]** In any of the methods provided herein, the therapeutic nucleic acid can be a therapeutic RNA. The therapeutic RNA can be an inhibitor of mRNA translation, agent of RNA interference (RNAi), catalytically active RNA molecule (ribozyme), transfer RNA (tRNA) or an RNA that binds an mRNA transcript (ASO), protein or other molecular ligand (aptamer). In any of the methods provided herein, the agent of RNAi can be a double-stranded RNA, single-stranded RNA, micro RNA, short interfering RNA, short hairpin RNA, or a triplex-forming oligonucleotide.

**[00133]** According to some embodiments, the therapeutic nucleic acid is a closed ended double stranded DNA, *e.g.*, a ceDNA. According to some embodiments, the expression and/or production of a therapeutic protein in a cell is from a non-viral DNA vector, *e.g.*, a ceDNA vector. 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 heterologous nucleic acid sequences encoding a desired protein. Thus, even a large therapeutic protein can be expressed from a single ceDNA vector. Thus, ceDNA vectors can be used to express a therapeutic protein in a subject in need thereof.

**[00134]** In general, a ceDNA vector for expression of a therapeutic protein as disclosed herein, comprises in the 5' to 3' direction: a first adeno-associated virus (AAV) inverted terminal repeat (ITR), a nucleotide 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.

### **III. Closed ended DNA (ceDNA) Vectors**

**[00135]** Described herein are novel non-viral, capsid-free DNA vectors with covalently-closed ends (ceDNA) administered in a composition in conjunction with one or more modified dexamethasone compounds. Also provided herein are methods of inhibiting innate immune reactions that occur upon administration of a ceDNA vector to a cell or subject by further administering a modified dexamethasone compound. In certain embodiments, the modified dexamethasone compound is dexamethasone palmitate.

**[00136]** The non-viral capsid free DNA vectors are produced in permissive host cells from an expression construct (*e.g.*, a plasmid, a Bacmid, a baculovirus, or an integrated cell-line) containing a heterologous nucleic acid, *e.g.* a transgene positioned between two inverted terminal repeat (ITR) sequences. In some embodiments, at least one of the ITRs is modified by deletion, insertion, and/or substitution as compared to a wild-type ITR sequence (*e.g.* AAV ITR); and at least one of the ITRs comprises a functional terminal resolution site (TRS) and a Rep binding site. In one embodiment, at least one of the ITRs has at least one polynucleotide deletion, insertion, or substitution with respect to a corresponding AAV ITR (*e.g.* SEQ ID NO:1, or SEQ ID NO:2, for wild type 3' and 5' ITRs respectively for AAV2) to induce replication of the DNA vector in a host cell in the presence of Rep protein. As discussed above, it is envisioned that any ITR can be used. For exemplary purposes, the ITRs in the ceDNA constructs in Table 1A and the Examples are a modified ITR and a WT ITR and are an example of an asymmetric ITR pair. However, encompassed herein are ceDNA vectors that contain a heterologous nucleic acid sequence (*e.g.*, a transgene) 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 (see *e.g.*, FIGS 1A-1E). A ceDNA vector comprising a NLS 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.

**[00137]** In some embodiments, the methods and compositions described herein relate to the use of a modified dexamethasone compound as disclosed herein for co-administration with any ceDNA vector, including but not limited to, a ceDNA vector comprising asymmetric ITRs as disclosed in International Patent Application PCT/US18/49996, filed on September 7, 2018 (see, *e.g.*, Examples 1-4); a ceDNA vector for gene editing as disclosed on the International Patent Application PCT/US18/64242 filed on December 6, 2018 (see, *e.g.*, Examples 1-7), or a ceDNA vector for production of antibodies or fusion proteins, as disclosed in the International Patent Application PCT/US19/18016, filed on February 14, 2019, (*e.g.*, see Examples 1-4), or a ceDNA vector for controlled transgene expression, as disclosed in International Patent Application PCT/US19/18927 filed on February 22, 2019, each of which are incorporated herein in their entirety by reference. In some embodiments, it is also envisioned that the methods and compositions described herein using a modified dexamethasone compound, as disclosed herein can be used with a synthetically produced ceDNA vector, *e.g.*, a ceDNA vector produced in a cell free or insect-free system of ceDNA

production, as disclosed in International Application PCT/US19/14122, filed on January 18, 2019, incorporated by reference in its entirety herein.

**[00138]** The ceDNA vector is preferably duplex, or self-complementary, over at least a portion of the molecule, *e.g.* the transgene. The ceDNA vector has covalently closed ends, and thus is preferably resistant to exonuclease digestion (*e.g.* Exo I or Exo III) for over an hour at 37°C. The presence of Rep protein in the host cells (*e.g.* insect cells or mammalian cells) promotes replication of the ceDNA vector polynucleotide template that has the modified ITR inducing production of non-viral capsid free DNA vector with covalently closed ends. The covalently closed ended molecule continues to accumulate in permissive cells through replication and is preferably sufficiently stable over time in the presence of Rep protein under standard replication conditions, *e.g.* to accumulate at yields of at least 1 pg/cell, preferably at least 2 pg/cell, preferably at least 3 pg/cell, more preferably at least 4 pg/cell, even more preferably at least 5 pg/cell.

**[00139]** In particular, in one embodiment, DNA vectors are produced by providing cells (*e.g.* insect cells or mammalian cells *e.g.* 293 cells etc.) harboring a polynucleotide vector template (*e.g.*, expression construct) that comprises two different ITRs (*e.g.* AAV ITRs) and a nucleotide sequence of interest (a heterologous nucleic acid, expression cassette) positioned between the ITRs, wherein at least one of the ITRs is a modified ITR comprising an insertion, substitution, or deletion relative to the other ITR. The polynucleotide vector template described herein contains at least one functional ITR that comprises a Rep-binding site (RBS; *e.g.* 5'-GCGCGCTCGCTCGCTC-3' (SEQ ID NO: 60) for AAV2) and a functional terminal resolution site (trs; *e.g.* 5'-AGTT (SEQ ID NO: 62)). The cells do not express viral capsid proteins and the polynucleotide vector template is devoid of viral capsid coding sequences.

**[00140]** In the presence of Rep, the vector polynucleotide template having at least one modified ITR replicates to produce ceDNA. The ceDNA production undergoes two steps: first, excision (“rescue”) of template from the vector backbone (*e.g.* plasmid, bacmid, genome etc.) via Rep proteins, and second, Rep mediated replication of the excised vector genome. Rep proteins and Rep binding sites of the various AAV serotypes are well known to those of skill in the art. One of skill in the art understands to choose a Rep protein from a serotype that binds to and replicates the functional ITR.

**[00141]** The cells harboring the vector polynucleotide either already contain Rep (*e.g.* a cell line with inducible rep), or are transduced with a vector that contains Rep and are then grown under conditions permitting replication and release of ceDNA vector. The ceDNA vector DNA is then harvested and isolated from the cells. The presence of the capsid-free, non-viral DNA ceDNA vector 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 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. For example, Figure 6 is a gel confirming the production of ceDNA from multiple TTX plasmid constructs using one embodiment for producing

these vectors described in the Examples. The ceDNA is confirmed by a characteristic band pattern in the gel, as discussed with respect to FIG. 4D.

**[00142]** The vector polynucleotide expression template (*e.g.* TTX-plasmid, Bacmid *etc.*), and/or ii) a polynucleotide that encodes Rep can be introduced into cells using any means well known to those of skill in the art, including but not limited to transfection (*e.g.* calcium phosphate, nanoparticle, or liposome), or introduction by viral vectors, *e.g.* HSV or baculovirus. For example, the vector polynucleotide expression construct template used for generating the ceDNA vectors of the present invention can be a plasmid (*e.g.*, TTX-plasmids, *e.g.* see FIG. 4B), a Bacmid (*e.g.*, TTX-bacmid), and/or a baculovirus (*e.g.*, TTX-baculovirus). In one embodiment, the TTX-plasmid comprises a restriction cloning site (*e.g.* SEQ ID NO: 7) operably positioned between the ITRs where the heterologous nucleic acid (*e.g.* expression cassette comprising a reporter gene or a therapeutic nucleic acid) can be inserted.

**[00143]** In one preferred embodiment, the host cells used to make the ceDNA vectors described herein are insect cells. In another preferred embodiment, baculovirus is used to deliver both the polynucleotide that encodes Rep protein and the non-viral DNA vector polynucleotide expression construct template for ceDNA. Examples of such processes for obtaining and isolating ceDNA vectors are described in **Example 1** below.

**[00144]** According to some embodiments, synthetic ceDNA is produced *via* excision from a double-stranded DNA molecule. 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).

**[00145]** In some embodiments, a construct to make a ceDNA vector comprises a regulatory switch as described herein.

**[00146]** 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, incorporated by reference in its entirety herein, shows an exemplary method of ligating a 5' ITR oligonucleotide and a 3' ITR oligonucleotide to a double stranded polynucleotide comprising an expression cassette.

**[00147]** An exemplary method of producing a ceDNA vector using a synthetic method is provided in Example 4 of PCT/US19/14122, incorporated by reference in its entirety herein, 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.

**[00148]** In yet another aspect, the invention provides for host cell lines that have stably integrated the DNA vector polynucleotide expression template (ceDNA template) described herein, into their own genome for use in production of the non-viral DNA vector. Methods for producing such cell lines are described in Lee, L. *et al.* (2013) Plos One 8(8): e69879, which is herein incorporated by reference in its entirety. Preferably, the Rep protein (*e.g.* as described in Example 1) is added to host cells at an MOI of 3. In one embodiment, the host cell line is an invertebrate cell line, preferably insect Sf9 cells. When the host cell line is a mammalian cell line, preferably 293 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.

**[00149]** Any promoter can be operably linked to the heterologous nucleic acid (*e.g.* reporter nucleic acid or therapeutic transgene) of the vector polynucleotide. The expression cassette can contain a synthetic regulatory element, such as CAG promoter. The CAG promoter comprises (i) the cytomegalovirus (CMV) early enhancer element, (ii) the promoter, the first exon and the first intron of the chicken beta actin gene, and (ii) the splice acceptor of the rabbit beta globin gene. Alternatively, expression cassette can contain an Alpha-1-antitrypsin (AAT) promoter, a liver specific (LP1) promoter, or Human elongation factor-1 alpha (EF1- $\alpha$ ) promoter. In some embodiments, the expression cassette includes one or more constitutive promoters, for example, the retroviral Rous sarcoma virus (RSV) LTR promoter (optionally with the RSV enhancer), cytomegalovirus (CMV) immediate early promoter (optionally with the CMV enhancer). Alternatively, an inducible or repressible promoter, a native promoter for a transgene, a tissue-specific promoter, or various promoters known in the art can be used. Suitable transgenes for gene therapy are well known to those of skill in the art.

**[00150]** The capsid-free ceDNA vectors can also be produced from vector polynucleotide expression constructs that further comprise cis-regulatory elements, or combination of cis regulatory elements, a non-limiting example include a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) and BGH polyA, or *e.g.* beta-globin polyA. Other posttranscriptional processing elements include, *e.g.* the thymidine kinase gene of herpes simplex virus, or hepatitis B virus (HBV). The expression cassettes can include any poly-adenylation sequence known in the art or a variation thereof, such as a naturally occurring isolated from bovine BGHpA or a virus SV40pA, or synthetic.

Some expression cassettes can also include SV40 late polyA signal upstream enhancer (USE) sequence. The, USE can be used in combination with SV40pA or heterologous poly-A signal.

**[00151]** The time for harvesting and collecting DNA 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 DNA-vectors (*e.g.*, TTX-vectors) but before a majority of cells start to die because of the viral 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.

**[00152]** 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.

**[00153]** In one embodiment, the capsid free non-viral DNA vector comprises or is obtained from a plasmid comprising a polynucleotide template comprising in this order: a first adeno-associated virus (AAV) inverted terminal repeat (ITR), a nucleotide sequence of interest (for example an expression cassette of an exogenous DNA) and a modified AAV ITR, wherein said template nucleic acid molecule is devoid of AAV capsid protein coding. In a further embodiment, the nucleic acid template of the invention 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 template nucleic acid molecule is also devoid of AAV Rep protein coding sequences. Accordingly, in a preferred embodiment, the nucleic acid molecule of the invention is devoid of both functional AAV cap and AAV rep genes.

**[00154]** In one embodiment, ceDNA can include an ITR structure that is mutated with respect to the wild type AAV2 ITR disclosed herein, but still retains an operable RBE, *TRS* and RBE' portion. In some embodiments, the ceDNA vectors do not have an ITR that comprises any sequence selected from SEQ ID NOs: 500-529.

#### **IV. Pharmaceutical Compositions**

**[00155]** The present invention contemplates pharmaceutical compositions and formulations comprising a therapeutic nucleic acid and one or more inhibitors of the immune response (*e.g.*, the innate immune response, *e.g.*, dexamethasone or dexamethasone palmitate) as described herein. In some embodiments, the pharmaceutical composition comprising a therapeutic nucleic acid and one or more inhibitors of the immune response (*e.g.*, the innate immune response) may include a pharmaceutically acceptable excipient or carrier.

**[00156]** The DNA-vectors, *e.g.*, ceDNA vectors 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 the DNA-

vectors disclosed herein and a pharmaceutically acceptable carrier. For example, the TTX-vectors of the invention 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 intraarterial 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 TTX-vector concentration. Sterile injectable solutions can be prepared by incorporating the TTX- 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.

**[00157]** Pharmaceutically active compositions comprising a TTX-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 therein. The composition can also include a pharmaceutically acceptable carrier.

**[00158]** The compositions and vectors provided herein can be used to deliver a transgene for various purposes. In some embodiments, the transgene encodes a protein or functional RNA 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 transgene product. In another example, the transgene encodes a protein or functional RNA that is intended to be used to create an animal model of disease. In some embodiments, the transgene encodes one or more peptides, polypeptides, or proteins, which are useful for the treatment or prevention of disease states in a mammalian subject. The transgene can be transferred (*e.g.*, expressed in) to a patient in a sufficient amount to treat a disease associated with reduced expression, lack of expression or dysfunction of the gene. In some embodiments, the transgene is a gene editing molecule (*e.g.*, nuclease). In certain embodiments, the nuclease is a CRISPR-associated nuclease (Cas nuclease).

**[00159]** Pharmaceutical compositions for therapeutic purposes typically must be sterile and stable under the conditions of manufacture and storage. 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.

**[00160]** In certain circumstances, it will be desirable to deliver a ceDNA composition as disclosed herein in suitably formulated pharmaceutical compositions disclosed herein either subcutaneously, intraoperatively, intranasally, parenterally, intravenously, intramuscularly, intrathecally, systemic administration, or orally, intraperitoneally, or by inhalation.

**[00161]** The technology described herein is directed in general to methods for administering closed-ended DNA vectors to a subject, exemplified particularly by ceDNA vectors. Close-ended DNA vectors include but are not limited to, ceDNA vectors as disclosed herein, and minicircle DNA, dog-bone DNA, dumbbell DNA and the like. In some embodiments, the closed-ended DNA vector is a ceDNA vector, as described herein. In alternative embodiments, the closed-ended DNA vector is,

*e.g.*, a dumbbell DNA vector or a dog-bone DNA vector (see *e.g.*, WO2010/0086626, the contents of which is incorporated by reference herein in its entirety).

**[00162]** In another aspect, pharmaceutical compositions are provided. The pharmaceutical composition comprises a closed-ended DNA vector, *e.g.*, ceDNA vector as described herein and a pharmaceutically acceptable carrier or diluent.

**[00163]** A closed-ended DNA vector, including a ceDNA vector, as described 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, such a closed-ended DNA vector, *e.g.*, a ceDNA vector 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 closed-ended DNA vector, *e.g.*, ceDNA vector concentration. Sterile injectable solutions can be prepared by incorporating the closed-ended DNA vector, *e.g.*, 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.

**[00164]** Pharmaceutically active compositions comprising a closed-ended DNA vector, including a ceDNA vector as described herein can be formulated to deliver a transgene for various purposes to the cell, *e.g.*, cells of a subject.

**[00165]** 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 closed-ended DNA vector, *e.g.* ceDNA vector concentration. Sterile injectable solutions can be prepared by incorporating closed-ended DNA vector, *e.g.*, 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.

**[00166]** A closed-ended DNA vector, including a ceDNA vector, as described herein 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.

#### *Unit Dosage*

**[00167]** According to some embodiments, the pharmaceutical compositions can 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 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 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.

#### **V. Administration and Dosing**

**[00168]** In some aspects, the methods provided herein comprise delivering one or more closed-ended DNA vector, including a ceDNA vector, as described 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) and lipofection reagents are sold commercially (*e.g.*, Transfectam™ and Lipofectin™). Delivery can be to cells (*e.g.*, *in vitro* or *ex vivo* administration) or target tissues (*e.g.*, *in vivo* administration).

**[00169]** According to some embodiments, the inhibitors of the innate immune response and the nucleic acids can be administered to the subject or patient in any combination. For example, one or more inhibitors of the immune response (*e.g.*, dexamethasone / dexamethasone palmitate) may be administered. According to some embodiments, the subject or patient is administered an inhibitor of the immune response (*e.g.*, the innate immune response) as described herein, and the nucleic acids (*e.g.*, minicircle, minigene, ministring covalently closed DNA, doggybone (dbDNA™) DNA, dumbbell shaped DNA, linear closed-ended duplex DNA (ceDNA and CELiD), plasmid based circular vector, antisense oligonucleotide (ASO), RNAi, siRNA, mRNA, etc.).

**[00170]** In some embodiments, a subject may be administered one or more inhibitors of the immune response (*e.g.*, dexamethasone / dexamethasone palmitate) and one or more nucleic acids (*e.g.*, minicircle, minigene, ministring covalently closed DNA, doggybone (dbDNA<sup>TM</sup>) DNA, dumbbell shaped DNA, linear closed-ended duplex DNA (ceDNA and CELiD), plasmid based circular vector, antisense oligonucleotide (ASO), RNAi, siRNA, mRNA, etc.) concomitantly. For example, the method may comprise administering to a subject an inhibitor of the immune response (*e.g.*, dexamethasone / dexamethasone palmitate) and a nucleic acid therapeutic as two separate formulations but concomitantly. In another example, the method may comprise simultaneously administering to a subject an inhibitor of the immune response (*e.g.*, dexamethasone / dexamethasone palmitate) and a therapeutic nucleic acid in one formulation at the same time.

**[00171]** In some embodiment, a subject may be administered one or more inhibitors of the immune response (*e.g.*, innate immune response) and one or more nucleic acids (*e.g.*, minicircle, minigene, ministring covalently closed DNA, doggybone (dbDNA<sup>TM</sup>) DNA, dumbbell shaped DNA, linear closed-ended duplex DNA (ceDNA and CELiD), plasmid based circular vector, antisense oligonucleotide (ASO), RNAi, siRNA, mRNA, etc.) sequentially. For example, the inhibitor of the immune response may be administered prior to administration of a therapeutic nucleic acid.

**[00172]** In cases of sequential administration, there may be a delay period between administration of the one or more inhibitor of the immune response (*e.g.*, dexamethasone / dexamethasone palmitate) and TNAs. For example, the inhibitor of the immune response (*e.g.*, innate immune response, *e.g.*, dexamethasone / dexamethasone palmitate) may be administered hours, days, or weeks prior to administration of the TNA (*e.g.*, at least 30 minutes, at least 1 hour, at least 2 hours, at least 3 hours, at least 4 hours, at least 5 hours, at least 6 hours, at least 7 hours, at least 8 hours, at least 9 hours, at least 10 hours, at least 11 hours, at least 12 hours, at least 13 hours, at least 14 hours, at least 15 hours, at least 16 hours, at least 17 hours, at least 18 hours, at least 19 hours, at least 20 hours, at least 21 hours, at least 22 hours, at least 23 hours, at least 24 hours, at least about 2 days, at least about 3 days, at least about 4 days, at least about 5 days, at least about 6 days, at least about 1 week, at least about 2 weeks, at least about 3 weeks, and at least about 4 weeks prior to the administration of a nucleic acid). In some embodiments, an inhibitor of the immune response (*e.g.*, dexamethasone / dexamethasone palmitate) may be administered about thirty (30) minutes prior to the administration of a TNA. In some embodiments, an inhibitor of the immune response (*e.g.*, innate immune response, *e.g.*, dexamethasone / dexamethasone palmitate) may be administered about one (1) hour prior to the administration of a nucleic acid. In some embodiments, an inhibitor of the immune response (*e.g.*, innate immune response, *e.g.*, dexamethasone / dexamethasone palmitate) can be administered about two (2) hours prior to the administration of a nucleic acid. In some embodiments, an inhibitor of the immune response (*e.g.*, innate immune response, *e.g.*, dexamethasone / dexamethasone palmitate) can be administered about three (3) hours prior to the administration of a nucleic acid. In some embodiments, an inhibitor of the immune response (*e.g.*, innate immune response, *e.g.*,

dexamethasone / dexamethasone palmitate) can be administered about four (4) hours prior to the administration of a nucleic acid. In some embodiments, an inhibitor of the immune response (*e.g.*, innate immune response, *e.g.*, dexamethasone / dexamethasone palmitate) can be administered about five (5) hours prior to the administration of a nucleic acid. In some embodiments, an inhibitor of the immune response (*e.g.*, innate immune response, *e.g.*, dexamethasone / dexamethasone palmitate) can be administered about six (6) hours prior to the administration of a nucleic acid. In some embodiments, an inhibitor of the immune response (*e.g.*, innate immune response, *e.g.*, dexamethasone / dexamethasone palmitate) can be administered about seven (7) hours prior to the administration of a nucleic acid. In some embodiments, an inhibitor of the immune response (*e.g.*, innate immune response, *e.g.*, dexamethasone / dexamethasone palmitate) can be administered about eight (8) hours prior to the administration of a nucleic acid. In some embodiments, an inhibitor of the immune response (*e.g.*, innate immune response, *e.g.*, dexamethasone / dexamethasone palmitate) can be administered about nine (9) hours prior to the administration of a nucleic acid. In some embodiments, an inhibitor of the immune response (*e.g.*, innate immune response) can be administered about ten (10) hours prior to the administration of a nucleic acid.

**[00173]** In one embodiment, an inhibitor of the immune response (*e.g.*, innate immune response, *e.g.*, dexamethasone / dexamethasone palmitate) is administered no more than about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12 hours, about 13 hours, about 14 hours, about 15 hours, about 16 hours, about 17 hours, about 18 hours, about 19 hours, about 20 hours, about 21 hours, about 22 hours, about 23 hours, or 24 hours before the administration of a nucleic acid. In some embodiments, an inhibitor of the immune response (*e.g.*, innate immune response, *e.g.*, dexamethasone / dexamethasone palmitate) can be administered no more than about 1 day, about 2 days, about 3 days, about 4 days, about 6 days, or about 7 days before the administration of a nucleic acid.

**[00174]** In some embodiments, an inhibitor of the immune response (*e.g.*, innate immune response, *e.g.*, dexamethasone / dexamethasone palmitate) can be administered about 30 minutes, about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12 hours, about 13 hours, about 14 hours, about 15 hours, about 16 hours, about 17 hours, about 18 hours, about 19 hours, about 20 hours, about 21 hours, about 22 hours, about 23 hours, or 24 hours after the administration of a nucleic acid. In some embodiments, an inhibitor of the immune response (*e.g.*, dexamethasone or dexamethasone palmitate) can be administered about 1 day, about 2 days, about 3 days, about 4 days, about 6 days, or about 7 days after the administration of a nucleic acid.

**[00175]** In one embodiment, an inhibitor of the immune response (*e.g.*, innate immune response, *e.g.*, dexamethasone / dexamethasone palmitate) is administered no more than about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about

9 hours, about 10 hours, about 11 hours, about 12 hours, about 13 hours, about 14 hours, about 15 hours, about 16 hours, about 17 hours, about 18 hours, about 19 hours, about 20 hours, about 21 hours, about 22 hours, about 23 hours, or 24 hours after the administration of a nucleic acid. In some embodiments, an inhibitor of the immune response (*e.g.*, innate immune response, *e.g.*, dexamethasone / dexamethasone palmitate) can be administered no more than about 1 day, about 2 days, about 3 days, about 4 days, about 6 days, or about 7 days after the administration of a nucleic acid.

**[00176]** In some embodiments, one or more inhibitor of the immune response (*e.g.*, innate immune response, *e.g.*, dexamethasone / dexamethasone palmitate) can be administered multiple times before, concurrently with, and/or after the administration of a nucleic acid.

**[00177]** In some embodiments, a nucleic acid (*e.g.*, a ceDNA vector) can be administered as a single dose or as multiple doses. According to some embodiments, more than one dose can be administered to a subject. Multiple doses can be administered as needed, because the ceDNA vector does not elicit an anti-capsid host immune response due to the absence of a viral capsid. According to some embodiments the number of doses administered can, for example, be between 2-10 or more doses, for example 2, 3, 4, 5, 6, 7, 8, 9, 10 or more.

**[00178]** In some embodiments, a nucleic acid can be administered and re-dosed multiple times in conjunction with one or more inhibitors of the immune response (*e.g.*, innate immune response, *e.g.*, dexamethasone / dexamethasone palmitate) disclosed herein. For example, the therapeutic nucleic acid can be administered on day 0 with one or more inhibitors of the immune response that is administered before, after or at the same time with the administration the nucleic acid in a first dosing regimen. Following the initial treatment at day 0, a second dosing (re-dose) can be performed in about 1 week, about 2 weeks, about 3 weeks, about 4 weeks, about 5 weeks, about 6 weeks, about 7 weeks, about 8 weeks, or about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, or about 1 year, about 2 years, about 3 years, about 4 years, about 5 years, about 6 years, about 7 years, about 8 years, about 9 years, about 10 years, about 11 years, about 12 years, about 13 years, about 14 years, about 15 years, about 16 years, about 17 years, about 18 years, about 19 years, about 20 years, about 21 years, about 22 years, about 23 years, about 24 years, about 25 years, about 26 years, about 27 years, about 28 years, about 29 years, about 30 years, about 31 years, about 32 years, about 33 years, about 34 years, about 35 years, about 36 years, about 37 years, about 38 years, about 39 years, about 40 years, about 41 years, about 42 years, about 43 years, about 44 years, about 45 years, about 46 years, about 47 years, about 48 years, about 49 years or about 50 years after the initial treatment with the nucleic acid, preferably with one or more inhibitors of the immune response (*e.g.*, innate immune response, *e.g.*, dexamethasone / dexamethasone palmitate) disclosed herein.

**[00179]** According to some embodiments, re-dosing of the nucleic acid results in an increase in expression of the nucleic acid. According to some embodiments, the increase of expression of the

nucleic acid after re-dosing, compared to the expression of the nucleic acid after the first dose is about 0.5-fold to about 10-fold, about 1-fold to about 5-fold, about 1-fold to about 2-fold, or about 0.5-fold, about 1-fold, about 2-fold, about 3-fold, about 4-fold, about 5-fold, about 6-fold, about 7-fold, about 8-fold, about 9-fold or about 10-fold higher after re-dosing of the nucleic acid.

**[00180]** According to some embodiments, more than one administration (*e.g.*, two, three, four or more administrations) of a nucleic acid (*e.g.*, a ceDNA vector) for expression of a protein as disclosed herein may be employed to achieve a desired level of gene expression over a period of various intervals, *e.g.*, daily, weekly, monthly, yearly, etc.

**[00181]** According to any of the embodiments disclosed herein, the nucleic acid may be a therapeutic nucleic acid.

**[00182]** Generally, the dosage will vary with the particular characteristics of the ceDNA vector, expression efficiency and with the age, condition, and sex of the patient. The dosage can be determined by one of skill in the art and, unlike traditional AAV vectors, can also be adjusted by the individual physician in the event of any complication because ceDNA vectors do not comprise immune activating capsid proteins that prevent repeat dosing.

**[00183]** Various techniques and methods are known in the art for delivering nucleic acids to cells. For example, a closed-ended DNA vector, including a ceDNA vector, as described herein 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).

**[00184]** Another method for delivering a closed-ended DNA vector, including a ceDNA vector, as described herein 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.

**[00185]** Nucleic acids and closed-ended DNA vector, including a ceDNA vector, as described herein 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®), OLIGOFECTION™ (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.

**[00186]** A closed-ended DNA vector, including a ceDNA vector, 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.

**[00187]** Methods for introduction of a closed-ended DNA vector, including a ceDNA vector, as described 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, incorporated by reference in its entirety herein.

**[00188]** A closed-ended DNA vector, including a ceDNA vector, as described herein 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 typically 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 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”, the contents of each of which are incorporated by reference in their entireties herein.

**[00189]** Various delivery methods known in the art or modifications thereof can be used to deliver a closed-ended DNA vector, including a ceDNA vector, as described herein *in vitro* or *in vivo*. For example, in some embodiments, ceDNA vectors 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 skin, thymus, cardiac muscle, skeletal muscle, or liver cells. In some cases, a ceDNA vector is delivered by gene gun. Gold or tungsten spherical particles (1–3  $\mu\text{m}$  diameter) coated with capsid-free AAV vectors can be accelerated to high speed by pressurized gas to penetrate into target tissue cells.

**[00190]** Compositions comprising a closed-ended DNA vector, including a ceDNA vector, as described herein 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.

**[00191]** In some cases, a closed-ended DNA vector, including a ceDNA vector as described herein 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.

**[00192]** In some cases, a closed-ended DNA vector, including a ceDNA vector, as described herein is 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 the closed-ended DNA vector have a great role in efficiency of the system. In some cases, closed-ended DNA vectors, including a ceDNA vector, as described herein are delivered by magnetofection by using magnetic fields to concentrate particles containing nucleic acid into the target cells.

**[00193]** 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:*

**[00194]** In some embodiments, a closed-ended DNA vector, including a ceDNA vector, as described 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 invention.

*B. Microparticle/Nanoparticles:*

**[00195]** In some embodiments, a closed-ended DNA vector, including a ceDNA vector and/or an immunosuppressant, as described 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.

**[00196]** 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 other embodiments, the lipid particles comprising a therapeutic nucleic acid and/or an immunosuppressant typically have a mean diameter of from about 20 nm to about 100 nm, 30 nm to about 150 nm, from about 40 nm to about 150 nm, from about 50 nm to about 150 nm, from about 60 nm to about 130 nm, from about 70 nm to about 110 nm, from about 70 nm to about 100 nm, from about 80 nm to about 100 nm, from about 90 nm to about 100 nm, from about 70 to about 90 nm, from about 80 nm to about 90 nm, from about 70 nm to about 80 nm, or about 30 nm, 35 nm, 40 nm, 45 nm, 50 nm, 55 nm, 60 nm, 65 nm, 70 nm, 75 nm, 80 nm, 85 nm, 90 nm, 95 nm, 100 nm, 105 nm, 110 nm, 115 nm, 120 nm, 125 nm, 130 nm, 135 nm, 140 nm, 145 nm, or 150 nm to ensure effective delivery. Nucleic acid containing lipid particles and their method of preparation are disclosed in, *e.g.*, PCT/US18/50042,

U.S. Patent Publication Nos. 20040142025 and 20070042031, the disclosures of which are herein incorporated by reference in their entirety for all purposes. 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.

**[00197]** According to some embodiments, a liquid pharmaceutical composition comprising a nucleic acid (*e.g.*, a therapeutic nucleic acid, a nucleic acid used for research purposes) and/or inhibitor of the immune response (*e.g.*, innate immune response) of the present invention may be formulated in lipid particles. In some embodiments, the lipid particle comprising a nucleic acid can be formed from a cationic lipid. In some other embodiments, the lipid particle comprising a nucleic acid can be formed from non-cationic lipid. In a preferred embodiment, the lipid particle of the invention is a nucleic acid containing lipid particle, which is formed from a cationic lipid comprising a nucleic acid selected from the group consisting of mRNA, antisense RNA and oligonucleotide, ribozymes, aptamer, interfering RNAs (RNAi), Dicer-substrate dsRNA, small hairpin RNA (shRNA), asymmetrical interfering RNA (aiRNA), microRNA (miRNA), 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”).

**[00198]** Various lipid nanoparticles known in the art can be used to deliver a closed-ended DNA vector, including a ceDNA vector as described 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.

**[00199]** In some embodiments, a closed-ended DNA vector, including a ceDNA vector and/or inhibitor of the immune response (*e.g.*, innate immune response), as described 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, the contents of which is incorporated by reference in its entirety herein.

### C. Conjugates

**[00200]** In some embodiments, a closed-ended DNA vector, including a ceDNA vector and/or inhibitor of the immune response (*e.g.*, innate immune response), as described herein 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.

**[00201]** In some embodiments, a closed-ended DNA vector, including a ceDNA vector and/or inhibitor of the immune response (*e.g.*, innate immune response), as described herein 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, incorporated by reference in its entirety herein. In some embodiments, a ceDNA vector as disclosed herein is conjugated to a poly(amide) polymer, for example as described by U.S. Patent No. 8,987,377, incorporated by reference in its entirety herein. 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, incorporated by reference in its entirety herein.

**[00202]** In some embodiments, a closed-ended DNA vector, including a ceDNA vector and/or inhibitor of the immune response (*e.g.*, innate immune response), as described herein as disclosed herein is conjugated to a carbohydrate, for example as described in U.S. Patent No. 8,450,467, the contents of which is incorporated by reference in its entirety herein.

**[00203]** In some embodiments, the lipid nanoparticles may be conjugated with other moieties to prevent aggregation. Such lipid conjugates include, but are not limited to, PEG-lipid conjugates such as, *e.g.*, PEG coupled to dialkylxypropyls (*e.g.*, PEG-DAA conjugates), PEG coupled to diacylglycerols (*e.g.*, PEG-DAG conjugates), PEG coupled to cholesterol, PEG coupled to phosphatidylethanolamines, and PEG conjugated to ceramides (see, *e.g.*, U.S. Pat. No. 5,885,613), cationic PEG lipids, polyoxazoline (POZ)-lipid conjugates (*e.g.*, POZ-DAA conjugates; see, *e.g.*, U.S. Provisional Application No. 61/294,828, filed Jan. 13, 2010, and U.S. Provisional Application No. 61/295,140, filed Jan. 14, 2010), polyamide oligomers (*e.g.*, ATTA-lipid conjugates), and mixtures thereof. Additional examples of POZ-lipid conjugates are described in PCT Publication No. WO 2010/006282, the contents of which is incorporated by reference in its entirety herein. PEG or POZ can be conjugated directly to the lipid or may be linked to the lipid via a linker moiety. Any linker moiety suitable for coupling the PEG or the POZ to a lipid can be used including, *e.g.*, non-ester containing linker moieties and ester-containing linker moieties. In certain preferred embodiments, non-ester containing linker moieties, such as amides or carbamates, are used. The disclosures of each of the above patent documents are herein incorporated by reference in their entirety for all purposes.

#### *D. Nanocapsule*

**[00204]** Alternatively, nanocapsule formulations of a closed-ended DNA vector, including a ceDNA vector and/or inhibitor of the immune response (*e.g.*, innate immune response), as described herein 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  $\mu\text{m}$ ) 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*

**[00205]** A closed-ended DNA vector, including a ceDNA vector and/or inhibitor of the immune response (*e.g.*, innate immune response), as described herein 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 typically 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.

**[00206]** The formation and use of liposomes is 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, the contents of each of which are incorporated by reference in its entirety herein).

#### *F. Exemplary liposome and Lipid Nanoparticle (LNP) Compositions*

**[00207]** A closed-ended DNA vector, including a ceDNA vector and/or inhibitor of the immune response (*e.g.*, innate immune response), as described herein 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 typically 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.

**[00208]** Lipid nanoparticles (LNPs) comprising ceDNA 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 each incorporated herein by reference in their entirety and envisioned for use in the methods and compositions as disclosed herein.

**[00209]** 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.

**[00210]** 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.

**[00211]** In some aspects, the liposome formulation comprises sphingomyelin and one or more lipids disclosed herein. In some aspects, the liposome formulation comprises optisomes.

**[00212]** 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 (palmitoyl-oleoylphosphatidylcholine); SM (sphingomyelin); MPEG (methoxy polyethylene glycol); DMPC (dimyristoyl phosphatidylcholine); DMPG (dimyristoyl phosphatidylglycerol); DSPG (distearoylphosphatidylglycerol); DEPC (dierucoylphosphatidylcholine); DOPE (dioleoyl-sn-glycero-phosphoethanolamine). cholesteryl sulphate (CS), dipalmitoylphosphatidylglycerol (DPPG), DOPC (dioleoyl-sn-glycero-phosphatidylcholine) or any combination thereof.

**[00213]** 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.

**[00214]** 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.

**[00215]** In some aspects, the disclosure provides for a liposome formulation further comprising one or more pharmaceutical excipients, *e.g.* sucrose and/or glycine.

**[00216]** 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.

**[00217]** 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.

**[00218]** In some aspects, the disclosure provides for a lipid nanoparticle comprising a DNA vector, including a ceDNA vector as described herein and/or inhibitor of the immune response (*e.g.*, innate immune response) 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 by reference in its entirety 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.

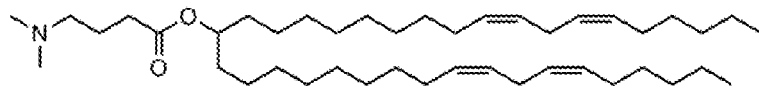
**[00219]** Generally, the lipid particles are prepared at a total lipid to ceDNA (mass or weight) ratio of from about 10:1 to 30:1. In some embodiments, the lipid to ceDNA ratio (mass/mass ratio; w/w ratio) can be in the range of from about 1:1 to about 25:1, from about 10:1 to about 14:1, from about 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. According to some embodiments of any of the aspects or embodiments herein, the composition has a total lipid to ceDNA ratio of about 15:1. According to some embodiments of any of the aspects or embodiments herein, the composition has a total lipid to ceDNA ratio of about 30:1. According to some embodiments of any of the aspects or embodiments herein, the composition has a total lipid to ceDNA ratio of about 40:1. According to some embodiments of any of the aspects or embodiments herein, the composition has a total lipid to ceDNA ratio of about 50:1. 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

**[00220]** 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.

**[00221]** 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 entirety.

[00222] 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")

[00223] 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.

[00224] 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.

[00225] In some embodiments, the ionizable lipid is (13Z,16Z)-N,N-dimethyl-3-nonyldocosa-13,16-dien-1-amine (Compound 32), as described in WO2012/040184, content of which is incorporated herein by reference in its entirety.

[00226] In some embodiments, the ionizable lipid is Compound 6 or Compound 22 as described in WO2015/199952, content of which is incorporated herein by reference in its entirety.

[00227] 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.

[00228] 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.

[00229] Exemplary non-cationic lipids envisioned for use in the methods and compositions comprising a DNA vector, including a ceDNA vector as described 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.

[00230] 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.

[00231] 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.

**[00232]** 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.

**[00233]** 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, contents of both of which are incorporated herein by reference in their entirety.

**[00234]** 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.

**[00235]** 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 1-(monomethoxy-polyethyleneglycol)-2,3-dimyristoylglycerol (PEG-DMG)), PEG-dialkylxypropyl (DAA), PEG-phospholipid, PEG-ceramide (Cer), a PEGylated phosphatidylethanolamine (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 entirety.

**[00236]** In some embodiments, a PEG-lipid is a compound disclosed in US2018/0028664, the content of which is incorporated herein by reference in its entirety.

**[00237]** 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.

**[00238]** The PEG-DAA conjugate can be, for example, PEG-dilauryloxypropyl, PEG-dimyristyloxypropyl, PEG-dipalmitoyloxypropyl, or PEG-distearoyloxypropyl. The PEG-lipid can be one or more of PEG-DMG, PEG-dilaurylglycerol, PEG-dipalmitoylglycerol, PEG-disteryl glycerol, 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-Ditetradecoxybenzyl-[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].

**[00239]** 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 entirety.

**[00240]** 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 cancer, the additional compound can be an anti-cancer agent (*e.g.*, a chemotherapeutic agent, a targeted cancer therapy (including, but not limited to, a small molecule, an antibody, or an antibody-drug conjugate). 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 invention.

[00241] 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.

[00242] Also provided herein is a pharmaceutical composition comprising the lipid nanoparticle-encapsulated ceDNA vector and a pharmaceutically acceptable carrier or excipient. In some embodiments, the pharmaceutical composition can further comprise a modified dexamethasone compound as disclosed herein. In alternative embodiments, a pharmaceutical composition comprising a lipid nanoparticle encapsulated ceDNA vector and a pharmaceutical acceptable carrier or excipient is co-administered to the subject with a pharmaceutical composition comprising a modified dexamethasone compound. In alternative embodiments, a pharmaceutical composition comprising a lipid nanoparticle encapsulated ceDNA vector and a pharmaceutical acceptable carrier or excipient comprises a modified dexamethasone compound, as described herein.

[00243] 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.

[00244] A closed-ended DNA vector, including a ceDNA vector, as described herein can be complexed with the lipid portion of the particle or encapsulated in the lipid position of the lipid nanoparticle. In some embodiments, a DNA vector, including a ceDNA vector as described herein 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, a DNA vector, including a ceDNA vector as described herein 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.

[00245] 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.

[00246] 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.

[00247] 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.

[00248] 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.

[00249] 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-naphthalene sulfonic acid (TNS).

## **VI. Methods and Compositions Comprising ceDNAs and Modified Dexamethasone Compounds**

[00250] Dexamethasone has long been known as an immunosuppressant, like other glucocorticoids. It has been approved for the treatment of osteoarthritis, bursitis, tendonitis, rheumatoid arthritis flares, epicondylitis, tenosynovitis, and gouty arthritis. Dexamethasone interacts with DUSP1 in the inhibition of proinflammatory signaling pathways, leading to the suppression of several proinflammatory genes including tumor necrosis factor, cyclooxygenase 2, NFκB and interleukin 1α and 1β (Chang *et al.*, *J. Surg. Res.* (1997) 72(2): 141-5; Abraham *et al.*, *J. Exp. Med.* (2006) 203(8): 1883-1889). While dexamethasone does not directly interact with the cGAS/STING, TLR9 or inflammasome pathways, it does inhibit the inflammatory mediators stimulated by many of those pathways. Dexamethasone, however, is difficult to encapsulate in LNP for co-delivery with ceDNA. One solution to this is to derivatize dexamethasone to increase its hydrophobicity, such as by attaching one or more fatty acid chains. In one embodiment, dexamethasone palmitate is used.

## **VII. Therapeutic Effect**

[00251] The efficacy of a ceDNA vector as described herein, or a ceDNA vector administered with an additional immunosuppressant (*e.g.* dexamethasone or dexamethasone palmitate), for suppressing or reducing the innate immune system, can be determined by the skilled clinician. According to some embodiments, a treatment is considered "effective treatment," as the term is used herein, if any one or all of the signs or symptoms of the innate immune system are reduced and/or 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% after treatment with a ceDNA vector encoding an inhibitor of the

immune response (*e.g.*, the innate immune response), as disclosed herein. Exemplary markers and symptoms are discussed in the Examples herein. 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.

**[00252]** Efficacy of an agent can be determined by assessing physical indicators that are particular to a given disease. Standard methods of analysis of disease indicators are known in the art. For example, physical indicators for the innate immune system include for example, without limitation, soluble CD14 (sCD14) and IL-18, IL-22, in the plasma or blood, inflammasome proteins, such as AIM2, NLRP3, NLRP1, ASC, and caspase-1 in the CSF or blood, activation of cytokine pathways can be used as functional readout of activation of the NLRP3 and/or AIM2 inflammasome pathway, or a caspase 1 activation, and include biomarkers such as, but not limited to: interleukin (IL)-1 $\beta$ , IL-6, IL-8, IL-18, interferon (IFN)- $\gamma$ , interferon (IFN)- $\alpha$ , monocyte chemoattractant protein (MCP)-1, and/or tumor necrosis factor (TNF)- $\alpha$ .

**[00253]** 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 invention 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.

**[00254]** 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.

**[00255]** 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.

**[00256]** 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 invention 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 invention, which is defined solely by the claims.

## EXAMPLES

**[00257]** 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**

**[00258]** 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 invention 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.

[00259] An exemplary method to produce ceDNA vectors is from a ceDNA-plasmid as described herein. Referring to **FIG. 1A and 1B**, the polynucleotide construct template of each of the ceDNA-plasmids includes both a left modified ITR and a right modified ITR with the following between the ITR sequences: (i) an enhancer/promoter; (ii) a cloning site for a transgene; (iii) a posttranscriptional response element (*e.g.* the woodchuck hepatitis virus posttranscriptional regulatory element (WPRE)); and (iv) a poly-adenylation signal (*e.g.* from bovine growth hormone gene (BGHpA). Unique restriction endonuclease recognition sites (R1-R6) (shown in **FIG. 1A** and **FIG. 1B**) were also introduced between each component to facilitate the introduction of new genetic components into the specific sites in the construct. R3 (PmeI) GTTTAAAC (SEQ ID NO: 123) and R4 (PacI) TTAATTAA (SEQ ID NO: 124) enzyme sites are engineered into the cloning site to introduce an open reading frame of a transgene. These sequences were cloned into a pFastBac HT B plasmid obtained from ThermoFisher Scientific.

[00260] Production of ceDNA-bacmids:

[00261] 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* ( $\Phi$ 80dlacZ $\Delta$ M15 marker provides  $\alpha$ -complementation of the  $\beta$ -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  $\beta$ -galactoside indicator gene were picked and cultured in 10 ml of media.

[00262] 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  $\mu$ m filter, separating the infectious baculovirus particles from cells or cell debris.

[00263] 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  $\mu$ m (from a naïve diameter of 14-15  $\mu$ m), and a density of  $\sim 4.0 \times 10^6$  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  $\mu$ m filter.

[00264] 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  $2.5 \times 10^6$  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.

**[00265]** 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 (SEQ ID NO: 131 or 133) and Rep52 (SEQ ID NO: 132) or Rep68 (SEQ ID NO: 130) and Rep40 (SEQ ID NO: 129). 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* ( $\Phi$ 80dlacZ $\Delta$ M15 marker provides  $\alpha$ -complementation of the  $\beta$ -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.

**[00266]** 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  $2.5 \times 10^6$  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.

**[00267]** ceDNA vector generation and characterization

**[00268]** With reference to **FIG. 4B**, 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 ( $2.5 \times 10^6$  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).

[00269] Yields of ceDNA vectors produced and purified from the Sf9 insect cells were initially determined based on UV absorbance at 260nm. The purified ceDNA vectors can be assessed for proper closed-ended configuration using the electrophoretic methodology described in Example 5.

[00270] 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 invention 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.

#### **EXAMPLE 2: Synthetic ceDNA production via excision from a double-stranded DNA molecule**

[00271] 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, incorporated by reference in its entirety herein).

[00272] In some embodiments, a construct to make a ceDNA vector comprises a regulatory switch as described herein.

[00273] 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.*, 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”.

**[00274]** 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.

**[00275]** 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.

**[00276]** 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.

**[00277]** 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**

**[00278]** 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.

**[00279]** As disclosed herein, the ITR oligonucleotides can comprise WT-ITRs (*e.g.*, see **FIG. 3A**, **FIG. 3C**), or modified ITRs (*e.g.*, see, **FIG. 3B** and **FIG. 3D**). (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, incorporated by reference in its entirety herein). 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**

**[00280]** Another exemplary method of producing a ceDNA vector using a synthetic method is provided in Example 4 of PCT/US19/14122, incorporated by reference in its entirety herein, 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.

**[00281]** 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.

**[00282]** 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.

**[00283]** 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.

**[00284]** 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**

[00285] 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,

[00286] The following is an exemplary method for confirming the identity of ceDNA vectors.

[00287] ceDNA vectors can be assessed by identified by agarose gel electrophoresis under native or denaturing conditions as illustrated in **FIG. 4D**, 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.

[00288] 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. 4C and 4D**, 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.

[00289] 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. 4E**).

[00290] 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 closed-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 ILUSTRATE™ 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.

**[00291]** 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 0.25μg 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: Expression and Host Response in CD-1 Mice**

**[00292]** *In vivo* experiments in mice were performed to assess the ability of dexamethasone palmitate to enhance the targeting of ceDNA to the liver and to decrease the innate immune response to the ceDNA vector itself.

**[00293]** A ceDNA vector encoding luciferase as the transgene (*e.g.*, SEQ ID NO: 56), with a wild-type AAV2 left ITR and a mutant right ITR and a hAAT promoter, methylated to eliminate free CpG, was used. The ceDNA vector was prepared as described above. LNP-encapsulated ceDNA vector samples were co-administered with either polyC or dexamethasone palmitate and intravenously administered via tail vein injection to ~ 4 week old male CD-1 mice at a dose level of 0.5 mg/kg in a volume of up to 5 mL/kg. Four replicates were included in each sample group. Body weights were recorded on days 0, 1, 2, 3, 7, 14, 21 and 28. In-life imaging was performed on days 4, 7, 14, 21, and 28 using an *in vivo* imaging system (IVIS). For the imaging, each mouse was injected with luciferin at 150 mg/kg via intraperitoneal injection at 2.5 mL/kg. After 15 minutes, each mouse was anaesthetized and imaged.

**[00294]** While body weight loss was observed in both groups, it was significantly attenuated in the dexamethasone palmitate group relative to the polyC group (Figure 10A). This finding correlated with the cytokine analysis results, which showed that ceDNA co-administered with dexamethasone palmitate treatment resulted in significantly less induction of IL-6, TNF-alpha and RANTES than with the ceDNA treatment alone (FIG. 6C). Equivalent levels of total flux (representative of luciferase expression from the introduced ceDNA vectors) were observed in both groups of ceDNA-administered animals at the day 4 and day 7 timepoints (FIG. 6B), indicating that the dexamethasone palmitate treatment has no impact on transgene expression itself. Dexamethasone palmitate treatment provides an avenue to protect the treated organism from the effects of inadvertently triggering one or more innate immune pathways, and to thus avoid unwanted immune reactions with concomitant negative side effects.

**[00295]** All references cited herein and throughout the specification and Examples are incorporated herein in their entirety by reference.

## CLAIMS

### What is claimed is:

1. A composition comprising (i) a linear, capsid-free DNA vector with covalently-closed ends (ceDNA vector), wherein the ceDNA vector comprises a heterologous nucleic acid sequence encoding a transgene operably positioned between two AAV inverted terminal repeat sequences (ITRs), and (ii) a modified dexamethasone compound.
2. The composition of claim 1, wherein the ceDNA vector when digested with a restriction enzyme having a single recognition site on the ceDNA vector has the presence of characteristic bands of linear and continuous DNA as compared to linear and non-continuous DNA controls when analyzed on a non-denaturing gel.
3. The composition of claim 1, wherein at least one of the ITRs comprises a functional AAV terminal resolution site (TRS) and a Rep binding site.
4. The composition of claim 1, wherein both ITRs are naturally occurring AAV ITRs from the same AAV strain.
5. The composition of claim 1, wherein one ITR comprises a deletion, insertion, or substitution relative to the other ITR.
6. The composition of claim 1, wherein one ITR comprises a deletion, insertion, or substitution relative to the other ITR and neither ITR is a naturally occurring AAV ITR.
7. The composition of any of claims 1-6, wherein the modified dexamethasone compound is dexamethasone palmitate.
8. The composition of any of claims 1-6, wherein the modified dexamethasone compound is co-encapsulated with the ceDNA vector.
9. The composition of any of claims 1-6, wherein the modified dexamethasone compound is not co-encapsulated with the ceDNA vector.
10. The composition of any of claims 1-9, wherein the composition further comprises at least one additional innate immune pathway inhibitor.
11. The composition of claim 10, wherein the at least one additional innate immune inhibitor is an inhibitor of one or more of the cGAS/STING pathway, the TLR9 pathway, or an inflammasome-mediated pathway.
12. A method for inhibiting an immune response when expressing a transgene in a cell, the method comprising: administering to a cell a composition comprising (i) a linear, capsid-free DNA vector with covalently-closed ends (ceDNA vector), wherein the ceDNA vector comprises a heterologous nucleic acid sequence encoding a transgene operably positioned between two AAV inverted terminal repeat sequences (ITRs), and (ii) a modified dexamethasone compound.

13. The method of claim 12, wherein one of the ITRs comprises a functional AAV terminal resolution site and a Rep binding site, and one of the ITRs comprises a deletion, insertion, or substitution relative to the other ITR.
14. The method of claim 12, wherein the ceDNA when digested with a restriction enzyme having a single recognition site on the ceDNA vector has the presence of characteristic bands of linear and continuous DNA as compared to linear and non-continuous DNA controls when analyzed on a non-denaturing gel.
15. The method of claim 12, wherein the modified dexamethasone compound is co-encapsulated with the ceDNA vector being administered to the cell.
16. The method of claim 12, wherein the modified dexamethasone compound is co-administered with the ceDNA vector being administered to the cell but is not co-encapsulated with the ceDNA vector.
17. The method of claim 16, wherein the modified dexamethasone compound is administered prior to, at the same time as, or after the administration of the ceDNA vector.
18. The method of any of claims 12-17, wherein the modified dexamethasone compound is dexamethasone palmitate.
19. The method of claim 12, wherein both ITRs are naturally occurring AAV ITRs from the same AAV strain.
20. The method of claim 12, wherein one ITR comprises a deletion, insertion, or substitution relative to the other ITR.
21. The method of claim 12, wherein one ITR comprises a deletion, insertion or substitution relative to the other ITR and neither ITR is a naturally occurring AAV ITR.
22. The method of claim 20, wherein the two ITRs are a pair of ITRs selected from the group consisting of:
  - a. SEQ ID NO: 1 and SEQ ID NO: 4; and
  - b. SEQ ID NO: 3 and SEQ ID NO: 2.
23. The method of any one of claims 12-22, wherein the ceDNA vector is administered in combination with a pharmaceutically acceptable carrier.
24. The method of claim 12, wherein increasing the amount of the ceDNA vector in the cell increases expression of the transgene in the cell.
25. The method of claim 12, wherein the heterologous nucleic acid sequence encodes a therapeutic transgene and the desired level of expression of the transgene is a therapeutically effective amount.
26. The method of claim 12, wherein at least one additional innate immune inhibitor is co-administered with the ceDNA vector and the modified dexamethasone compound.

27. The method of claim 26, wherein the at least one additional innate immune inhibitor is an inhibitor of one or more of the cGAS/STING pathway, the TLR9 pathway, or an inflammasome-mediated pathway.
28. The method of any one of claims 12-27, wherein the ceDNA vector is obtained from a process comprising the steps of:
  - a. incubating a population of insect cells harboring a ceDNA vector polynucleotide, which is devoid of viral capsid coding sequences in the presence of Rep protein under conditions effective and for time sufficient to induce production of the closed-ended linear, capsid-free, DNA vector within the insect cells, wherein the insect cells do not comprise production of closed-ended linear, capsid-free, DNA within the insect cells; and
  - b. harvesting and isolating the closed-ended linear capsid-free, DNA from the insect cells.
29. The method of claim 28, wherein the presence of the linear, capsid-free, DNA isolated from the insect cells can be confirmed by digesting DNA isolated from the insect cells with a restriction enzyme having a single recognition site on the DNA 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.
30. The method of any one of claims 12-27, wherein the ceDNA vector is obtained by cell-free synthesis.
31. The method of any of claims 12-30, wherein the ceDNA vector is encapsulated.
32. A method for treating a disease in a subject, the method comprising:  
administering to a subject in need thereof a composition comprising (i) a linear, capsid-free DNA vector with covalently-closed ends (ceDNA vector), wherein the ceDNA vector comprises a heterologous nucleic acid sequence encoding a transgene operably positioned between two AAV inverted terminal repeat sequences (ITRs), and (ii) a modified dexamethasone compound.
33. The method of claim 32, wherein one of the ITRs comprises a functional AAV terminal resolution site and a Rep binding site, and one of the ITRs comprises a deletion, insertion, or substitution relative to the other ITR.
34. The method of claim 32, wherein the modified dexamethasone compound is co-encapsulated with the ceDNA vector being administered to the cell.
35. The method of claim 32, wherein the modified dexamethasone compound is co-administered with the ceDNA vector being administered to the cell but is not co-encapsulated with the ceDNA vector.
36. The method of claim 32, wherein the modified dexamethasone compound is administered prior to, at the same time as, or after the administration of the ceDNA vector.

37. The method of any of claims 32-36, wherein the modified dexamethasone compound is dexamethasone palmitate.
38. The method of claim 32 wherein both ITRs are naturally occurring AAV ITRs from the same AAV strain.
39. The method of claim 32, wherein one ITR comprises a deletion, insertion, or substitution relative to the other ITR.
40. The method of claim 32, wherein one ITR comprises a deletion, insertion or substitution relative to the other ITR and neither ITR is a naturally occurring AAV ITR.
41. The method of claim 27, wherein the two ITRs are a pair of ITRs selected from the group consisting of:
  - a. SEQ ID NO: 1 and SEQ ID NO: 4; and
  - b. SEQ ID NO: 3 and SEQ ID NO: 2.
42. The method of any one of claims 32-41, wherein the ceDNA vector is administered in combination with a pharmaceutically acceptable carrier.
43. The method of claim 32, wherein increasing the amount of the ceDNA vector in the cell increases expression of the transgene in the cell.
44. The method of claim 32, wherein the heterologous nucleic acid sequence encodes a therapeutic transgene and the desired level of expression of the transgene is a therapeutically effective amount.
45. The method of claim 32, wherein at least one additional innate immune inhibitor is co-administered with the ceDNA vector and the modified dexamethasone compound.
46. The method of claim 45, wherein the at least one additional innate immune inhibitor is an inhibitor of one or more of the cGAS/STING pathway, the TLR9 pathway, or an inflammasome-mediated pathway.
47. The method of any one of claims 32-46, wherein the ceDNA vector is obtained from a process comprising the steps of:
  - a. incubating a population of insect cells harboring a ceDNA vector polynucleotide, which is devoid of viral capsid coding sequences in the presence of Rep protein under conditions effective and for time sufficient to induce production of the closed-ended, linear, capsid-free DNA vector within the insect cells, wherein the insect cells do not comprise production of closed-ended linear, capsid-free DNA within the insect cells; and
  - b. harvesting and isolating the closed-ended linear, capsid-free DNA from the insect cells.
48. The method of claim 47, wherein the presence of the closed ended linear, capsid-free, non-viral DNA isolated from the insect cells can be confirmed by digesting DNA isolated from the insect cells with a restriction enzyme having a single recognition site on the DNA 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.

49. The method of any of claims 32-47, wherein the ceDNA vector is obtained by cell-free synthesis.
50. The method of any of claims 32-49, wherein the ceDNA vector is encapsulated.

FIG. 1A

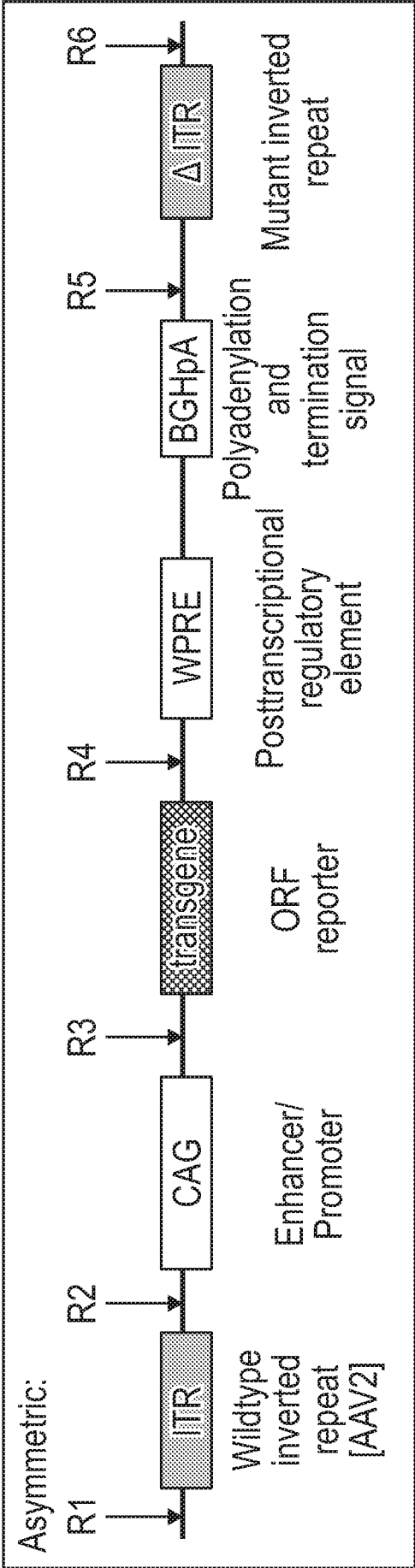


FIG. 1B

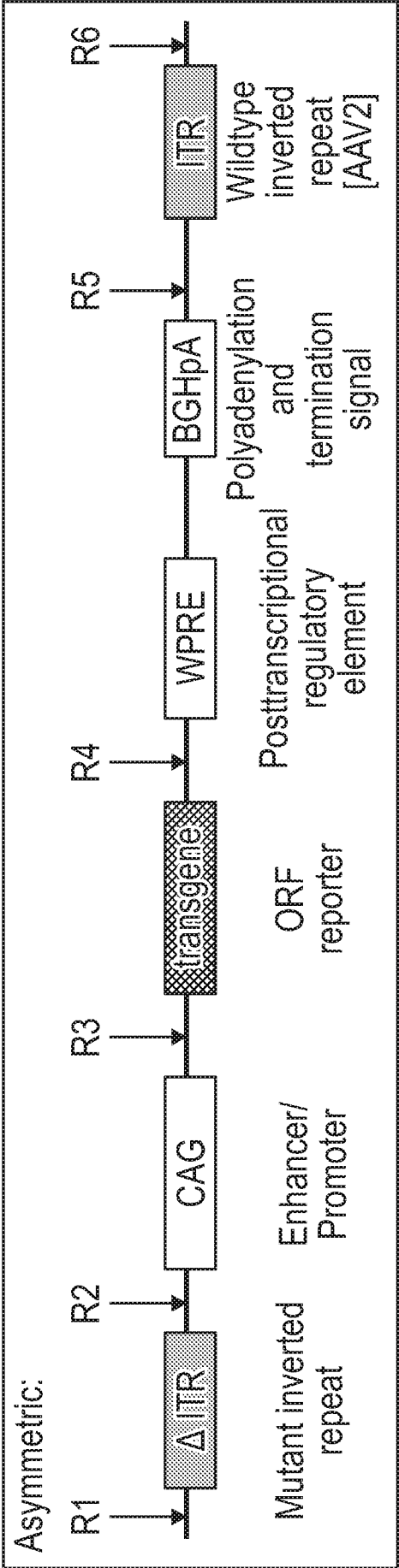


FIG. 1C

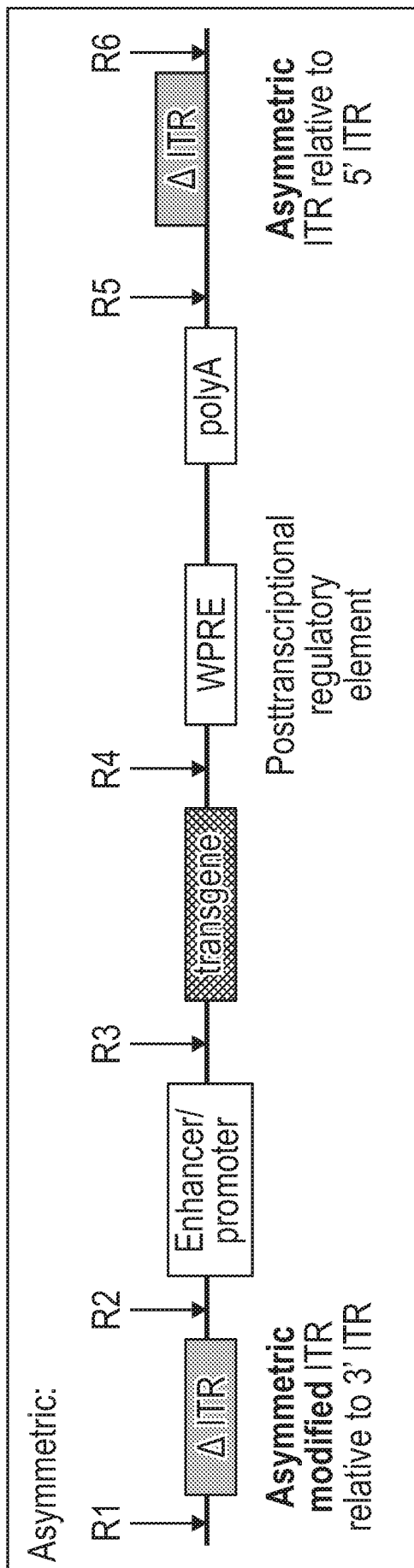


FIG. 1D

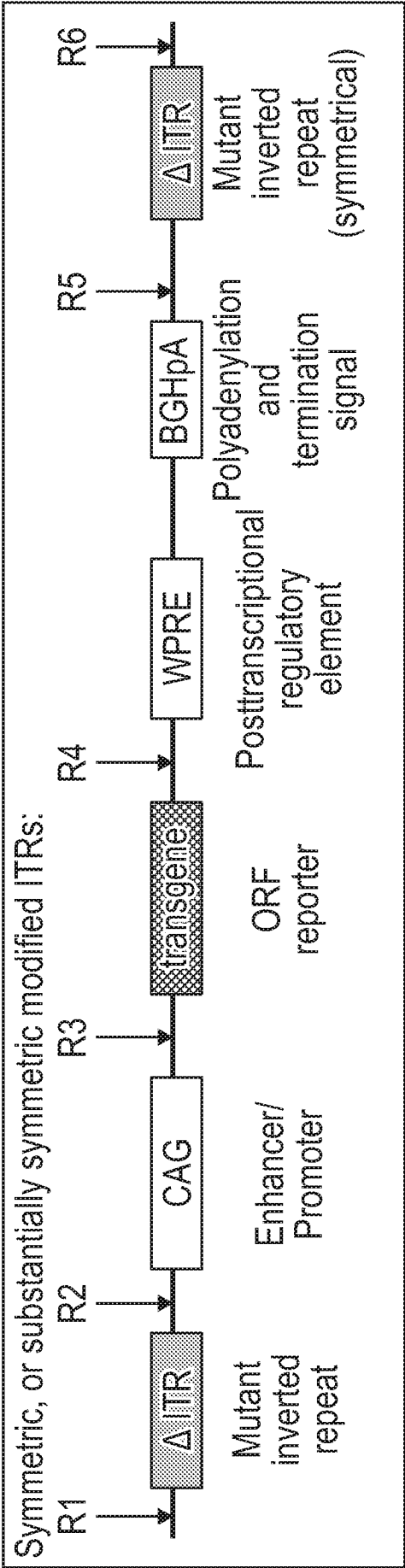


FIG. 1E

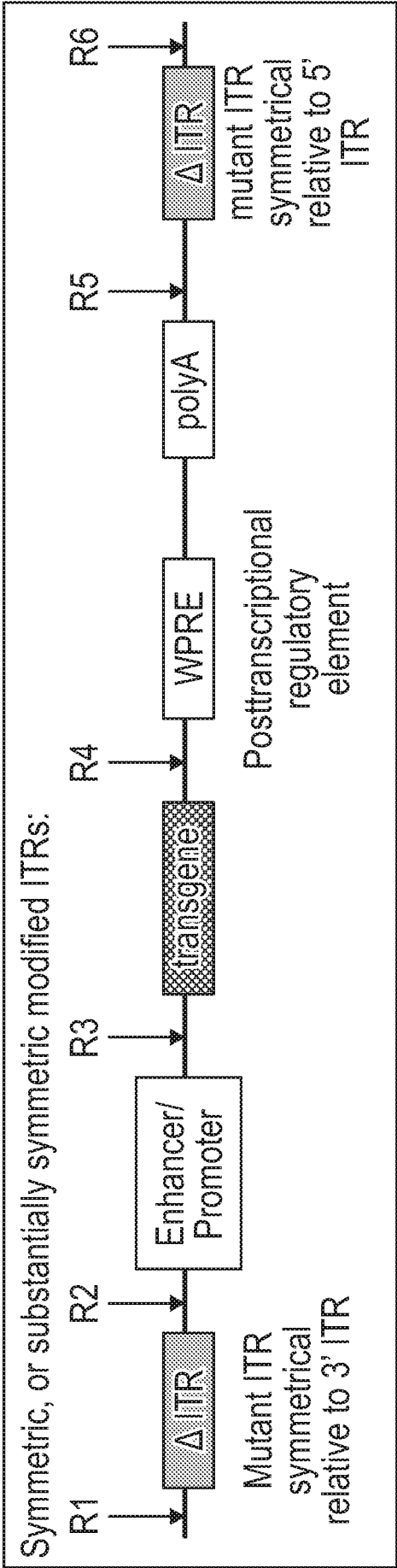


FIG. 1F

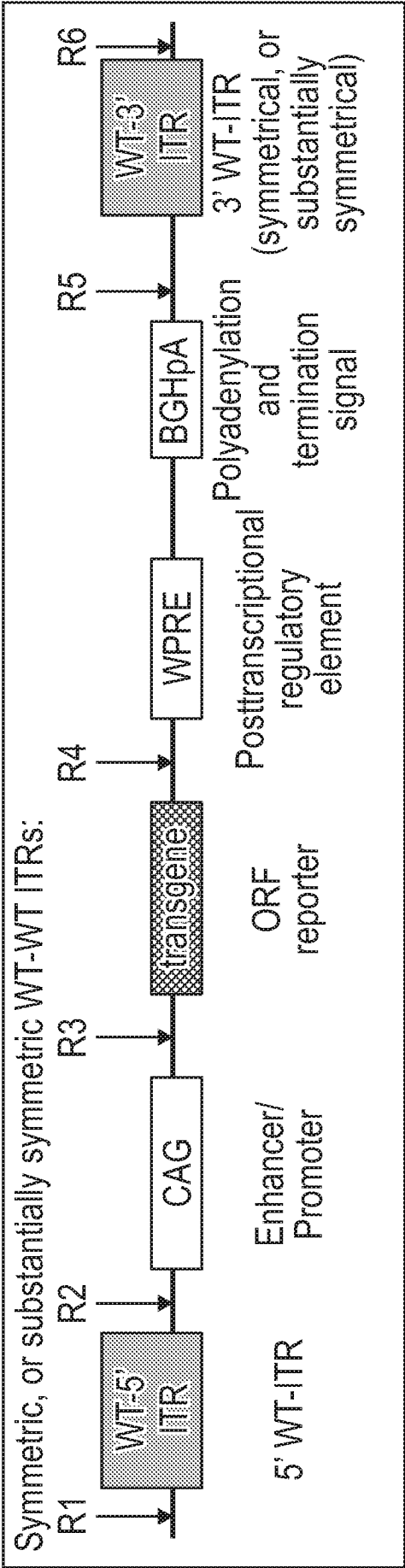
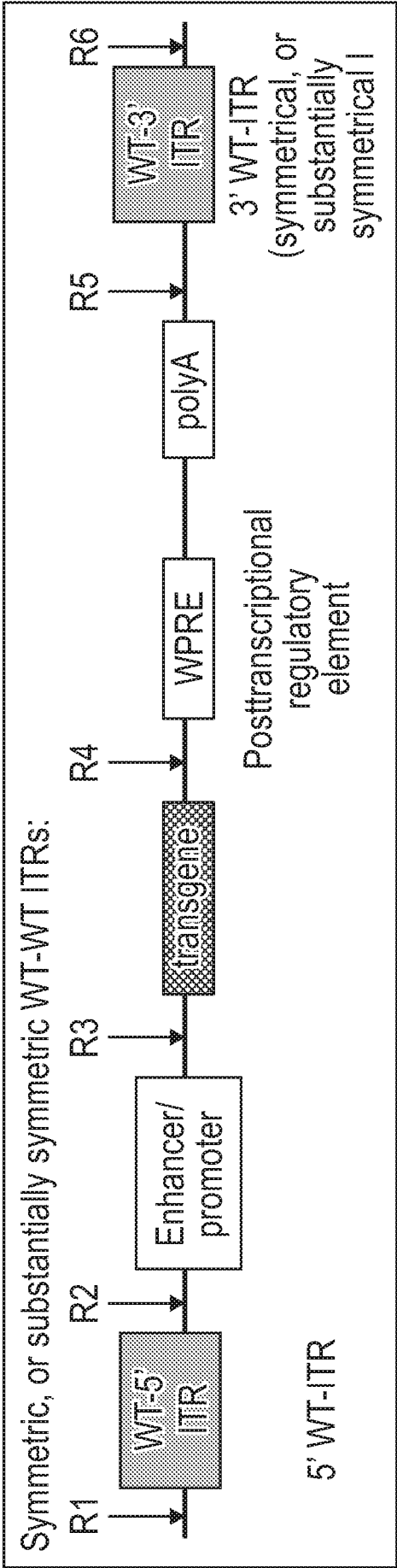


FIG. 1G



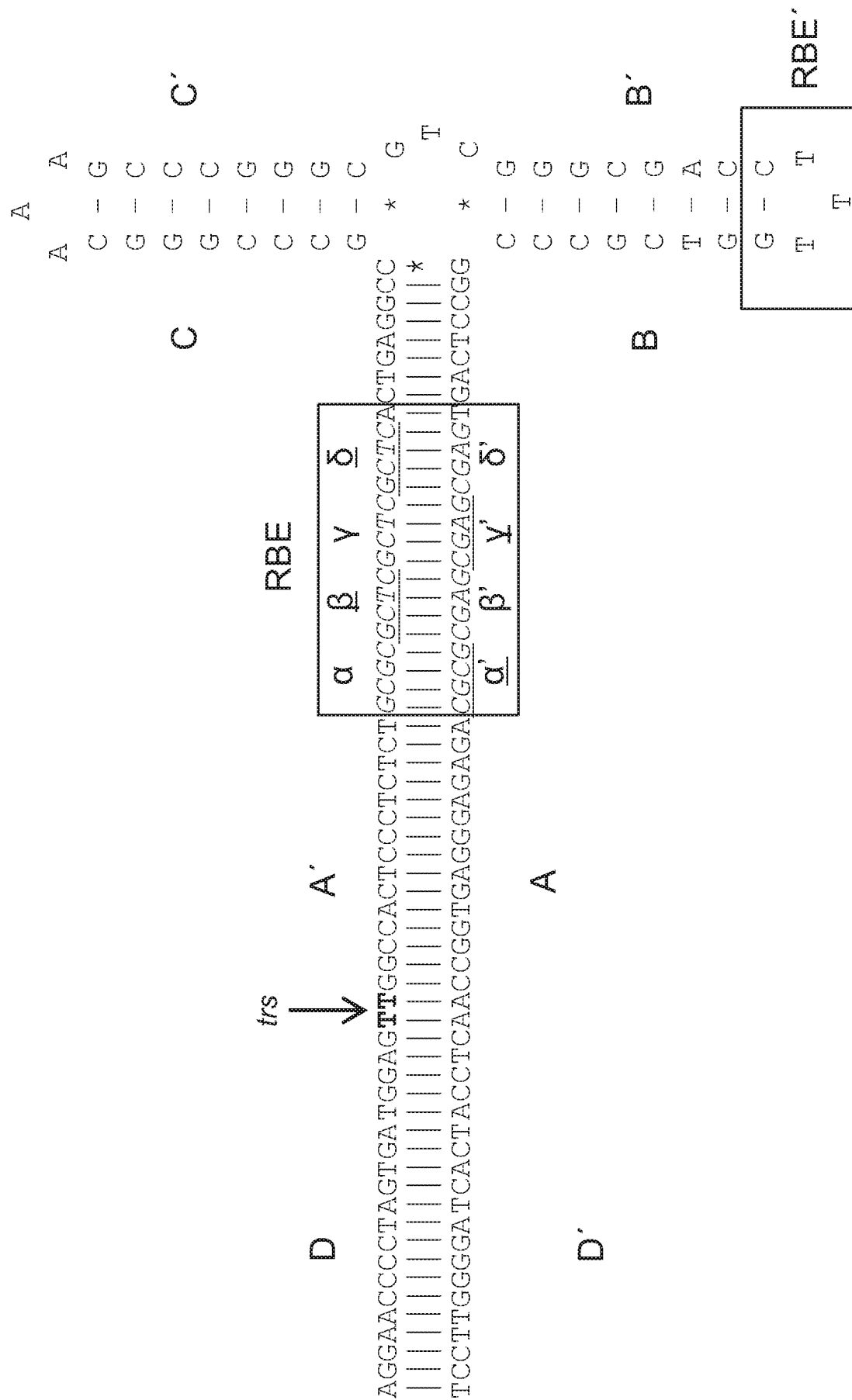
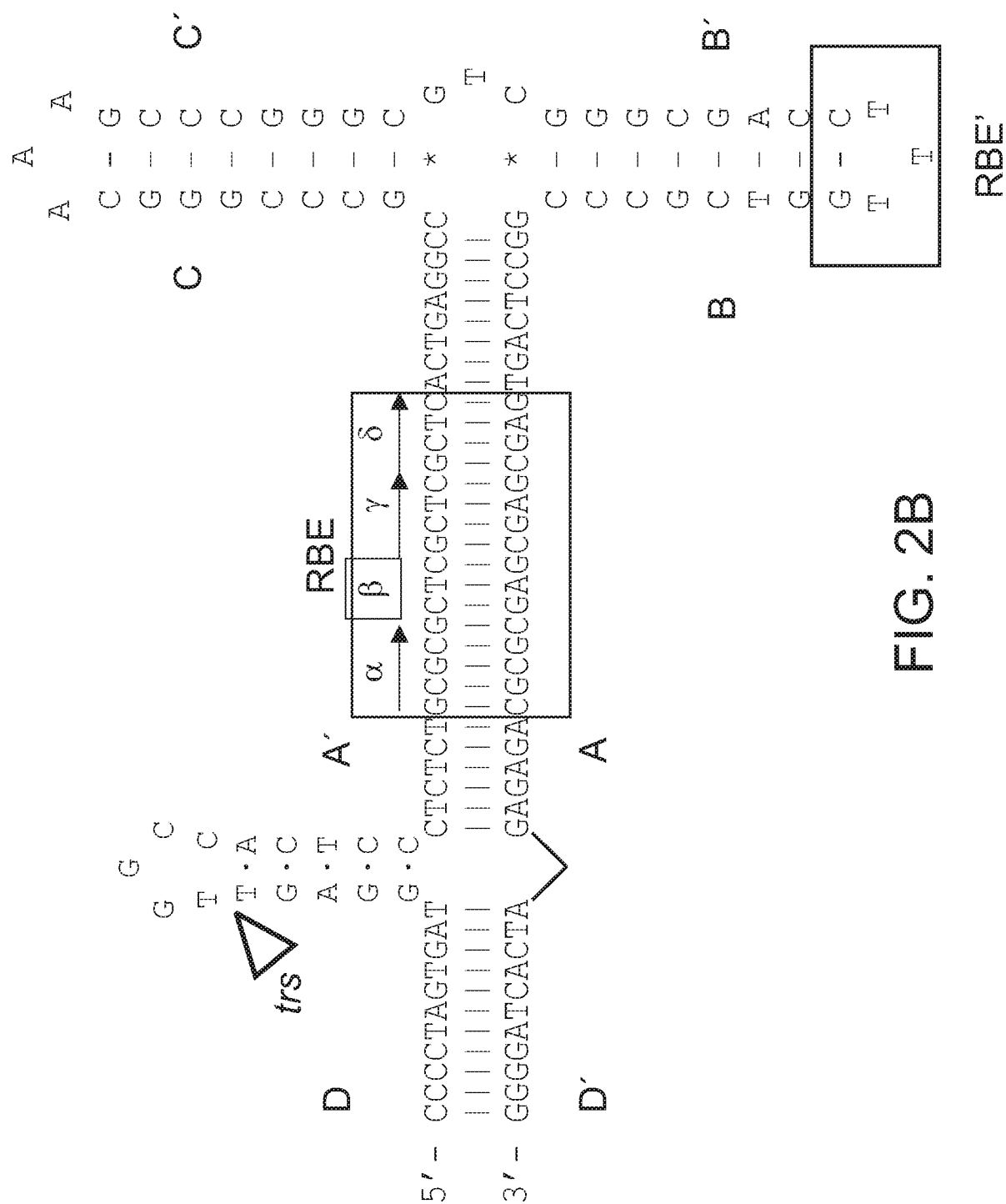
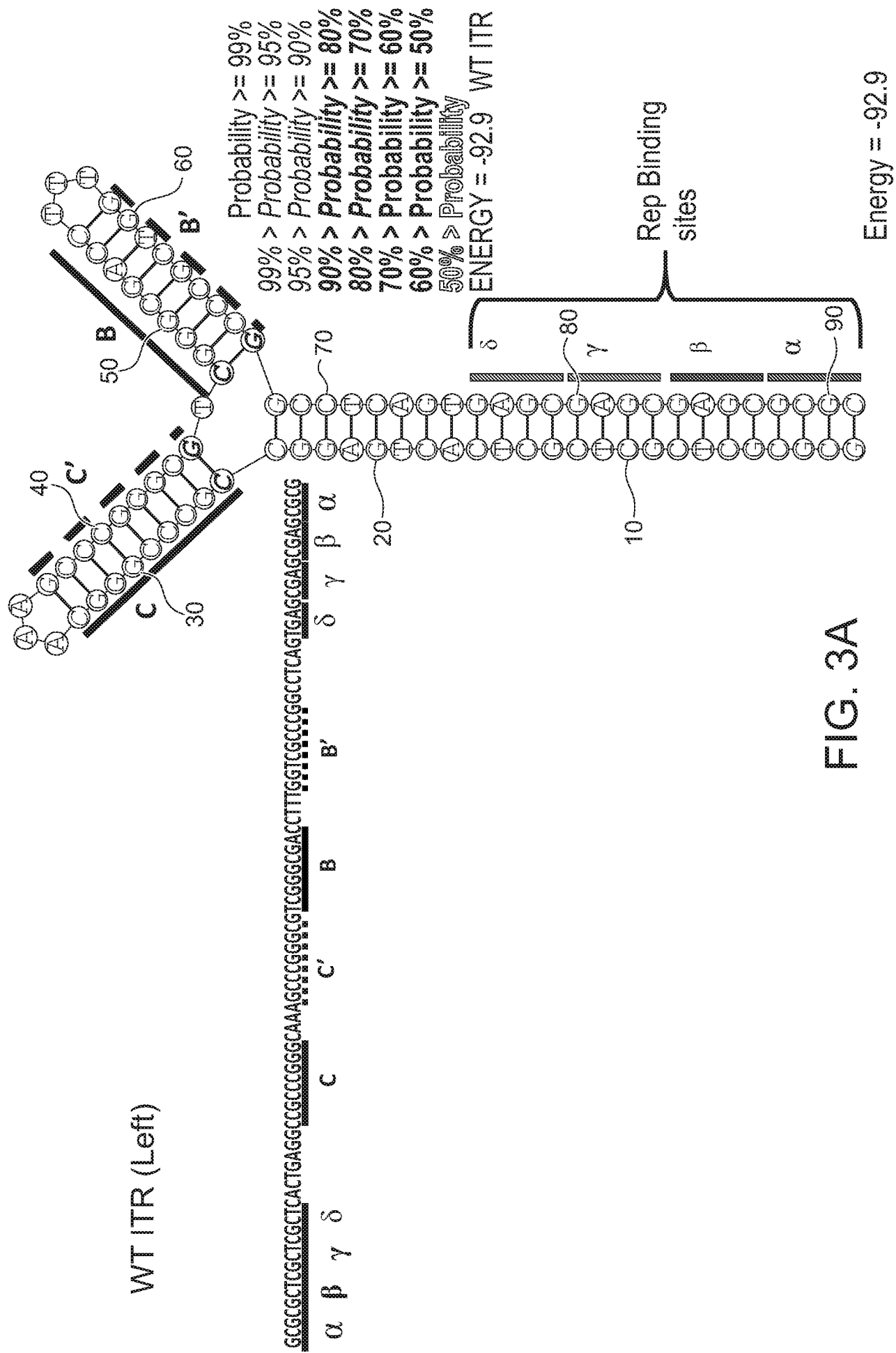
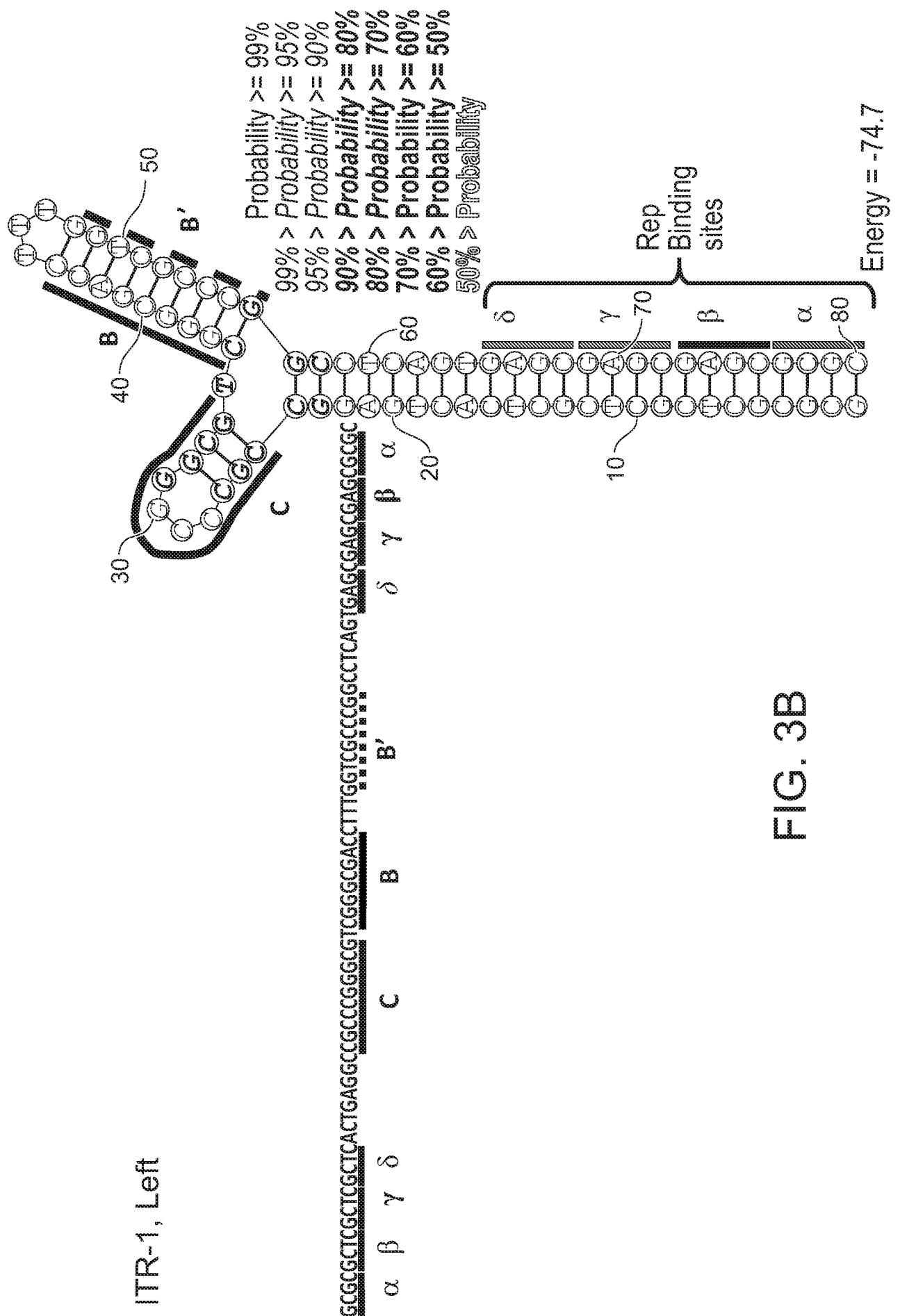


FIG. 2A







மேல்

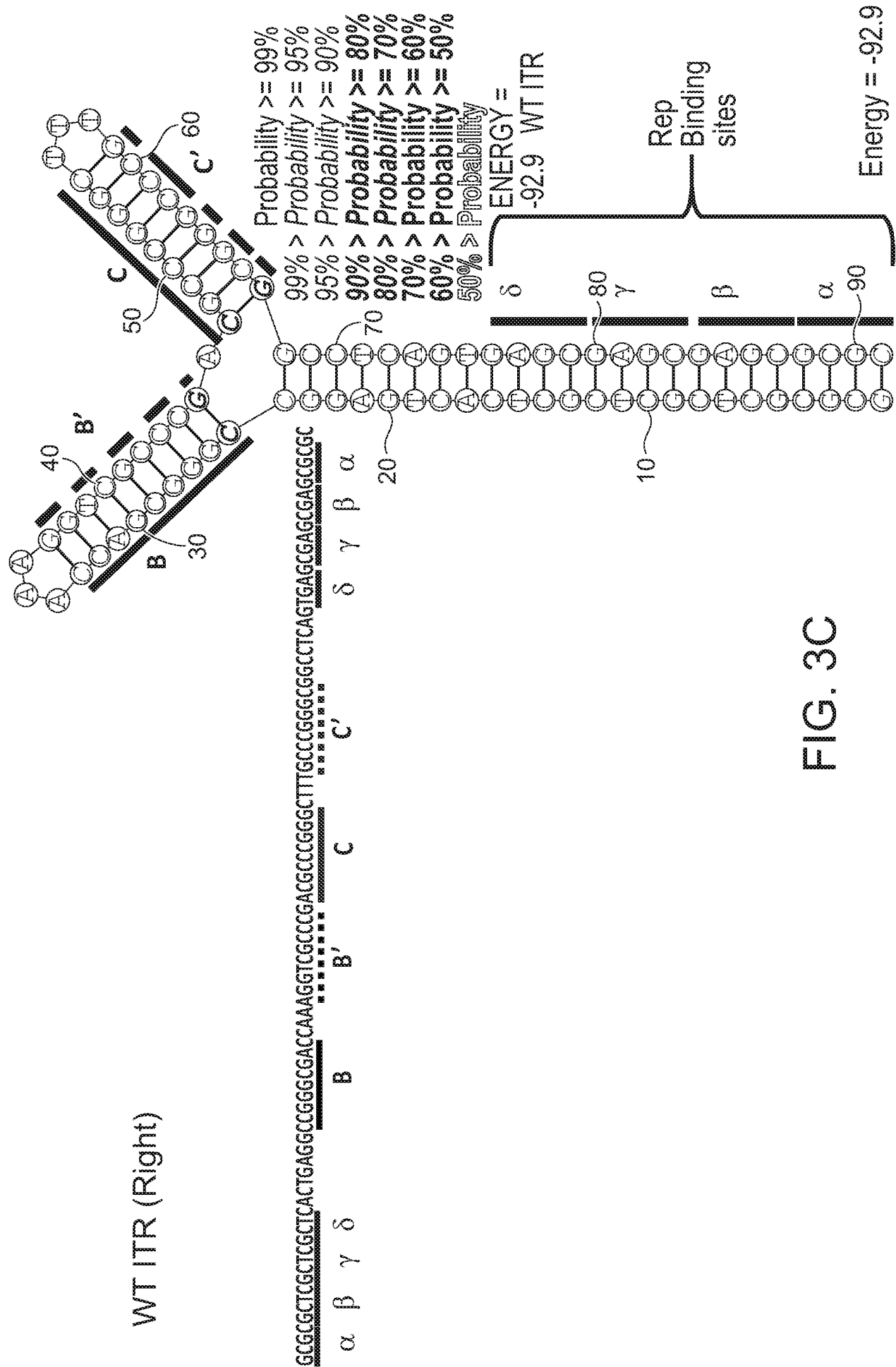
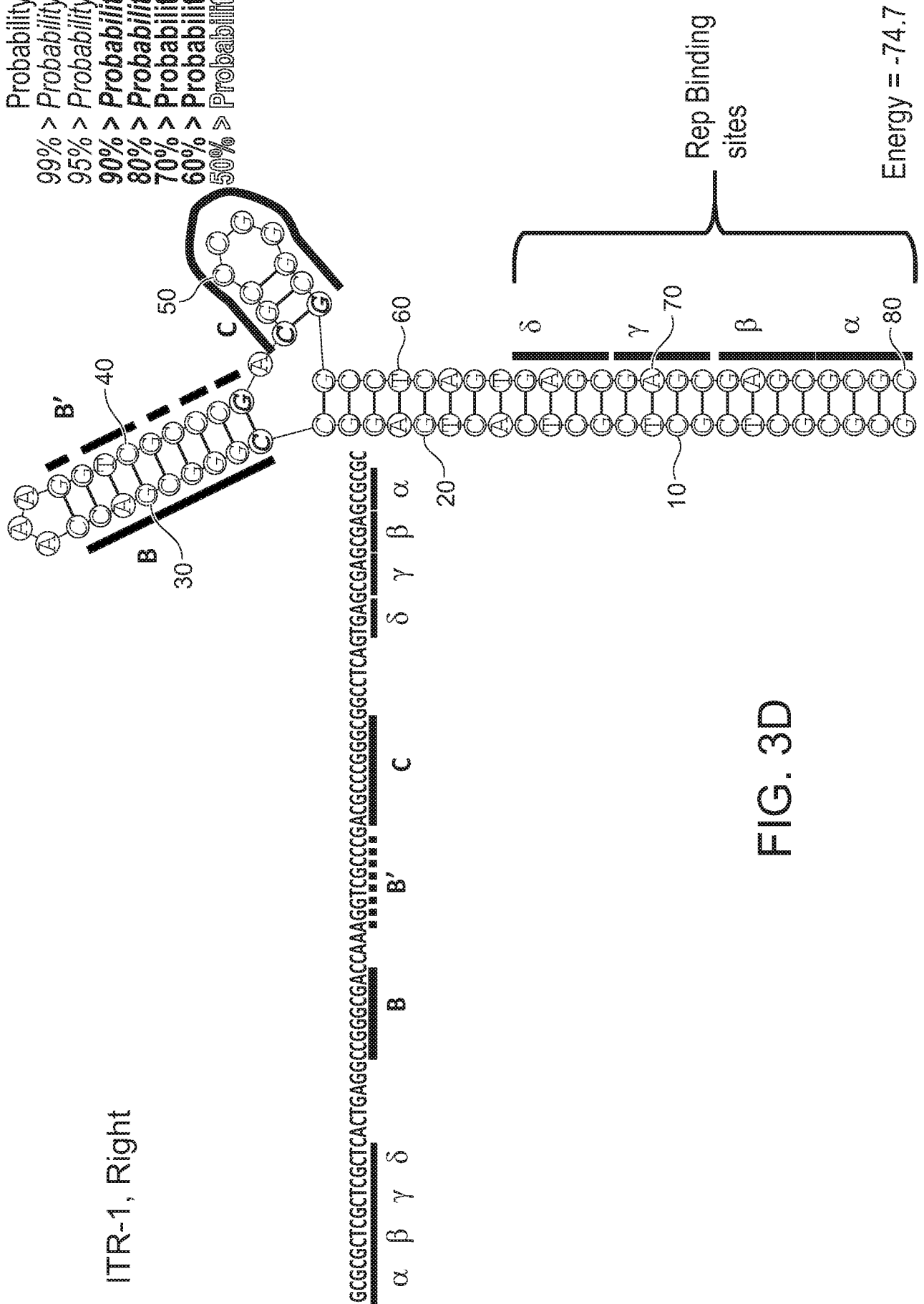


FIG. 3C

Probability >= 99%  
99% > Probability >= 95%  
95% > Probability >= 90%  
90% > Probability >= 80%  
80% > Probability >= 70%  
70% > Probability >= 60%  
60% > Probability >= 50%  
50% > Probability



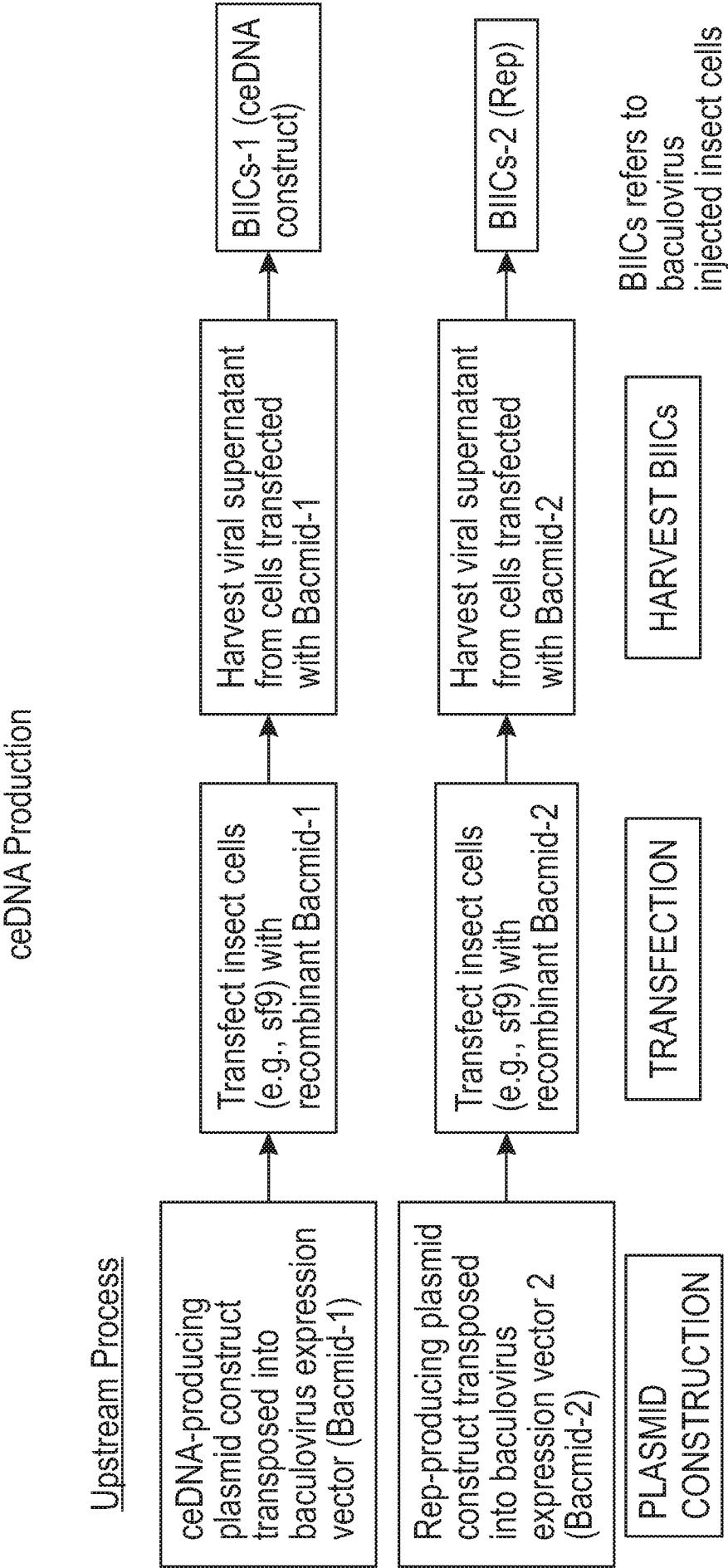


FIG. 4A

ceDNA Production

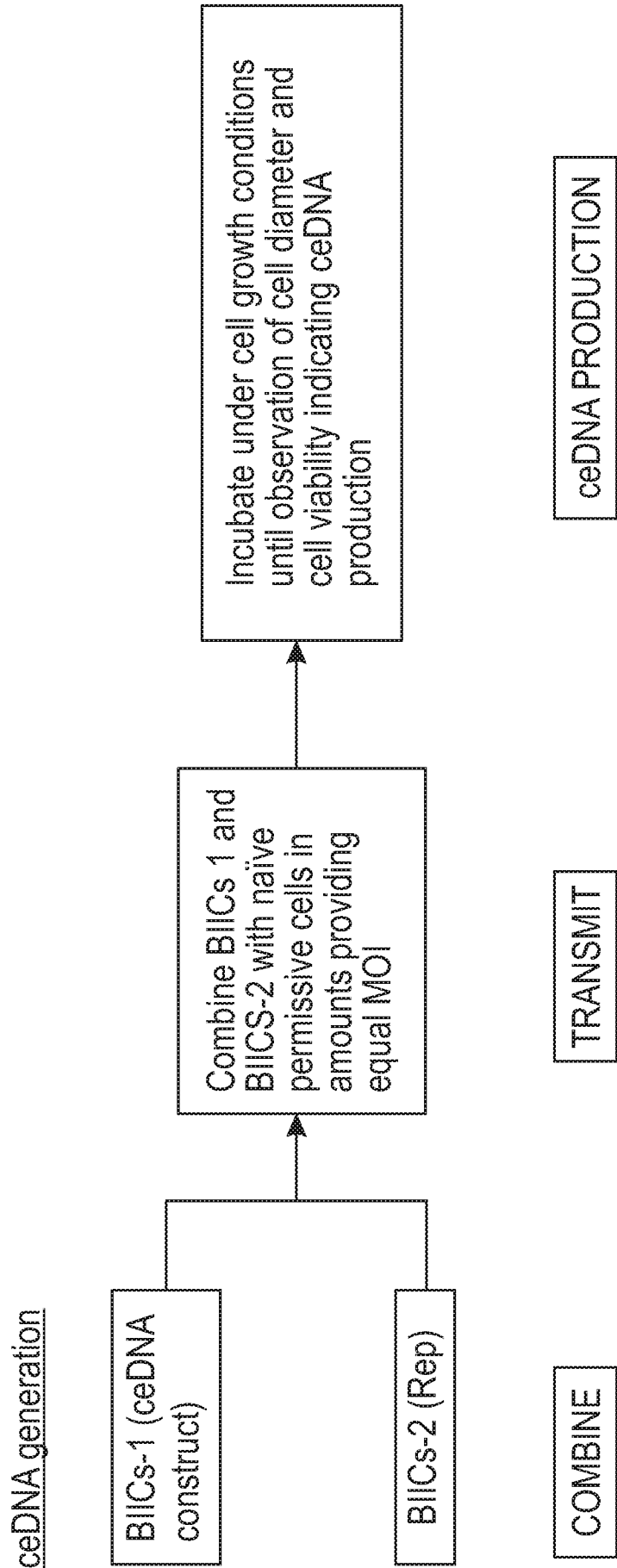


FIG. 4B

ceDNA Production

Downstream Process

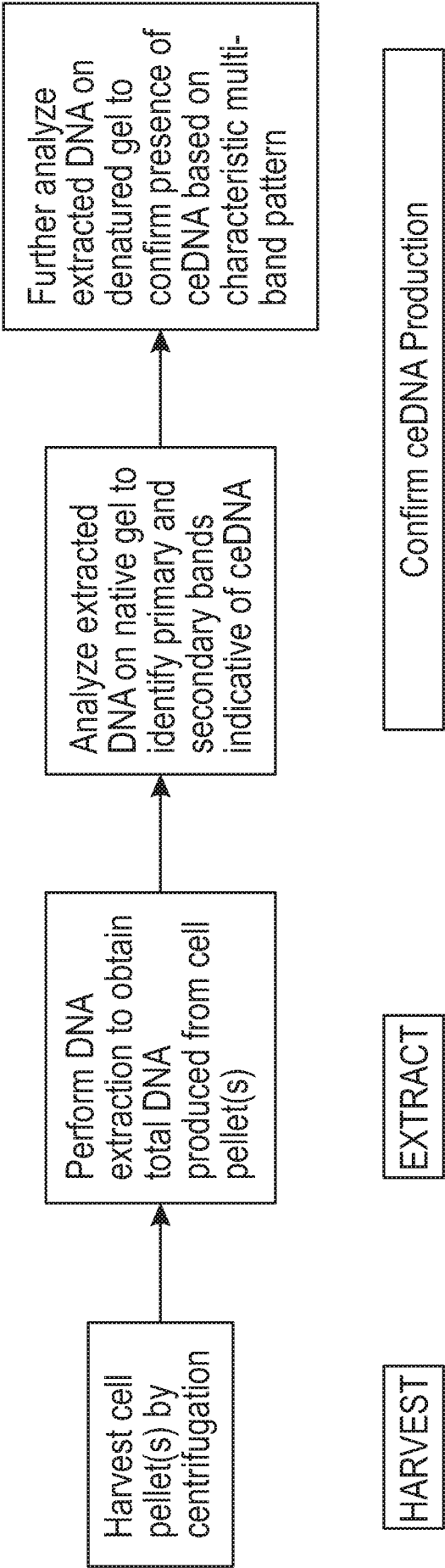


FIG. 4C

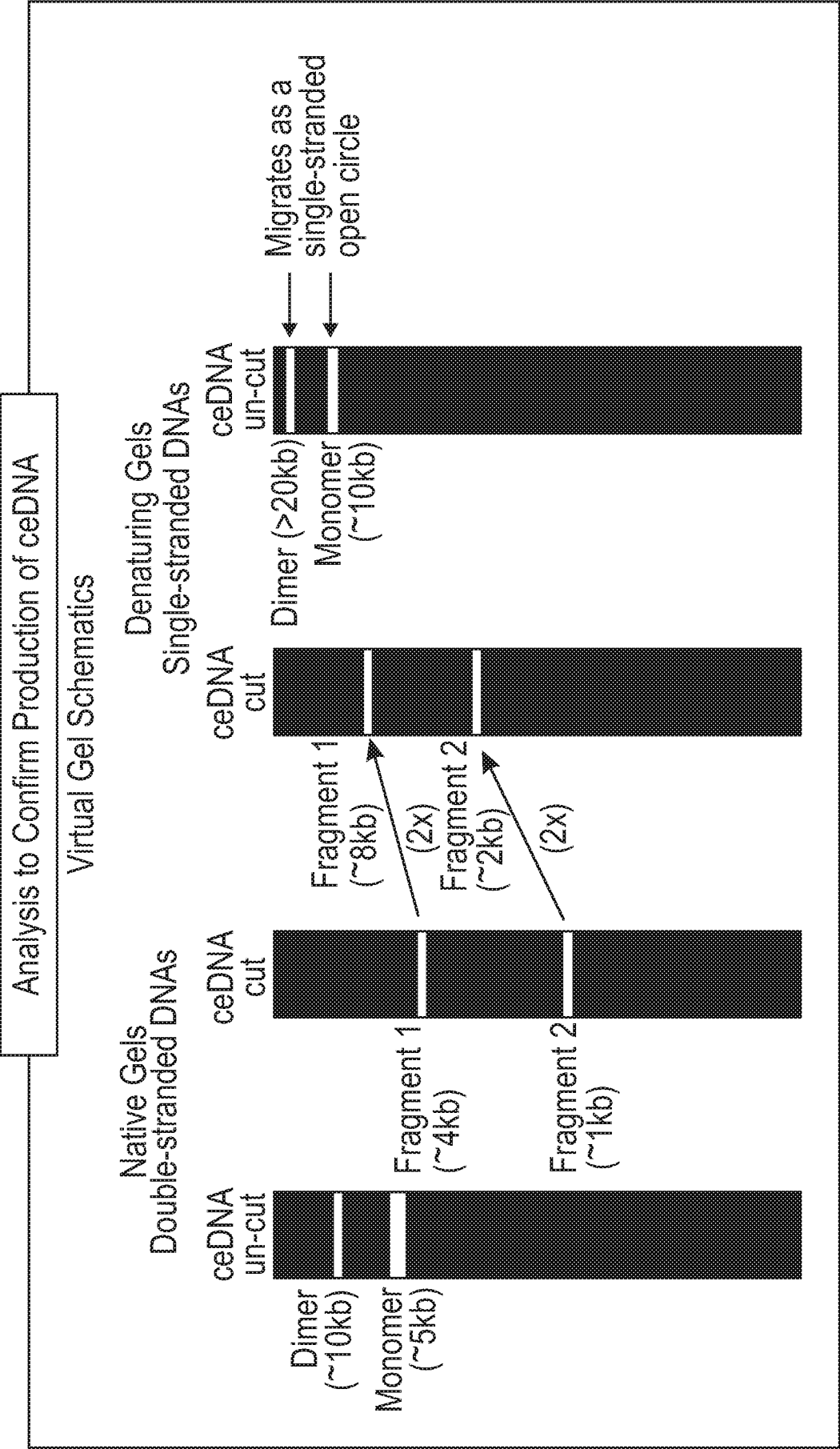
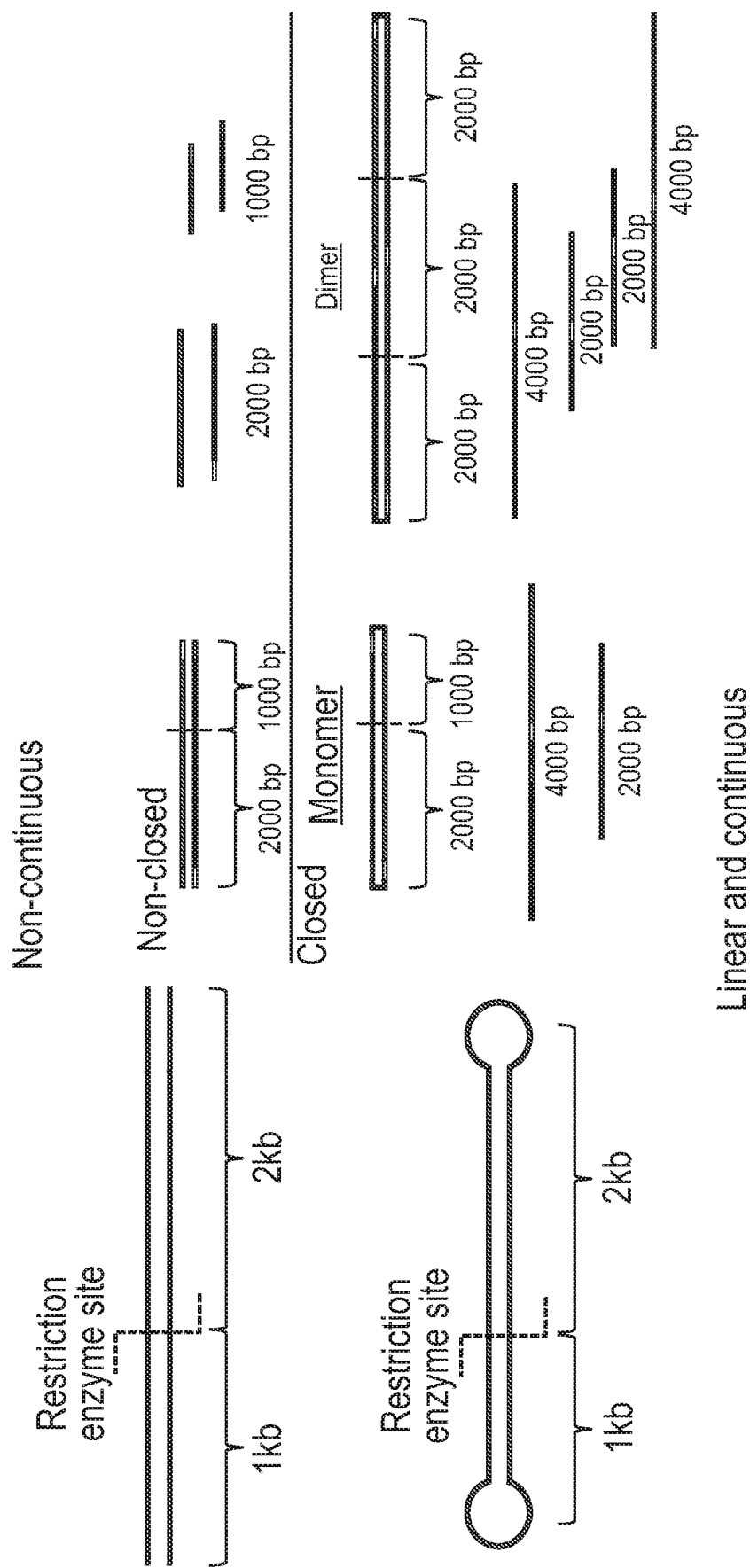


FIG. 4D

FIG. 4E



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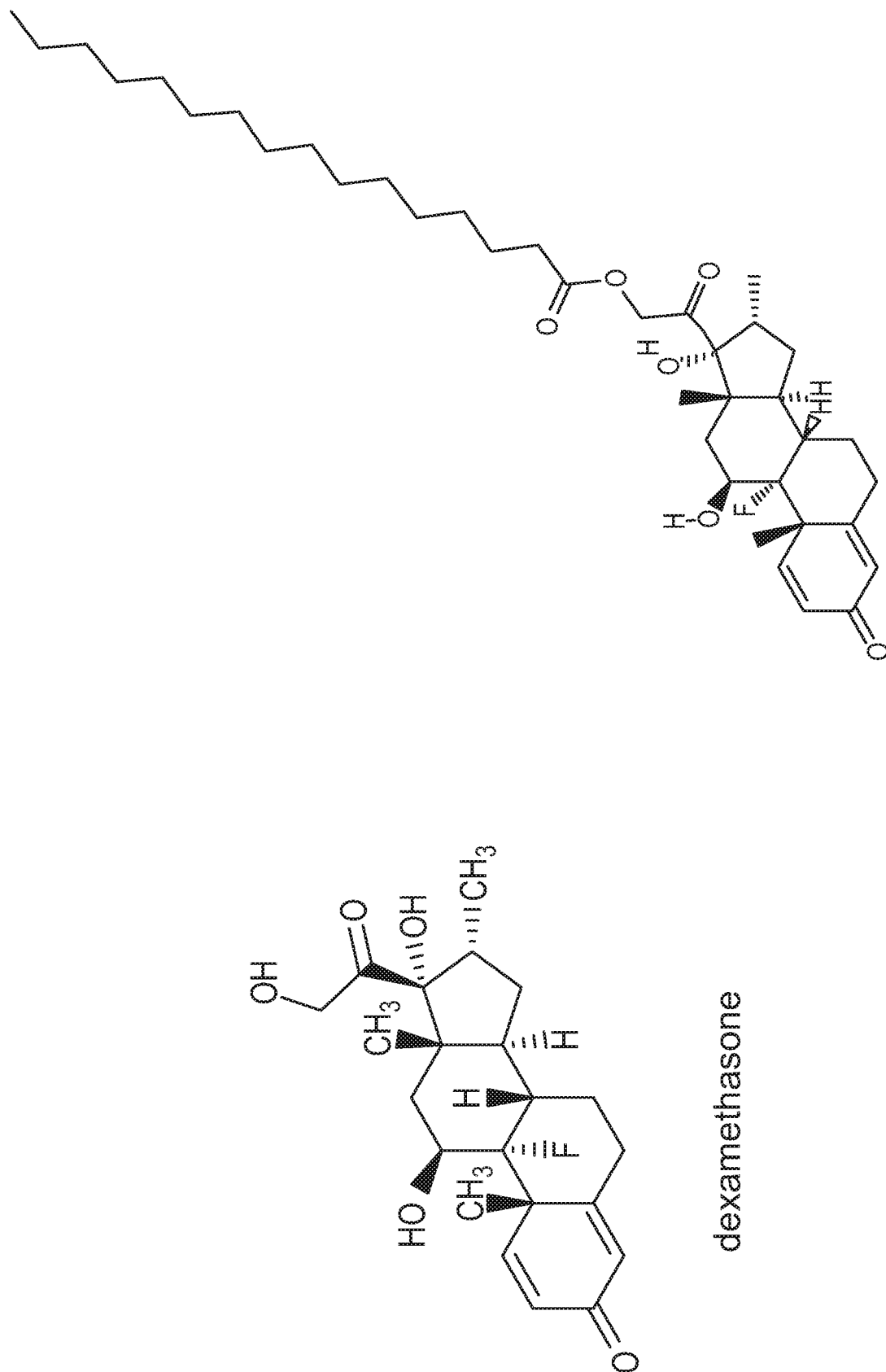


FIG. 5

FIG. 6A

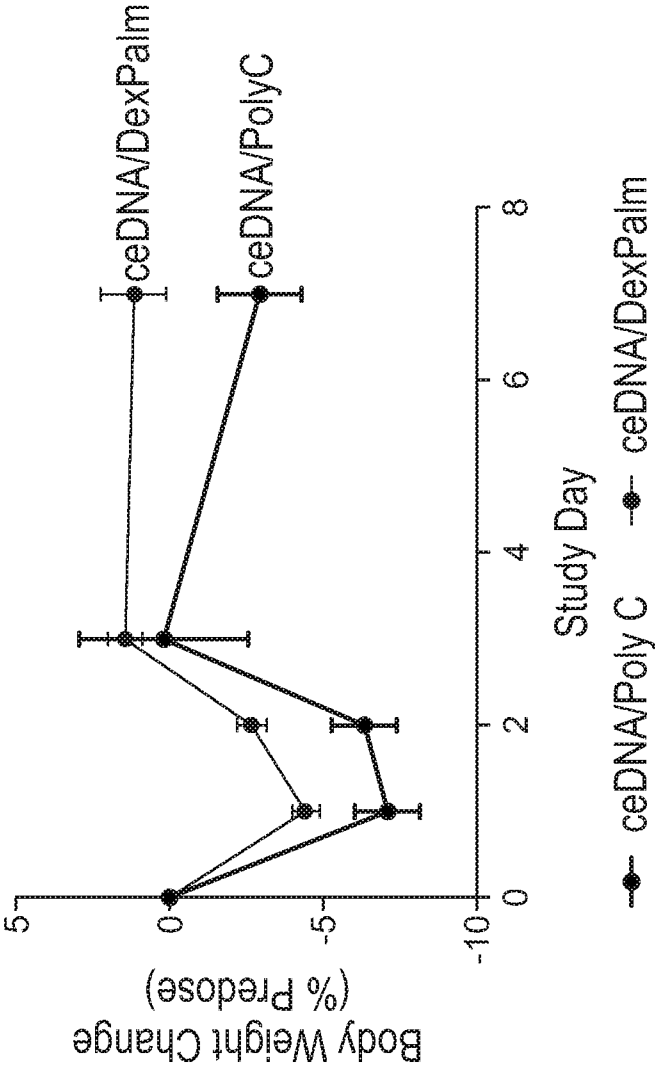


FIG. 6B

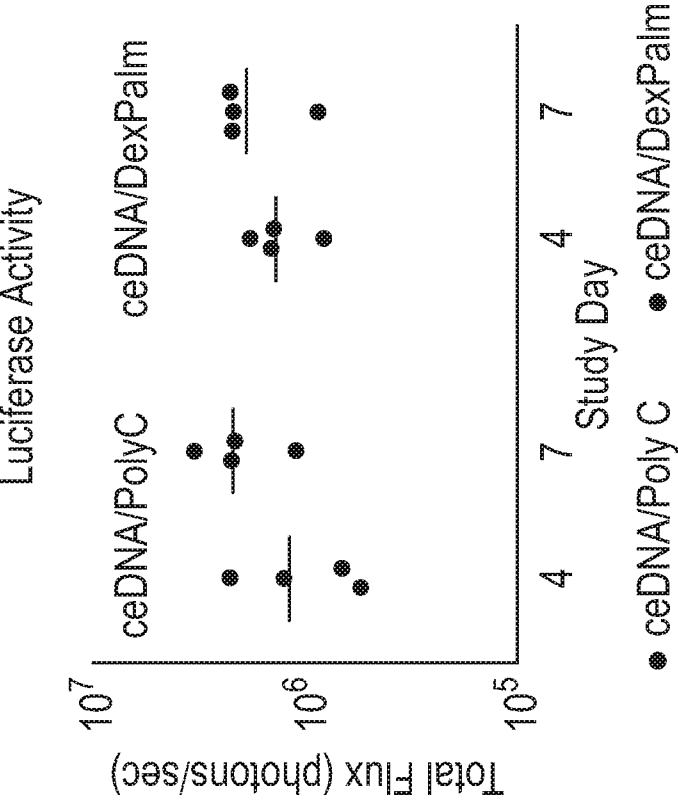
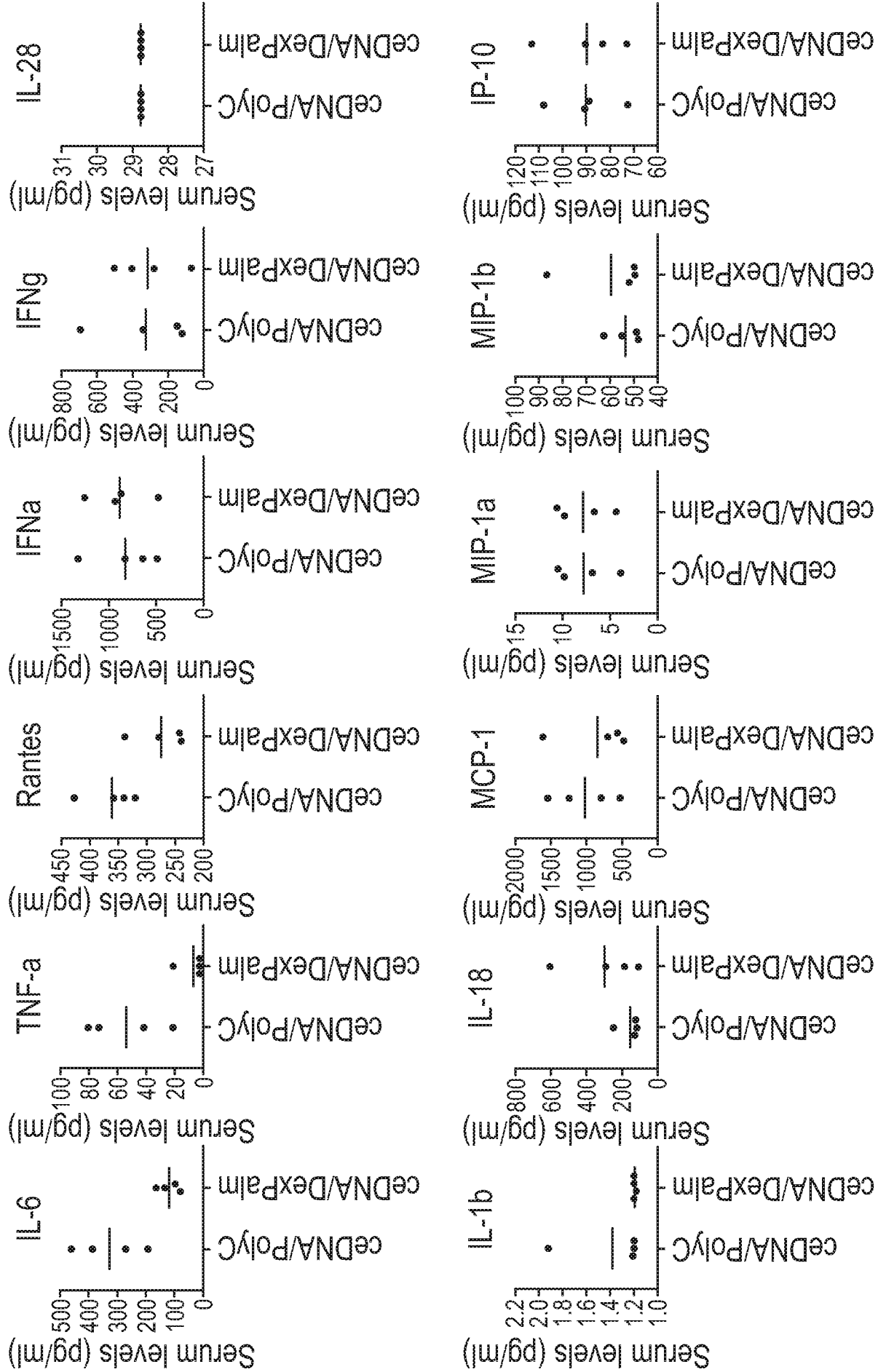


FIG. 6C



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polynucleotide

<400> 23

aggaaccctt agtgatggag ttggccactc cctctctgcg cgctcgctcg ctactgagg 60  
ccgggcgacc aaaggctgcc cgacgcccgg gtttcccggg cggcctcagt gagcgagcga 120  
gcgcgcagct gcctgcagg 139

<210> 24

<211> 137

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
polynucleotide

<400> 24  
aggaaccct agtgatggag ttggccactc cctctctgcg cgctcgctcg ctactgagg 60  
ccgggcgacc aaaggtcgcc cgacgcccg tttccgggcg gcctcagtga gcgagcgagc 120  
gcgcagctgc ctgcagg 137

<210> 25  
<211> 135  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
polynucleotide

<400> 25  
aggaaccct agtgatggag ttggccactc cctctctgcg cgctcgctcg ctactgagg 60  
ccgggcgacc aaaggtcgcc cgacgcccg tttccgggcg gcctcagtga gcgagcgagc 120  
gcagctgcct gcagg 135

<210> 26  
<211> 133  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
polynucleotide

<400> 26  
aggaaccct agtgatggag ttggccactc cctctctgcg cgctcgctcg ctactgagg 60  
ccgggcgacc aaaggtcgcc cgacgccctt tggggcgcc cagtgcgga gcgagcgagc 120  
agctgcctgc agg 133

<210> 27  
<211> 131  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
polynucleotide

<400> 27  
aggaaccct agtgatggag ttggccactc cctctctgcg cgctcgctcg ctactgagg 60  
ccgggcgacc aaaggtcgcc cgacgccctt ggccgacctca gtgagcgagc gagcgcgagc 120  
ctgcctgcag g 131

<210> 28  
<211> 129  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
polynucleotide

<400> 28  
 aggaaccct agtgatggag ttggccactc cctctctgcg cgctcgctcg ctactgagg 60  
 ccgggcgacc aaaggtcgcc cgacgctttg gcgcctcagt gagcgagcga gcgcgcagct 120  
 gcctgcagg 129

<210> 29  
 <211> 127  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic  
 polynucleotide

<400> 29  
 aggaaccct agtgatggag ttggccactc cctctctgcg cgctcgctcg ctactgagg 60  
 ccgggcgacc aaaggtcgcc cgacgctttg gcctcagtga gcgagcgagc gcgcagctgc 120  
 ctgcagg 127

<210> 30  
 <211> 122  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic  
 polynucleotide

<400> 30  
 aggaaccct agtgatggag ttggccactc cctctctgcg cgctcgctcg ctactgagg 60  
 ccgggcgacc aaaggtcgcc cgacggcctc agtgagcgag cgagcgcgca gctgcctgca 120  
 gg 122

<210> 31  
 <211> 130  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic  
 polynucleotide

<400> 31  
 aggaaccct agtgatggag ttggccactc cctctctgcg cgctcgctcg ctactgagg 60  
 ccgggcgacc aaaggtcgcc cgacgcccgg gcggcctcag tgagcgagcg agcgcgagc 120  
 tgcctgcagg 130

<210> 32  
 <211> 120  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic  
 polynucleotide

<400> 32

cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccgggaaacc cgggcgtgcg 60

cctcagttag cgagcgagcg cgagagagg gaggggccaa ctccatcact aggggttcct 120

<210> 33

<211> 122

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 33

cctgcaggca gctgcgcgct cgctcgctca ctgaggccgt cgggcgacct ttggtcgccc 60

ggcctcagtg agcgagcgag cgcgagaga gggagtggcc aactccatca ctaggggttc 120

ct 122

<210> 34

<211> 122

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 34

cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccgggcaaag cccgggcgtc 60

ggcctcagtg agcgagcgag cgcgagaga gggagtggcc aactccatca ctaggggttc 120

ct 122

<210> 35

<211> 129

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 35

cctgcaggca gctgcgcgct cgctcgctca ctgaggcgcc cgggcgtcgg gcgacctttg 60

gtcgcccgcc ctcagttagc gagcgagcgc gcagagaggg agtggccaac tccatcacta 120

ggggttcct 129

<210> 36

<211> 101

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 36

cctgcaggca gctgcgcgct cgctcgctca ctgaggcaaa gcctcagtga gcgagcgagc 60

gcgcagagag ggagtggcca actccatcac taggggttcc t 101

<210> 37  
<211> 139  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
polynucleotide

<400> 37  
cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccgggcaaag cccgggcgtc 60  
gggcgacttt gtcgcccggc ctcagtgagc gagcgagcgc gcagagaggg agtggccaac 120  
tccatcacta ggggttcct 139

<210> 38  
<211> 137  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
polynucleotide

<400> 38  
cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccgggcaaag cccgggcgtc 60  
gggcgatttt cgcccggcct cagtgagcga gcgagcgcgc agagagggag tggccaactc 120  
catcactagg ggttcct 137

<210> 39  
<211> 135  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
polynucleotide

<400> 39  
cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccgggcaaag cccgggcgtc 60  
gggcgtttcg cccggcctca gtgagcgagc gagcgcgcag agaggagtg gccaaactcca 120  
tcactagggg ttctt 135

<210> 40  
<211> 133  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
polynucleotide

<400> 40  
cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccgggcaaag cccgggcgtc 60  
gggctttgcc cggcctcagt gagcgagcga gcgcgagag agggagtggc caactccatc 120  
actaggggtt cct 133

<210> 41  
<211> 139  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
polynucleotide

<400> 41  
cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccgggaaacc cgggcgtcgg 60  
gcgacctttg gtcgcccggc ctcaagtgcg gagcgagcgc gcagagaggg agtggccaac 120  
tccatcacta ggggttcct 139

<210> 42  
<211> 137  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
polynucleotide

<400> 42  
cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccggaaaccg ggcgtcgggc 60  
gaccttttgt cgcccgccct cagtgcgcga gcgagcgcgc agagagggag tggccaactc 120  
catcactagg ggttcct 137

<210> 43  
<211> 135  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
polynucleotide

<400> 43  
cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccgaaacggg cgtcgggcga 60  
cctttgtgtc cccggcctca gtgagcgagc gagcgcgcag agaggagtg gccaaactcca 120  
tcactagggg ttcct 135

<210> 44  
<211> 133  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
polynucleotide

<400> 44  
cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccaaaggcg tcgggcgacc 60  
tttggtcgcc cggcctcagt gagcgagcga gcgcgcagag agggagtggc caactccatc 120  
actaggggtt cct 133

<210> 45  
 <211> 131  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence: Synthetic polynucleotide  
  
 <400> 45  
 cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc caaaggcgtc gggcgacctt 60  
 tggtcgccccg gcctcagtga gcgagcgagc gcgcagagag ggagtggcca actccatcac 120  
 taggggttcc t 131  
  
 <210> 46  
 <211> 129  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence: Synthetic polynucleotide  
  
 <400> 46  
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 gtcgcccggc ctcagtgagc gagcgagcgc gcagagaggg agtggccaac tccatcacta 120  
 ggggttcct 129  
  
 <210> 47  
 <211> 127  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence: Synthetic polynucleotide  
  
 <400> 47  
 cctgcaggca gctgcgcgct cgctcgctca ctgaggccga aacgtcgggc gacctttggt 60  
 cgcccggcct cagtgagcga gcgagcgcgc agagagggag tggccaactc catcactagg 120  
 ggttcct 127  
  
 <210> 48  
 <211> 122  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence: Synthetic polynucleotide  
  
 <400> 48  
 aggaaccctt agtgatggag ttggccactc cctctctgcg cgctcgctcg ctactgagg 60  
 ccgggcgacc aaaggtcgcc cgacggcctc agtgagcgag cgagcgcgca gctgcctgca 120  
 gg 122  
  
 <210> 49

<211> 12  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence: Synthetic  
         oligonucleotide  
  
 <400> 49  
 cgatcgttcg at 12  
  
  
 <210> 50  
 <211> 12  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence: Synthetic  
         oligonucleotide  
  
 <400> 50  
 atcgaaccat cg 12  
  
  
 <210> 51  
 <211> 12  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence: Synthetic  
         oligonucleotide  
  
 <400> 51  
 atcgaacgat cg 12  
  
  
 <210> 52  
 <211> 165  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence: Synthetic  
         polynucleotide  
  
 <400> 52  
 aggaaccct agtgatggag ttggccactc cctctctgcg cgctcgctcg ctactgagg 60  
 ccgcccgggc aaagcccggg cgtcgggcga cctttggtcg cccggcctca gtgagcgagc 120  
 gagcgcgag agagggagtg gccaaactca tctagggg ttcct 165  
  
  
 <210> 53  
 <211> 140  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence: Synthetic  
         polynucleotide  
  
 <400> 53  
 cccctagtga tggagttggc cactccctct ctgcgcgctc gctcgctcac tgaggccgcc 60  
 cgggcaaagc ccgggcgtcg ggcgacctt ggctgcccgg cctcagttag cgagcgagcg 120

cgcagagaga tcactagggg 140

<210> 54  
<211> 91  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
oligonucleotide

<400> 54  
gcgcgctcgc tcgctcactg aggccgccc ggcaaagccc gggcgtcggg cgacctttgg 60  
tcgcccggcc tcagtgagcg agcgagcgcg c 91

<210> 55  
<211> 91  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
oligonucleotide

<400> 55  
gcgcgctcgc tcgctcactg aggccgggcg accaaaggtc gcccgacgcc cgggctttgc 60  
ccgggcggcc tcagtgagcg agcgagcgcg c 91

<210> 56  
<211> 1662  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
polynucleotide

<400> 56  
gccgccacca tggaagacgc caaaaacata aagaaaggcc cggcgccatt ctatccgctg 60  
gaagatggaa ccgctggaga gcaactgcat aaggctatga agagatacgc cctggttcct 120  
ggaacaattg cttttacaga tgcacatatc gaggtggaca tcacttacgc tgagtacttc 180  
gaaatgtccg ttcggttggc agaagctatg aaacgatatg ggctgaatac aaatcacaga 240  
atcgctgtat gcagtgaaaa ctctcttcaa ttctttatgc cgggtgttggg cgcgttattt 300  
atcggagttg cagttgcgcc gcggaacgac atttataatg aacgtgaatt gctcaacagt 360  
atgggcattt cgcagcctac cgtggtgttc gtttccaaaa aggggttgca aaaaattttg 420  
aacgtgcaaa aaaagctccc aatcatccaa aaaattatta tcatggattc taaaacggat 480  
taccagggat ttcagtcgat gtacacgttc gtcacatctc atctacctcc cggttttaat 540  
gaatacgatt ttgtgccaga gtccttcgat agggacaaga caattgcact gatcatgaac 600  
tcctctggat ctactggtct gcctaaaggt gtcgctctgc ctcatagaac tgcctgcgtg 660  
agattctcgc atgccagaga tcctatTTTT ggcaatcaaa tcattccgga tactgcgatt 720  
ttaagtgttg ttccattcca tcacggtttt ggaatgttta ctacactcgg atatttgata 780

|                                                                    |      |
|--------------------------------------------------------------------|------|
| tgtggatttc gagtcgtctt aatgtataga tttgaagaag agctgtttct gaggagcctt  | 840  |
| caggattaca agattcaaag tgcgctgctg gtgccaaccc tattctcctt cttcgccaaa  | 900  |
| agcactctga ttgacaaata cgatttatct aatttacacg aaattgcttc tgggtggcgct | 960  |
| cccctctcta aggaagtcgg ggaagcggtt gccaaagagt tccatctgcc aggtatcagg  | 1020 |
| caaggatatg ggctcactga gactacatca gctattctga ttacaccga gggggatgat   | 1080 |
| aaaccgggcg cggtcggtaa agttgttcca ttttttgaag cgaaggttgt ggatctggat  | 1140 |
| accgggaaaa cgctgggcgt taatcaaaga ggcgaactgt gtgtgagagg tcctatgatt  | 1200 |
| atgtccggtt atgtaaaca tccggaagcg accaacgcct tgattgaca ggatggatgg    | 1260 |
| ctacattctg gagacatagc ttactgggac gaagacgaac acttcttcat cgttgaccgc  | 1320 |
| ctgaagtctc tgattaagta caaaggctat cagggtggctc ccgctgaatt ggaatccatc | 1380 |
| ttgtccaac accccaacat cttcgacgca ggtgtcgag gtcttcccga cgatgacgcc    | 1440 |
| ggtgaacttc ccgccccgt tgttgttttg gagcacggaa agacgatgac ggaaaaagag   | 1500 |
| atcgtggatt acgtcgccag tcaagtaaca accgcgaaaa agttgcgcgg aggagtgtg   | 1560 |
| tttgtggacg aagtaccgaa aggtcttacc ggaaaactcg acgcaagaaa aatcagagag  | 1620 |
| atcctcataa aggccaagaa gggcggaaag atcgccgtgt aa                     | 1662 |

<210> 57

<211> 453

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polypeptide

<220>

<221> MOD\_RES

<222> (1)..(1)

<223> Any amino acid

<400> 57

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Xaa | Val | Gln | Leu | Val | Glu | Ser | Gly | Gly | Gly | Val | Val | Gln | Pro | Gly | Arg |
| 1   |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Leu | Arg | Leu | Ser | Cys | Ala | Ala | Ser | Gly | Phe | Ala | Phe | Ser | Ser | Tyr |
|     |     | 20  |     |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gly | Met | His | Trp | Val | Arg | Gln | Ala | Pro | Gly | Lys | Gly | Leu | Glu | Trp | Val |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Val | Ile | Trp | Phe | Asp | Gly | Thr | Lys | Lys | Tyr | Tyr | Thr | Asp | Ser | Val |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | Gly | Arg | Phe | Thr | Ile | Ser | Arg | Asp | Asn | Ser | Lys | Asn | Thr | Leu | Tyr |
| 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     | 80  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Gln | Met | Asn | Thr | Leu | Arg | Ala | Glu | Asp | Thr | Ala | Val | Tyr | Tyr | Cys |
|     |     |     | 85  |     |     |     |     |     | 90  |     |     |     |     | 95  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Arg | Asp | Arg | Gly | Ile | Gly | Ala | Arg | Arg | Gly | Pro | Tyr | Tyr | Met | Asp | 100 | 105 | 110 |     |
| Val | Trp | Gly | Lys | Gly | Thr | Thr | Val | Thr | Val | Ser | Ser | Ala | Ser | Thr | Lys | 115 | 120 | 125 |     |
| Gly | Pro | Ser | Val | Phe | Pro | Leu | Ala | Pro | Ser | Ser | Lys | Ser | Thr | Ser | Gly | 130 | 135 | 140 |     |
| Gly | Thr | Ala | Ala | Leu | Gly | Cys | Leu | Val | Lys | Asp | Tyr | Phe | Pro | Glu | Pro | 145 | 150 | 155 | 160 |
| Val | Thr | Val | Ser | Trp | Asn | Ser | Gly | Ala | Leu | Thr | Ser | Gly | Val | His | Thr | 165 | 170 | 175 |     |
| Phe | Pro | Ala | Val | Leu | Gln | Ser | Ser | Gly | Leu | Tyr | Ser | Leu | Ser | Ser | Val | 180 | 185 | 190 |     |
| Val | Thr | Val | Pro | Ser | Ser | Ser | Leu | Gly | Thr | Gln | Thr | Tyr | Ile | Cys | Asn | 195 | 200 | 205 |     |
| Val | Asn | His | Lys | Pro | Ser | Asn | Thr | Lys | Val | Asp | Lys | Arg | Val | Glu | Pro | 210 | 215 | 220 |     |
| Lys | Ser | Cys | Asp | Lys | Thr | His | Thr | Cys | Pro | Pro | Cys | Pro | Ala | Pro | Glu | 225 | 230 | 235 | 240 |
| Leu | Leu | Gly | Gly | Pro | Ser | Val | Phe | Leu | Phe | Pro | Pro | Lys | Pro | Lys | Asp | 245 | 250 | 255 |     |
| Thr | Leu | Met | Ile | Ser | Arg | Thr | Pro | Glu | Val | Thr | Cys | Val | Val | Val | Asp | 260 | 265 | 270 |     |
| Val | Ser | His | Glu | Asp | Pro | Glu | Val | Lys | Phe | Asn | Trp | Tyr | Val | Asp | Gly | 275 | 280 | 285 |     |
| Val | Glu | Val | His | Asn | Ala | Lys | Thr | Lys | Pro | Arg | Glu | Glu | Gln | Tyr | Asn | 290 | 295 | 300 |     |
| Ser | Thr | Tyr | Arg | Val | Val | Ser | Val | Leu | Thr | Val | Leu | His | Gln | Asp | Trp | 305 | 310 | 315 | 320 |
| Leu | Asn | Gly | Lys | Glu | Tyr | Lys | Cys | Lys | Val | Ser | Asn | Lys | Ala | Leu | Pro | 325 | 330 | 335 |     |
| Ala | Pro | Ile | Glu | Lys | Thr | Ile | Ser | Lys | Ala | Lys | Gly | Gln | Pro | Arg | Glu | 340 | 345 | 350 |     |
| Pro | Gln | Val | Tyr | Thr | Leu | Pro | Pro | Ser | Arg | Glu | Glu | Met | Thr | Lys | Asn | 355 | 360 | 365 |     |

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile  
370 375 380

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr  
385 390 395 400

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys  
405 410 415

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys  
420 425 430

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu  
435 440 445

Ser Leu Ser Pro Gly  
450

<210> 58

<211> 214

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
polypeptide

<400> 58

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly  
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Tyr  
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile  
35 40 45

Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly  
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro  
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Ser Thr Pro Leu  
85 90 95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala  
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly  
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala  
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln  
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser  
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr  
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser  
195 200 205

Phe Asn Arg Gly Glu Cys  
210

<210> 59

<211> 1310

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
polynucleotide

<400> 59

|                                                                     |      |
|---------------------------------------------------------------------|------|
| ggagccgaga gtaattcata caaaaggagg gatcgcttc gcaaggggag agcccaggga    | 60   |
| ccgtccctaa attctcacag acccaaatcc ctgtagccgc cccacgacag cgcgaggagc   | 120  |
| atgcgctcag ggctgagcgc ggggagagca gagcacacaa gctcatagac cctggtcgtg   | 180  |
| ggggggaggga ccggggagct ggcgcggggc aaactgggaa agcgggtgtcg tgtgctggct | 240  |
| ccgccctctt cccgaggggtg ggggagaacg gtatataagt gcggcagtcg ccttggacgt  | 300  |
| tctttttcgc aacgggtttg ccgtcagaac gcaggtgagg ggcggggtgtg gcttccgcgg  | 360  |
| gccgccgagc tggaggtcct gctccgagcg ggccggggccc cgctgtcgtc ggcggggatt  | 420  |
| agctgcgagc attcccgtt cgagttgcgg gcggcgcggg aggcagagtg cgaggcctag    | 480  |
| cggcaacccc gtagcctcgc ctctgtgtccg gcttgaggcc tagcgtggtg tccgcgccgc  | 540  |
| cgccgcgtgc tactccggcc gcaactctggt cttttttttt tttgttgttg ttgccctgct  | 600  |
| gccttcgatt gccgttcagc aataggggct aacaaaggga ggggtgcgggg cttgctcgcc  | 660  |
| cggagcccgg agaggtcatg gttggggagg aatggaggga caggagtggc ggctggggcc   | 720  |
| cgcccgccctt cggagcacat gtccgacgcc acctggatgg ggcgaggcct ggggtttttc  | 780  |
| ccgaagcaac caggctgggg ttagcgtgcc gaggccatgt ggccccagca cccggcacga   | 840  |
| tctggcttgg cggcgccgcg ttgccctgcc tccctaacta gggtagggcc atcccgtccg   | 900  |
| gcaccagttg cgtgcgtgga aagatggccg ctcccgggcc ctgttgcaag gagctcaaaa   | 960  |
| tggaggacgc ggagcccgg tggagcgggc gggtagtca cccacacaaa ggaagagggc     | 1020 |
| ctggtccctc accggctgct gcttcctgtg accccgtggt cctatcggcc gcaatagtca   | 1080 |
| cctcgggctt ttgagcacgg ctagtcgcgg cggggggagg ggatgtaatg gcgttgaggt   | 1140 |

|                                                                   |      |
|-------------------------------------------------------------------|------|
| ttgttcacat ttggtgggtg gagactagtc aggccagcct ggcgctggaa gtcatttttg | 1200 |
| gaatttgtcc ctttgagttt tgagcggagc taattctcgg gcttcttagc ggttcaaagg | 1260 |
| tatcttttaa accctttttt aggtgttgtg aaaaccaccg ctaattcaaa            | 1310 |

<210> 60  
 <211> 16  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic  
 oligonucleotide

|                   |    |
|-------------------|----|
| <400> 60          |    |
| gcgcgctcgc tcgctc | 16 |

<210> 61  
 <211> 6  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic  
 oligonucleotide

|          |   |
|----------|---|
| <400> 61 |   |
| ggttga   | 6 |

<210> 62  
 <211> 4  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic  
 oligonucleotide

|          |   |
|----------|---|
| <400> 62 |   |
| agtt     | 4 |

<210> 63  
 <211> 6  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic  
 oligonucleotide

|          |   |
|----------|---|
| <400> 63 |   |
| ggttgg   | 6 |

<210> 64  
 <211> 6  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic  
 oligonucleotide

<400> 64  
agttgg 6

<210> 65  
<211> 6  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
oligonucleotide

<400> 65  
agttga 6

<210> 66  
<211> 6  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
oligonucleotide

<400> 66  
rrttrr 6

<210> 67  
<211> 581  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
polynucleotide

<400> 67  
gagcatctta ccgccattta ttcccatatt tgttctgttt ttcttgattt gggatatacat 60  
ttaaatgtta ataaaacaaa atgggtggggc aatcatttac atttttaggg atatgtaatt 120  
actagttcag gtgtattgcc acaagacaaa catgttaaga aactttcccg ttatttacgc 180  
tctgttcctg ttaatcaacc tctggattac aaaatttgtg aaagattgac tgatattctt 240  
aactatgttg ctctttttac gctgtgtgga tatgctgctt tatagcctct gtatctagct 300  
attgcttccc gtacggcttt cgttttctcc tccttgata aatcctgggt gctgtctctt 360  
ttagaggagt tgtggcccg 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> 68  
<211> 225  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
polynucleotide

<400> 68  
 tgtgccttct agttgccagc catctgttgt ttgccctcc cccgtgcctt ccttgaccct 60  
 ggaagggtgcc actcccactg tcctttccta ataaaatgag gaaattgcat cgattgtct 120  
 gagtaggtgt cattctattc tggggggtgg ggtggggcag gacagcaagg gggaggattg 180  
 ggaagacaat agcaggcatg ctggggatgc ggtgggctct atggc 225

<210> 69  
 <211> 8  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic  
 oligonucleotide

<400> 69  
 actgaggc 8

<210> 70  
 <211> 8  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic  
 oligonucleotide

<400> 70  
 gcctcagt 8

<210> 71  
 <211> 16  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic  
 oligonucleotide

<400> 71  
 gagcgagcga gcgcgc 16

<210> 72  
 <211> 1923  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic  
 polynucleotide

<400> 72  
 tcaatattgg ccattagcca tattattcat tggttatata gcataaatca atattggcta 60  
 ttggccattg catacgttgt atctatatca taatatgtac atttatattg gtcattgtcc 120  
 aatatgaccg ccatgttggc attgattatt gactagttat taatagtaat caattacggg 180  
 gtcattagtt catagcccat atatggagtt ccgcgttaca taacttacgg taaatggccc 240  
 gcctggctga ccgccaacg acccccgccc attgacgtca ataatgacgt atgttcccat 300

|                                                                    |      |
|--------------------------------------------------------------------|------|
| agtaacgcca atagggactt tccattgacg tcaatgggtg gagtatttac ggtaaactgc  | 360  |
| ccacttggca gtacatcaag tgtatcatat gccaaagtccg cccctattg acgtcaatga  | 420  |
| cggtaaatgg cccgcctggc attatgcccc gtacatgacc ttacgggact ttcctacttg  | 480  |
| gcagtacatc tacgtattag tcatcgctat taccatggtc gaggtgagcc ccacgttctg  | 540  |
| cttcactctc cccatctccc cccctcccc accccaatt ttgtatttat ttatTTTTTA    | 600  |
| attatTTTTgt gcagcgatgg gggcgggggg gggggggggg cgcgcgccag gcggggcggg | 660  |
| gcggggcgag gggcggggcg gggcgaggcg gagagggtcg gcggcagcca atcagagcgg  | 720  |
| cgcgctccga aagtttcctt ttatggcgag gcggcgggcg cgcgggccct ataaaaagcg  | 780  |
| aagcgcgcg gcggcgggag tcgctgcgac gctgccttcg ccccggtccc cgctccgccg   | 840  |
| ccgcctcgcg ccgcccggc cggtcttgac tgaccgcgtt actccacag gtgagcgggc    | 900  |
| gggacggccc ttctcctccg ggctgtaatt agcgcttggt ttaatgacgg cttgtttctt  | 960  |
| ttctgtggct gcgtgaaagc cttgaggggc tccgggaggg ccctttgtgc gggggggagc  | 1020 |
| ggctcggggg gtgcgtgcgt gtgtgtgtgc gtggggagcg ccgctgcgg cccgcgctgc   | 1080 |
| ccggcggtg tgagcgctgc gggcgggcg cggggctttg tgcgctccgc agtgtgcgcg    | 1140 |
| aggggagcgc ggccgggggc ggtgccccgc ggtgcggggg gggctgcgag gggaacaaag  | 1200 |
| gctgcgtgcg ggggtgtgtc gtgggggggt gagcaggggg tgtgggcgcg gcggtcgggc  | 1260 |
| tgtaaccccc ccctgcaccc ccctccccga gttgctgagc acggcccggc ttcgggtgcg  | 1320 |
| gggctccgta cggggcgtag cgcggggctc gccgtgccgg gcgggggggtg gcggcaggtg | 1380 |
| ggggtgccgg gcggggcggg gccgcctcgg gccggggagg gctcggggga ggggcgcggc  | 1440 |
| ggcccccgga gcgcccggcg ctgtcgaggc gcggcgagcc gcagccattg ctttttatgg  | 1500 |
| taatcgtagc agagggcgca gggacttcct ttgtccaaa tctgtgcgga gccgaaatct   | 1560 |
| gggaggcgcc gccgcacccc ctctagcggg cgcggggcga agcggtagcg cgccggcagg  | 1620 |
| aaggaaatgg gcggggaggg ccttcgtgcg tcgccgcgcc gccgtcccct tctccctctc  | 1680 |
| cagcctcggg gctgtccgcg gggggacggc tgccttcggg ggggacgggg cagggcgggg  | 1740 |
| ttcggcttct ggctgttgac cgcggtctt agagcctctg ctaaccatgt tttagccttc   | 1800 |
| ttctttttcc tacagctcct gggcaacgtg ctggttattg tgctgtctca tcatttgtcg  | 1860 |
| acagaattcc tcgaagatcc gaaggggttc aagcttgga ttccggtact gttggtaaag   | 1920 |
| cca                                                                | 1923 |

<210> 73

<211> 1272

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 73

|                                                                   |    |
|-------------------------------------------------------------------|----|
| aggctcagag gcacacagga gtttctgggc tcaccctgcc cccttccaac ccctcagttc | 60 |
|-------------------------------------------------------------------|----|

|                                                                    |      |
|--------------------------------------------------------------------|------|
| ccatcctcca gcagctgttt gtgtgctgcc tctgaagtcc acactgaaca aacttcagcc  | 120  |
| tactcatgtc cctaaatgg gcaaacattg caagcagcaa acagcaaaca cacagccctc   | 180  |
| cctgcctgct gaccttggag ctggggcaga ggtcagagac ctctctgggc ccatgccacc  | 240  |
| tccaacatcc actcgacccc ttggaatttc ggtggagagg agcagagggt gtcctggcgt  | 300  |
| ggtttaggta gtgtgagagg gtccgggttc aaaaccactt gctgggtggg gagtcgtcag  | 360  |
| taagtggcta tgccccgacc ccgaagcctg tttccccatc tgtacaatgg aaatgataaa  | 420  |
| gacgcccatac 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 gcctgtgcc cccaccccgg ctaatttttt   | 720  |
| ctatttttga cagggacggg gtttcacat gttggtcagg ctggtctaga ggtaccgat    | 780  |
| cttgctacca gtggaacagc cactaaggat tctgcagtga gagcagaggg ccagctaagt  | 840  |
| ggtactctcc cagagactgt ctgactcacg ccaccccctc caccttggac acaggacgct  | 900  |
| gtggtttctg agccaggtag aatgactcct ttcggtaagt gcagtggaag ctgtacactg  | 960  |
| cccaggcaaa gcgtccgggc agcgtaggcg ggcgactcag atcccagcca gtggacttag  | 1020 |
| cccctgtttg ctctccgat aactgggggtg accttggtta atattcacca gcagcctccc  | 1080 |
| ccgttgcccc tctggatcca ctgcttaaat acggacgagg acagggccct gtctcctcag  | 1140 |
| cttcaggcac caccactgac ctgggacagt gaatccggac tctaaggtaa atataaaatt  | 1200 |
| tttaagtgtg taatgtgtta aactactgat tctaattgtt tctctctttt agattccaac  | 1260 |
| ctttggaact ga                                                      | 1272 |

<210> 74

<211> 1177

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 74

|                                                                     |     |
|---------------------------------------------------------------------|-----|
| ggctcagagg ctgagaggca cacaggagtt tctgggctca ccctgcccc ttccaacccc    | 60  |
| tcagttccca tcctccagca gctgtttgtg tgctgcctct gaagtccaca ctgaacaaac   | 120 |
| ttcagcctac tcatgtccct aaaatgggca aacattgcaa gcagcaaaca gcaaacacac   | 180 |
| agccctccct gcctgctgac cttggagctg gggcagaggt cagagacctc tctgggcca    | 240 |
| tgccacctcc aacatccact cgaccccttg gaatttcggt ggagaggagc agaggttgtc   | 300 |
| ctggcgtgggt ttaggtagtg tgagagggtc cgggttcaaa accacttgct ggggtggggag | 360 |
| tcgtcagtaa gtggctatgc ccgaccccc aagcctgttt ccccatctgt acaatggaaa    | 420 |
| tgataaagac gcccatctga tagggttttt gtggcaaata aacatttggt ttttttgttt   | 480 |

|                                                                   |      |
|-------------------------------------------------------------------|------|
| tgttttgttt tgttttttga gatggagggt tgctctgtcg cccaggctgg agtgcagtga | 540  |
| cacaatctca tctcaccaca accttcccct gcctcagcct cccaagtagc tgggattaca | 600  |
| agcatgtgcc accacacctg gctaattttc tatttttagt agagacgggt ttctccatgt | 660  |
| tggtcagcct cagcctccca agtaactggg attacaggcc tgtgccacca caccggcta  | 720  |
| attttttcta tttttgacag ggacgggggt tcaccatgtt ggtcaggctg gtctagaggt | 780  |
| accggatctt gctaccagtg gaacagccac taaggattct gcagtgagag cagaggcca  | 840  |
| gctaagtggg actctcccag agactgtctg actcacgcca cccctccac cttggacaca  | 900  |
| ggacgtgtg gtttctgagc caggtacaat gactcctttc ggtaagtga gtggaagtg    | 960  |
| tacactgccc aggcaaagcg tccgggcagc gtaggcgggc gactcagatc ccagccagtg | 1020 |
| gacttagccc ctgtttgctc ctccgataac tggggtgacc ttggttaata ttcaccagca | 1080 |
| gcctcccccg ttgcccctct ggatccactg cttaaatacg gacgaggaca gggccctgtc | 1140 |
| tcctcagctt caggcaccac cactgacctg ggacagt                          | 1177 |

<210> 75  
 <211> 547  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic polynucleotide

|                                                                               |     |
|-------------------------------------------------------------------------------|-----|
| <400> 75<br>ccctaaaatg ggcaaacatt gcaagcagca aacagcaaac acacagccct ccctgcctgc | 60  |
| tgaccttgga gctggggcag aggtcagaga cctctctggg cccatgccac ctccaacatc             | 120 |
| cactcgaccc cttggaattt ttcggtggag aggagcagag gttgtcctgg cgtggtttag             | 180 |
| gtagtgtgag aggggaatga ctcttttcgg taagtgcagt ggaagctgta cactgcccag             | 240 |
| gcaaagcgtc cgggcagcgt aggcgggcga ctcagatccc agccagtgga cttagcccct             | 300 |
| gtttgctcct ccgataactg gggtgacctt ggtaaatatt caccagcagc ctccccgtt              | 360 |
| gcccctctgg atccactgct taaatacggg cgaggacagg gccctgtctc ctgagcttca             | 420 |
| ggcaccacca ctgacctggg acagtgaatc cggactctaa ggtaaataata aaatttttaa            | 480 |
| gtgtataatg tgtaaacta ctgattctaa ttgtttctct ctttttagatt ccaacctttg             | 540 |
| gaactga                                                                       | 547 |

<210> 76  
 <211> 556  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic polynucleotide

|                                                                               |    |
|-------------------------------------------------------------------------------|----|
| <400> 76<br>ccctaaaatg ggcaaacatt gcaagcagca aacagcaaac acacagccct ccctgcctgc | 60 |
|-------------------------------------------------------------------------------|----|

|                                                                    |     |
|--------------------------------------------------------------------|-----|
| tgaccttgga gctggggcag aggtcagaga cctctctggg cccatgccac ctccaacatc  | 120 |
| cactcgaccc cttggaattt cgggtggagag gagcagaggt tgtcctggcg tggtttaggt | 180 |
| agtgtgagag gggaatgact cctttcggta agtgcagtgg aagctgtaca ctgcccaggc  | 240 |
| aaagcgtagc ggcagcgtag gcgggcgact cagatcccag ccagtggact tagcccctgt  | 300 |
| ttgctcctcc gataactggg gtgaccttgg ttaatatcca ccagcagcct cccccgttgc  | 360 |
| ccctctggat ccactgctta aatacggacg aggacactcg agggccctgt ctcctcagct  | 420 |
| tcaggcacca ccactgacct gggacagtga atccggacat cgattctaag gtaaataataa | 480 |
| aatttttaag tgtataattt gttaaactac tgattctaat tgtttctctc ttttagattc  | 540 |
| caacctttgg aactga                                                  | 556 |

<210> 77

<211> 1179

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 77

|                                                                    |      |
|--------------------------------------------------------------------|------|
| ggctccggtg cccgtcagtg ggcagagcgc acatcgccca cagtccccga gaagttgggg  | 60   |
| ggaggggtcg gcaattgaac cgggtgcctag agaaggtggc gcggggtaaa ctgggaaagt | 120  |
| gatgtcgtgt actggctccg cttttttccc gaggggtggg gagaaccgta tataagtgca  | 180  |
| gtagtcgccg tgaacgttct ttttcgcaac gggtttgccg ccagaacaca ggtaagtgcc  | 240  |
| gtgtgtggtt cccgcgggcc tggcctcttt acgggttatg gcccttgctg gccttgaatt  | 300  |
| acttcacact ggctgcagta cgtgattctt gatcccagac ttcgggttgg aagtgggtgg  | 360  |
| gagagttcga ggccttgccg ttaaggagcc ctttcgcctc gtgcttgagt tgaggcctgg  | 420  |
| cctgggcgct ggggccgccg cgtgcgaatc tgggtggcacc ttcgcgcctg tctcgtgct  | 480  |
| ttcgataagt ctctagccat ttaaaatttt tgatgacctg ctgcgacgct ttttttctgg  | 540  |
| caagatagtc ttgtaaatgc gggccaagat ctgcacactg gtatttcggt ttttggggcc  | 600  |
| gcgggcggcg acggggcccc tgcgtcccag cgcacatgtt cggcgaggcg gggcctgcga  | 660  |
| gcgcggccac cgagaatcgg acgggggtag tctcaagctg gccggcctgc tctggtgcct  | 720  |
| ggtctcgcgc cgccgtgtat cgccccgcc tgggcggcaa ggctggcccc gtcggcacca   | 780  |
| gttgcgtgag cggaaagatg gccgcttccc ggccctgctg caggagctc aaaatggagg   | 840  |
| acgcggcgct cgggagagcg ggcgggtgag tcaccacac aaaggaaaag ggcctttccg   | 900  |
| tcctcagccg tcgcttcatt tgactccacg gaggaccggg cgccgtccag gcacctgat   | 960  |
| tagttctcga gcttttgag tacgtcgtct ttaggttggg gggaggggtt ttatgcgatg   | 1020 |
| gagtttccc aactgagtg ggtggagact gaagttaggc cagcttggca cttgatgtaa    | 1080 |
| ttctccttgg aatttgcct ttttgagttt ggatcttggc tcatttctaa gcctcagaca   | 1140 |
| gtggttcaaa gtttttttct tccatttcag gtgtcgtga                         | 1179 |

<210> 78  
 <211> 141  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence: Synthetic polynucleotide  
  
 <400> 78  
 aataaacgat aacgccgttg gtggcgtgag gcatgtaaaa ggttacatca ttatcttggt 60  
 cgccatccgg ttggtataaa tagacgttca tgttggtttt tgtttcagtt gcaagttggc 120  
 tgcggcgcgc gcagcacctt t 141

<210> 79  
 <211> 317  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence: Synthetic polynucleotide  
  
 <400> 79  
 ggtgtggaaa gtccccaggc tccccagcag gcagaagtat gcaaagcatg catctcaatt 60  
 agtcagcaac caggtgtgga aagtccccag gctccccagc aggcagaagt atgcaaagca 120  
 tgcatctcaa ttagtcagca accatagtcc cgcccctaac tccgcccatac ccgcccctaa 180  
 ctccgcccag ttccgcccatac tctccgcccc atggctgact aatTTTTTTT atttatgcag 240  
 aggccgaggc cgctcggcc tctgagctat tccagaagta gtgaggaggc ttttttggag 300  
 gcctaggctt ttgcaaa 317

<210> 80  
 <211> 241  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence: Synthetic polynucleotide  
  
 <400> 80  
 gagggcctat ttcccatgat tccttcatat ttgcatatac gatacaaggc tgtagagag 60  
 ataattggaa ttaatttgac tgtaaacaca aagatattag tacaaaatac gtgacgtaga 120  
 aagtaataat ttcttgggta gtttgcagtt taaaattat gttttaaaat ggactatcat 180  
 atgcttaccg taacttgaaa gtatttcgat ttcttggctt tatatatctt gtggaaagga 240  
 c 241

<210> 81  
 <211> 215  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence: Synthetic

polynucleotide

<400> 81

|                                                                     |     |
|---------------------------------------------------------------------|-----|
| gaacgctgac gtcacatcaacc cgctccaagg aatcgcgggc ccagtgtcac taggcgggaa | 60  |
| caccagcgc gcgtgcgccc tggcaggaag atggctgtga gggacagggg agtggcgccc    | 120 |
| tgcaatatatt gcatgtcgtc atgtgttctg ggaaatcacc ataaacgtga aatgtctttg  | 180 |
| gatttgggaa tcgtataaga actgtatgag accac                              | 215 |

<210> 82

<211> 546

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 82

|                                                                    |     |
|--------------------------------------------------------------------|-----|
| ccctaaaatg ggcaaacatt gcaagcagca aacagcaaac acacagccct ccctgcctgc  | 60  |
| tgaccttgga gctggggcag aggtcagaga cctctctggg cccatgccac ctccaacatc  | 120 |
| cactcgaccc cttggaattt ttcggtggag aggagcagag gttgtcctgg cgtggtttag  | 180 |
| gtagtgtgag aggggaatga ctcttttcgg taagtgcagt ggaagctgta cactgcccag  | 240 |
| gcaaagcgtc cgggcagcgt aggcgggcga ctcatatccc agccagtgga cttagcccct  | 300 |
| gtttgctcct ccgataactg gggtagacctt ggttaatat caccagcagc ctccccggt   | 360 |
| gccccctctgg atccactgct taaatacggg cgaggacagg gccctgtctc ctacgcttca | 420 |
| ggcaccacca ctgacctggg acagtgaatc cggactctaa ggtaaatata aaatttttaa  | 480 |
| gtgtataatg tgtaaaacta ctgattctaa ttgtttctct ctttttagatt ccaacctttg | 540 |
| gaactg                                                             | 546 |

<210> 83

<211> 576

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 83

|                                                                   |     |
|-------------------------------------------------------------------|-----|
| tagtaatcaa ttacgggggtc attagttcat agcccatata tggagtccg cgttacataa | 60  |
| cttacggtaa atggcccgcc tggctgaccg cccaacgacc cccgcccatt gacgtcaata | 120 |
| atgacgtatg ttcccatagt aacgccaata gggactttcc attgacgtca atgggtggag | 180 |
| tatttacggt aaactgccc cttggcagta catcaagtgt atcatatgcc aagtacgccc  | 240 |
| cctattgacg tcaatgacgg taaatggccc gcctggcatt atgcccagta catgacctta | 300 |
| tgggactttc ctacttgga gtacatctac gtattagtca tcgctattac catggtgatg  | 360 |
| cggttttggc agtacatcaa tgggcgtgga tagcggtttg actcacgggg atttccaagt | 420 |
| ctccacccca ttgacgtcaa tgggagtttg ttttggcacc aaaatcaac ggactttcca  | 480 |

aaatgtcgta acaactccgc cccattgacg caaatgggcg gtaggcgtgt acggtgggag 540

gtctatataa gcagagctgg tttagtgaac cgtcag 576

<210> 84

<211> 150

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 84

ataaacgata acgccgttgg tggcgtgagg catgtaaaag gttacatcat tatcttggtc 60

gccatccggt tggatataat agacgttcat gttgggtttt gtttcagttg caagttggct 120

gcggcgcgcg cagcaccttt gcggccatct 150

<210> 85

<211> 1313

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 85

ggagccgaga gtaattcata caaaaggagg gatcgccctt gcaaggggag agcccaggga 60

ccgtccctaa attctcacag acccaaattc ctgtagccgc cccacgacag cgcgaggagc 120

atgcgcccag ggctgagcgc gggtagatca gagcacacaa gctcacagtc cccggcgggtg 180

gggggagggg cgcgctgagc gggggccagg gagctggcgc ggggcaaact gggaaagtgg 240

tgctgtgtgc tggctccgcc ctcttcccga ggggtggggga gaacggtata taagtgcggt 300

agtcgccttg gacgttcttt ttcgcaacgg gtttgccgtc agaacgcagg tgagtggcgg 360

gtgtggcttc cgcgggcccc ggagctggag ccctgctctg agcgggcccgg gctgatatgc 420

gagtgtcgtc cgcagggttt agctgtgagc attcccactt cgagtggcgg gcggtgcggg 480

ggtgagagtg cgaggcctag cggcaacccc gtagcctcgc ctctgtgtccg gcttgaggcc 540

tagcgtgggtg tccgccgccg cgtgccactc cggccgcact atgcgttttt tgccttgct 600

gccctcgatt gccttcacgc agcatgggct aacaaaggga ggggtgtgggg ctactctta 660

aggagcccat gaagcttacg ttggatagga atggaagggc aggaggggcg actggggccc 720

gcccgccttc ggagcacatg tccgacgcca cctggatggg gcgaggcctg tggctttccg 780

aagcaatcgg gcgtgagttt agcctacctg ggccatgtgg ccctagcact gggcacggtc 840

tggcctggcg gtgccgcgtt cccttgctc ccaacaaggg tgaggccgtc ccgccggca 900

ccagttgctt gcgcggaaag atggccgctc ccggggccct gttgcaagga gctcaaaatg 960

gaggacgcgg cagcccgggtg gagcgggcgg gtgagtcacc cacacaaagg aagagggcct 1020

tgcccctcgc cggccgctgc ttcctgtgac cccgtggtct atcggccgca tagtcacctc 1080

gggcttctct tgagcaccgc tcgtcgcggc ggggggaggg gatctaattg cgttggagtt 1140

|                                                                   |      |
|-------------------------------------------------------------------|------|
| tgttcacatt tggtaggtgg agactagtca ggccagcctg gcgctggaag tcattcttgg | 1200 |
| aatttgcccc tttagatttg gagcgaggct aattctcaag cctcttagcg gttcaaaggt | 1260 |
| atatttctaaa ccggtttcca ggtgttgtga aagccaccgc taattcaaag caa       | 1313 |

<210> 86  
 <211> 213  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic polynucleotide

|                                                                     |     |
|---------------------------------------------------------------------|-----|
| <400> 86                                                            |     |
| taagatacat tgatgagttt ggacaaacca caactagaat gcagtgaaaa aaatgcttta   | 60  |
| tttgtgaaat ttgtgatgct attgctttat ttgtaaccat tataagctgc aataaacaag   | 120 |
| ttaacaacaa caattgcatt catTTTTatgt ttcaggttca gggggagggtg tgggagggtt | 180 |
| tttaaagcaa gtaaaacctc tacaatgtg gta                                 | 213 |

<210> 87  
 <211> 7  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic peptide

|                             |   |
|-----------------------------|---|
| <400> 87                    |   |
| Pro Lys Lys Lys Arg Lys Val |   |
| 1                           | 5 |

<210> 88  
 <211> 19  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic peptide

|                                                                 |         |
|-----------------------------------------------------------------|---------|
| <400> 88                                                        |         |
| Met Asp Trp Thr Trp Arg Ile Leu Phe Leu Val Ala Ala Ala Thr Gly |         |
| 1                                                               | 5 10 15 |

Ala His Ser

<210> 89  
 <211> 19  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic peptide

<400> 89

Met Leu Pro Ser Gln Leu Ile Gly Phe Leu Leu Leu Trp Val Pro Ala  
1 5 10 15

Ser Arg Gly

<210> 90  
<211> 7  
<212> PRT  
<213> Simian virus 40

<400> 90  
Pro Lys Lys Lys Arg Lys Val  
1 5

<210> 91  
<211> 21  
<212> DNA  
<213> Simian virus 40

<400> 91  
cccaagaaga agaggaaggt g 21

<210> 92  
<211> 16  
<212> PRT  
<213> Unknown

<220>  
<223> Description of Unknown:  
Nucleoplasmin bipartite NLS sequence

<400> 92  
Lys Arg Pro Ala Ala Thr Lys Lys Ala Gly Gln Ala Lys Lys Lys Lys  
1 5 10 15

<210> 93  
<211> 9  
<212> PRT  
<213> Unknown

<220>  
<223> Description of Unknown:  
C-myc NLS sequence

<400> 93  
Pro Ala Ala Lys Arg Val Lys Leu Asp  
1 5

<210> 94  
<211> 11  
<212> PRT  
<213> Unknown

<220>  
<223> Description of Unknown:  
C-myc NLS sequence

<400> 94  
Arg Gln Arg Arg Asn Glu Leu Lys Arg Ser Pro  
1 5 10

<210> 95  
<211> 38  
<212> PRT  
<213> Homo sapiens

<400> 95  
Asn Gln Ser Ser Asn Phe Gly Pro Met Lys Gly Gly Asn Phe Gly Gly  
1 5 10 15

Arg Ser Ser Gly Pro Tyr Gly Gly Gly Gly Gln Tyr Phe Ala Lys Pro  
20 25 30

Arg Asn Gln Gly Gly Tyr  
35

<210> 96  
<211> 42  
<212> PRT  
<213> Unknown

<220>  
<223> Description of Unknown:  
IBB domain from importin-alpha sequence

<400> 96  
Arg Met Arg Ile Glx Phe Lys Asn Lys Gly Lys Asp Thr Ala Glu Leu  
1 5 10 15

Arg Arg Arg Arg Val Glu Val Ser Val Glu Leu Arg Lys Ala Lys Lys  
20 25 30

Asp Glu Gln Ile Leu Lys Arg Arg Asn Val  
35 40

<210> 97  
<211> 8  
<212> PRT  
<213> Unknown

<220>  
<223> Description of Unknown:  
Myoma T protein sequence

<400> 97  
Val Ser Arg Lys Arg Pro Arg Pro  
1 5

<210> 98  
<211> 8  
<212> PRT  
<213> Unknown

<220>  
<223> Description of Unknown:  
Myoma T protein sequence

<400> 98  
Pro Pro Lys Lys Ala Arg Glu Asp  
1 5

<210> 99

<211> 8  
<212> PRT  
<213> Homo sapiens

<400> 99  
Pro Gln Pro Lys Lys Lys Pro Leu  
1 5

<210> 100  
<211> 12  
<212> PRT  
<213> Mus musculus

<400> 100  
Ser Ala Leu Ile Lys Lys Lys Lys Lys Met Ala Pro  
1 5 10

<210> 101  
<211> 70  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
oligonucleotide

<400> 101  
gcgcgctcgc tcgctcactg aggccgcccg ggaaacccgg gcgtgcgctt cagtgcgga 60  
gcgagcgcgc 70

<210> 102  
<211> 70  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
oligonucleotide

<400> 102  
gcgcgctcgc tcgctcactg aggcgcacgc ccgggtttcc cgggcggcct cagtgcgga 60  
gcgagcgcgc 70

<210> 103  
<211> 72  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
oligonucleotide

<400> 103  
gcgcgctcgc tcgctcactg aggccgtcgg gcgacctttg gtcgcccggc ctgcgtgagc 60  
gagcgcgcgc gc 72

<210> 104  
<211> 72  
<212> DNA  
<213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic  
 oligonucleotide

<400> 104  
 gcgcgctcgc tcgctcactg aggccgggcg accaaaggtc gcccgcggc ctcagtgagc 60  
 gagcgagcgc gc 72

<210> 105  
 <211> 72  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic  
 oligonucleotide

<400> 105  
 gcgcgctcgc tcgctcactg aggccgccc ggcaaagccc gggcgtcggc ctcagtgagc 60  
 gagcgagcgc gc 72

<210> 106  
 <211> 72  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic  
 oligonucleotide

<400> 106  
 gcgcgctcgc tcgctcactg aggccgacgc ccgggctttg cccgggcggc ctcagtgagc 60  
 gagcgagcgc gc 72

<210> 107  
 <211> 83  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic  
 oligonucleotide

<400> 107  
 gcgcgctcgc tcgctcactg aggccgccc ggcaaagccc gggcgtcggg ctttgcccgg 60  
 cctcagttag cgagcgagcg cgc 83

<210> 108  
 <211> 83  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic  
 oligonucleotide

<400> 108  
 gcgcgctcgc tcgctcactg aggccgggca aagcccgcg cccgggcttt gccggggcgg 60  
 cctcagttag cgagcgagcg cgc 83

<210> 109  
 <211> 77  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence: Synthetic  
         oligonucleotide  
  
 <400> 109  
 gcgcgctcgc tcgctcactg aggccgaaac gtcgggcgac ctttggtcgc ccggcctcag 60  
 tgagcgagcg agcgcg 77

<210> 110  
 <211> 77  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence: Synthetic  
         oligonucleotide  
  
 <400> 110  
 gcgcgctcgc tcgctcactg aggccgggcg accaaaggct gcccgacgtt tcggcctcag 60  
 tgagcgagcg agcgcg 77

<210> 111  
 <211> 51  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence: Synthetic  
         oligonucleotide  
  
 <400> 111  
 gcgcgctcgc tcgctcactg aggcaaagcc tcagtgcgagcg agcgagcgcg c 51

<210> 112  
 <211> 51  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence: Synthetic  
         oligonucleotide  
  
 <400> 112  
 gcgcgctcgc tcgctcactg aggctttgcc tcagtgcgagcg agcgagcgcg c 51

<210> 113  
 <211> 80  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence: Synthetic  
         oligonucleotide  
  
 <400> 113  
 gcgcgctcgc tcgctcactg aggccgcccg ggcgtcgggc gacctttggt cgcccggcct 60

cagtgagcga gcgagcgcgc 80

<210> 114  
<211> 80  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
oligonucleotide

<400> 114  
gcgcgctcgc tcgctcactg aggccgggcg accaaaggtc gcccgcgccc cgggcggcct 60

cagtgagcga gcgagcgcgc 80

<210> 115  
<211> 79  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
oligonucleotide

<400> 115  
gcgcgctcgc tcgctcactg aggcgcccgg gcgtcgggcg acctttggtc gcccggcctc 60

agtgagcgag cgagcgcgc 79

<210> 116  
<211> 79  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
oligonucleotide

<400> 116  
gcgcgctcgc tcgctcactg aggccgggcg accaaaggtc gcccgcgccc cgggcgcctc 60

agtgagcgag cgagcgcgc 79

<210> 117  
<211> 5  
<212> PRT  
<213> Influenza virus

<400> 117  
Asp Arg Leu Arg Arg  
1 5

<210> 118  
<211> 7  
<212> PRT  
<213> Influenza virus

<400> 118  
Pro Lys Gln Lys Lys Arg Lys  
1 5

<210> 119

<211> 10  
<212> PRT  
<213> Hepatitis delta virus

<400> 119  
Arg Lys Leu Lys Lys Lys Ile Lys Lys Leu  
1 5 10

<210> 120  
<211> 10  
<212> PRT  
<213> Mus musculus

<400> 120  
Arg Glu Lys Lys Lys Phe Leu Lys Arg Arg  
1 5 10

<210> 121  
<211> 20  
<212> PRT  
<213> Homo sapiens

<400> 121  
Lys Arg Lys Gly Asp Glu Val Asp Gly Val Asp Glu Val Ala Lys Lys  
1 5 10 15

Lys Ser Lys Lys  
20

<210> 122  
<211> 17  
<212> PRT  
<213> Homo sapiens

<400> 122  
Arg Lys Cys Leu Gln Ala Gly Met Asn Leu Glu Ala Arg Lys Thr Lys  
1 5 10 15

Lys

<210> 123  
<211> 8  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
oligonucleotide

<400> 123  
gtttaaac

8

<210> 124  
<211> 8  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
oligonucleotide

<400> 124

ttaattaa

8

<210> 125

<211> 141

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 125

aataaacgat aacgccgttg gtggcgtgag gcatgtaaaa ggttacatca ttatcttggt 60

cgccatccgg ttggtataaa tagacgttca tgttggtttt tgtttcagtt gcaagttggc 120

tgcggcgcgc gcagcacctt t 141

<210> 126

<211> 317

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 126

ggtgtggaaa gtccccaggc tccccagcag gcagaagtat gcaaagcatg catctcaatt 60

agtcagcaac caggtgtgga aagtccccag gctccccagc aggcagaagt atgcaaagca 120

tgcattctcaa ttagtcagca accatagtcc cgcccctaac tccgcccata ccgcccctaa 180

ctccgcccag ttccgcccata tctccgcccc atggctgact aatttttttt atttatgcag 240

aggccgaggc cgctcggcc tctgagctat tccagaagta gtgaggaggc ttttttggag 300

gcctaggctt ttgcaaa 317

<210> 127

<211> 72

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 127

gcgcgctcgc tcgctcactg aggccgggcg accaaaggct gcccgacggc ctcagtgagc 60

gagcgagcgc gc 72

<210> 128

<211> 60

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 128

gagacagaca cactcctgct atgggtactg ctgctctggg ttccaggttc cactggtgac 60

<210> 129

<211> 1260

<212> DNA

<213> Adeno-associated virus - 2

<400> 129

atggagctgg tcgggtggct cgtggacaag gggattacct cggagaagca gtggatccag 60  
gaggaccagg cctcatatcat ctcttcaat gcggcctcca actcgcggtc ccaaatcaag 120  
gctgccttgg acaatgcggg aaagattatg agcctgacta aaaccgcccc cgactacctg 180  
gtgggccagc agcccgtgga ggacatttcc agcaatcgga ttataaaaat ttggaacta 240  
aacgggtacg atccccaata tgcggcttcc gtctttctgg gatggggcac gaaaaagttc 300  
ggcaagagga acaccatctg gctgtttggg cctgcaacta ccgggaagac caacatcgcg 360  
gaggccatag cccacactgt gcccttctac gggcgcgtaa actggaccaa tgagaacttt 420  
cccttcaacg actgtgtcga caagatgggt atctgggtggg aggaggggaa gatgaccgcc 480  
aaggtcgtgg agtcggccaa agccattctc ggaggaagca aggtgcgcgt ggaccagaaa 540  
tgcaagtcct cggcccagat agaccgcact cccgtgatcg tcacctcaa caccaacatg 600  
tgcgccgtga ttgacgggaa ctcaacgacc ttcgaacacc agcagccgtt gcaagaccgg 660  
atgttcaa at ttgaactcac ccgccgtctg gatcatgact ttgggaaggt caccaagcag 720  
gaagtcaaag actttttccg gtgggcaaag gatcacgtgg ttgaggtgga gcatgaattc 780  
tacgtcaaaa aggggtggagc caagaaaaga cccgccccca gtgacgcaga tataagttag 840  
cccaaaccgg tgccgcgagtc agttgcgcag ccatcgacgt cagacgcgga agcttcgac 900  
aactacgcag acaggtacca aaacaaatgt tctcgtcacg tgggcatgaa tctgatgctg 960  
tttccctgca gacaatgcga gagaatgaat cagaattcaa atatctgctt cactcacgga 1020  
cagaaagact gtttagagtg ctttcccgtg tcagaatctc aaccgtttc tgcgtcaaaa 1080  
aaggcgtatc agaaactgtg ctacattcat catatcatgg gaaaggtgcc agacgcttgc 1140  
actgcctgcg atctgggtcaa tgtggatttg gatgactgca tctttgaaca ataatgatt 1200  
taaatcaggt atggctgccg atggttatct tccagattgg ctcgaggaca ctctctctga 1260

<210> 130

<211> 1932

<212> DNA

<213> Adeno-associated virus - 2

<400> 130

atgccggggt ttacagatg tgtgattaag gtccccagcg accttgacga gcatctgccc 60  
ggcatttctg acagctttgt gaactgggtg gccgagaagg aatgggagtt gccgccagat 120  
tctgacatgg atctgaatct gattgagcag gcacccctga ccgtggccga gaagctgcag 180  
cgcgactttc tgacggaatg gcgccgtgtg agtaaggccc cggaggccct tttctttgtg 240  
caatttgaga agggagagag ctacttccac atgcacgtgc tcgtggaaac caccggggtg 300  
aaatccatgg ttttgggacg tttcctgagt cagattcgcg aaaaactgat tcagagaatt 360

|                                                                      |      |
|----------------------------------------------------------------------|------|
| taccgcggga tcgagccgac tttgccaac tggttcgcgg tcacaaagac cagaaatggc     | 420  |
| gccggaggcg ggaacaaggt ggtggatgag tgctacatcc ccaattactt gctcccaaaa    | 480  |
| accagcctg agctccagt ggcgtggact aatatggaac agtattttaag cgcctgtttg     | 540  |
| aatctcacgg agcgtaaacg gttggtggcg cagcatctga cgcacgtgtc gcagacgcag    | 600  |
| gagcagaaca aagagaatca gaatcccaat tctgatgcgc cggatgatcag atcaaaaact   | 660  |
| tcagccagggt acatggagct ggtcgggtgg ctctgtggaca aggggattac ctcgagagaag | 720  |
| cagtggatcc aggaggacca ggcctcatac atctccttca atgcggcctc caactcgcgg    | 780  |
| tcccaaatca aggctgcctt ggacaatgcg ggaaagatta tgagcctgac taaaaccgcc    | 840  |
| cccgactacc tgggtgggcca gcagcccgtg gaggacattt ccagcaatcg gatttataaa   | 900  |
| attttgaac taaacgggta cgatcccaa tatgcggcctt ccgtctttct gggatgggccc    | 960  |
| acgaaaaagt tcggcaagag gaacaccatc tggctgtttt ggcctgcaac taccgggaag    | 1020 |
| accaacatcg cggaggccat agcccacact gtgcccttct acgggtgcgt aaactggacc    | 1080 |
| aatgagaact ttcccttcaa cgactgtgtc gacaagatgg tgatctggtg ggaggagggg    | 1140 |
| aagatgaccg ccaaggtcgt ggagtcggcc aaagccattc tcggaggaag caaggtgcgc    | 1200 |
| gtggaccaga aatgcaagtc ctcgcccag atagaccga ctcccgtgat cgtcacctcc      | 1260 |
| aacaccaaca tgtgcgccgt gattgacggg aactcaacga ccttcgaaca ccagcagccg    | 1320 |
| ttgcaagacc ggatgttcaa atttgaactc acccgccgtc tggatcatga ctttgggaag    | 1380 |
| gtcaccaagc aggaagtcaa agactttttc cgggtgggcaa aggatcacgt ggttgaggtg   | 1440 |
| gagcatgaat tctacgtcaa aaagggtgga gccaaagaaa gaccgcgcc cagtgcgca      | 1500 |
| gatataagt agcccaaacg ggtgcgcgag tcagttgcgc agccatcgac gtcagacgcg     | 1560 |
| gaagcttcga tcaactacgc agacaggtac caaaacaaat gttctcgtca cgtgggcatg    | 1620 |
| aatctgatgc tgtttccctg cagacaatgc gagagaatga atcagaattc aaatatctgc    | 1680 |
| ttcactcacg gacagaaaga ctgtttagag tgctttcccg tgtcagaatc tcaaccggtt    | 1740 |
| tctgtcgtca aaaaggcgta tcagaaactg tgctacattc atcatatcat gggaaagggtg   | 1800 |
| ccagacgctt gactgcctg cgatctggtc aatgtggatt tggatgactg catctttgaa     | 1860 |
| caataaatga tttaaatcag gtatggctgc cgatggttat cttccagatt ggctcgagga    | 1920 |
| cactctctct ga                                                        | 1932 |

<210> 131

<211> 1876

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 131

|                                                                 |    |
|-----------------------------------------------------------------|----|
| gcagaccacc atggcggggt ttacgagat tgtgattaag gtcccagcg accttgacgg | 60 |
|-----------------------------------------------------------------|----|

|                                                                   |     |
|-------------------------------------------------------------------|-----|
| gcactctgcc ggcatctctg acagctttgt gaactgggtg gccgagaagg aatgggagtt | 120 |
|-------------------------------------------------------------------|-----|

|                                                                    |      |
|--------------------------------------------------------------------|------|
| gccgccagat tctgacatgg atctgaatct gattgagcag gcacccctga ccgtggccga  | 180  |
| gaagctgcag cgcgactttc tgacggaatg gcgccgtgtg agtaaggccc cggaggccct  | 240  |
| tttctttgtg caatttgaga aggagagag ctacttccac atgcacgtgc tcgtggaaac   | 300  |
| caccggggtg aaatccatgg ttttgggacg tttcctgagt cagattcgcg aaaaactgat  | 360  |
| tcagagaatt taccgcggga tcgagccgac tttgccaaac tggttcgcgg tcacaaagac  | 420  |
| cagaaatggc gccggaggcg ggaacaaggt ggtggatgag tgctacatcc ccaattactt  | 480  |
| gctcccaaaa acccagcctg agctccagtg ggcgtggact aatatggaac agtatttaag  | 540  |
| cgcctgtttg aatctcacgg agcgtaaacg gttggtggcg cagcatctga cgcacgtgtc  | 600  |
| gcagacgcag gagcagaaca aagagaatca gaatcccaat tctgatgcgc cggatgatcag | 660  |
| atcaaaaact tcagccagggt acatggagct ggtcgggtgg ctcgtggaca aggggattac | 720  |
| ctcggagaag cagtggatcc aggaggacca ggcctcatac atctccttca atgcggcctc  | 780  |
| caactcgcgg tcccaaatca aggctgcctt ggacaatgcg ggaaagatta tgagcctgac  | 840  |
| taaaaccgcc cccgactacc tgggtgggcca gcagcccgtg gaggacattt ccagcaatcg | 900  |
| gatttataaa attttgaac taaacgggta cgatcccaa tatgcggctt ccgtctttct    | 960  |
| gggatgggcc acgaaaaagt tcggcaagag gaacaccatc tggctgtttg ggcctgcaac  | 1020 |
| taccgggaag accaaccatcg cggaggccat agcccacact gtgcccttct acgggtgcgt | 1080 |
| aaactggacc aatgagaact ttcccttcaa cgactgtgtc gacaagatgg tgatctggtg  | 1140 |
| ggaggagggg aagatgaccg ccaaggctgt ggagtcggcc aaagccattc tcggaggaag  | 1200 |
| caaggtgcgc gtggaccaga aatgcaatgc ctcggcccg atagaccga ctcccgtgat    | 1260 |
| cgtcacctcc aacaccaaca tgtgcgccgt gattgacggg aactcaacga ctttgaaca   | 1320 |
| ccagcagccg ttgcaagacc ggatgttcaa atttgaactc acccgccgtc tggatcatga  | 1380 |
| ctttgggaag gtcaccaagc aggaagtcaa agactttttc cggatgggcaa aggatcacgt | 1440 |
| ggttgaggtg gagcatgaat tctacgtcaa aaagggtgga gccaaagaaa gaccgcccc   | 1500 |
| cagtgcgcga gatataagtg agcccaaacg ggtgcgcgag tcagttgcgc agccatcgac  | 1560 |
| gtcagacgcg gaagcttcga tcaactacgc agacaggtac caaaacaaat gttctcgtca  | 1620 |
| cgtgggcatg aatctgatgc tgtttccctg cagacaatgc gagagaatga atcagaattc  | 1680 |
| aaatatctgc ttcactcacg gacagaaaga ctgttttagag tgctttcccg tgtcagaatc | 1740 |
| tcaaccggtt tctgtcgtca aaaaggcgta tcagaaactg tgctacattc atcatatcat  | 1800 |
| gggaaaggtg ccagacgctt gcactgcctg cgatctggtc aatgtggatt tggatgactg  | 1860 |
| catctttgaa caataa                                                  | 1876 |

<210> 132

<211> 1194

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic

polynucleotide

<400> 132

|                                                                    |      |
|--------------------------------------------------------------------|------|
| atggagctgg tcgggtggct cgtggacaag gggattacct cggagaagca gtggatccag  | 60   |
| gaggaccagg cctcatacat ctccttcaat gcggcctcca actcgcggtc ccaaatacaag | 120  |
| gctgccttgg acaatgcggg aaagattatg agcctgacta aaaccgcccc cgactacctg  | 180  |
| gtgggccagc agcccgtgga ggacatttcc agcaatcgga tttataaaat tttggaacta  | 240  |
| aacgggtacg atccccaata tgcggcttcc gtctttctgg gatgggccac gaaaaagttc  | 300  |
| ggcaagagga acaccatctg gctgtttggg cctgcaacta ccgggaagac caacatcgcg  | 360  |
| gaggccatag cccacactgt gcccttctac ggggtcgtaa actggaccaa tgagaacttt  | 420  |
| cccttcaacg actgtgtcga caagatggtg atctggtggg aggaggggaa gatgaccgcc  | 480  |
| aaggctcgtg agtcggccaa agccattctc ggaggaagca aggtgcgctg ggaccagaaa  | 540  |
| tgcaagtcct cggcccagat agaccgact cccgtgatcg tcacctcaa caccaacatg    | 600  |
| tgcgccgtga ttgacgggaa ctcaacgacc ttcgaacacc agcagccgtt gcaagaccgg  | 660  |
| atgttcaaat ttgaactcac ccgccgtctg gatcatgact ttgggaaggt caccaagcag  | 720  |
| gaagtcaaag actttttccg gtgggcaaag gatcacgtgg ttgaggtgga gcatgaattc  | 780  |
| tacgtcaaaa aggggtggagc caagaaaaga cccgccccca gtgacgcaga tataagtgag | 840  |
| cccaaacggg tgcgcgagtc agttgcgcag ccatcgacgt cagacgcgga agcttcgatc  | 900  |
| aactacgcag accgctacca aaacaaatgt tctcgtcacg tgggcatgaa tctgatgctg  | 960  |
| tttccctgca gacaatgcga gagaatgaat cagaattcaa atatctgctt cactcacgga  | 1020 |
| cagaaagact gtttagagtg ctttcccgtg tcagaatctc aaccgtttc tgtcgtcaaa   | 1080 |
| aaggcgtatc agaaactgtg ctacattcat catatcatgg gaaaggtgcc agacgcttgc  | 1140 |
| actgcctgcg atctggtcaa tgttgatttg gatgactgca tctttgaaca ataa        | 1194 |

<210> 133

<211> 1876

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
polynucleotide

<400> 133

|                                                                    |     |
|--------------------------------------------------------------------|-----|
| cgagccacc atggcggggt tttacgagat tgtgattaag gtccccagcg accttgacgg   | 60  |
| gcatctgccc ggcatcttctg acagctttgt gaactgggtg gccgagaagg aatgggagtt | 120 |
| gccgccagat tctgacatgg atctgaatct gattgagcag gcacccctga ccgtggccga  | 180 |
| gaagctgcag cgcgactttc tgacggaatg gcgccgtgtg agtaaggccc cggaggccct  | 240 |
| tttctttgtg caatttgaga agggagagag ctactttcac atgcacgtgc tcgttgaaac  | 300 |
| caccgggggtg aaatccatgg ttttgggacg tttcctgagt cagattcgcg aaaaactgat | 360 |
| tcagagaatt taccgcggga tcgagccgac tttgcaaac tggttcgagg tcacaaagac   | 420 |
| cagaaatggc gccggaggcg ggaacaaggt ggtggatgag tgctacatcc ccaattactt  | 480 |

|                                                                    |      |
|--------------------------------------------------------------------|------|
| gctcccaaaa acccagcctg agctccagtg ggcgtggact aatatggaac agtattttaag | 540  |
| cgcctgtttg aatctcacgg agcgtaaacg gttggtggcg cagcatctga cgcacgtgtc  | 600  |
| gcagacgcag gagcagaaca aagagaatca gaatcccaat tctgatgcgc cggatgatcag | 660  |
| atcaaaaact tcagccaggt acatggagct ggtcgggtgg ctctgtggaca aggggattac | 720  |
| ctcggagaag cagtggatcc aggaggacca ggcctcatat atctccttca atgcggcctc  | 780  |
| caactcgcgg tcccaaatca aggctgcctt ggacaatgcg ggaaagatta tgagcctgac  | 840  |
| taaaaccgcc cccgactacc tgggtgggcca gcagcccgtg gaggacattt ccagcaatcg | 900  |
| gatttataaa attttggaaac taaacgggta cgatcccaa tatgcggctt ccgtctttct  | 960  |
| gggatgggcc acgaaaaagt tcggcaagag gaacaccatc tggctgtttg ggcctgcaac  | 1020 |
| taccgggaag accaacaatcg cggaggccat agcccacact gtgcccttct acgggtgcgt | 1080 |
| aaactggacc aatgagaact ttcccttcaa cgactgtgtc gacaagatgg tgatctggtg  | 1140 |
| ggaggagggg aagatgaccg ccaaggtcgt ggagtcggcc aaagccattc tcggaggaag  | 1200 |
| caaggtgcgc gtggaccaga aatgcaagtc ctcggccag atagaccga ctcccgtgat    | 1260 |
| cgtcacctcc aacaccaaca tgtgcgccgt gattgacggg aactcaacga ctttgaaca   | 1320 |
| ccagcagccg ttgcaagacc ggatgttcaa atttgaactc acccgccgtc tggatcatga  | 1380 |
| ctttgggaag gtcaccaagc aggaagtcaa agactttttc cgggtgggcaa aggatcacgt | 1440 |
| ggttgagggtg gagcatgaat tctacgtcaa aaagggtgga gccaagaaaa gacccgcccc | 1500 |
| cagtgcgca gatataagtg agcccaaacg ggtgcgcgag tcagttgcgc agccatcgac   | 1560 |
| gtcagacgcg gaagcttcga tcaactacgc agacaggtac caaaacaaat gttctcgtca  | 1620 |
| cgtgggcatg aatctgatgc tgtttccctg cagacaatgc gagagaatga atcagaattc  | 1680 |
| aaatatctgc ttcactcacg gacagaaaga ctgttttagag tgctttcccg tgtcagaatc | 1740 |
| tcaaccggtt tctgtcgtca aaaaggcgta tcagaaactg tgctacattc atcatatcat  | 1800 |
| gggaaagggtg ccagacgctt gcactgcctg cgatctggtc aatgtggatt tggatgactg | 1860 |
| catctttgaa caataa                                                  | 1876 |

<210> 134  
 <211> 51  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic  
 oligonucleotide

|                                                        |    |
|--------------------------------------------------------|----|
| <400> 134                                              |    |
| ctaggccgcc cgggcaaagc ccgggcgtcg ggcgacctt ggtcgcccg c | 51 |

<210> 135  
 <211> 65  
 <212> DNA  
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
oligonucleotide

<400> 135  
ctaggactga ggccgcccgg gcaaagcccg ggcgtcgggc gacctttggt cgcccggcct 60  
cagtc 65

<210> 136  
<211> 67  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
oligonucleotide

<400> 136  
ggactgaggc cgcccgggca aagcccgggc gtcgggcgac ctttggtcgc ccggcctcag 60  
tcctgca 67

<210> 137  
<211> 41  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
oligonucleotide

<400> 137  
gtgcgggcga ccaaaggtcg cccgacgccc gggcgcactc a 41

<210> 138  
<211> 56  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
oligonucleotide

<400> 138  
ggactgaggc cgggcgacca aaggctgccc gacgcccggg cggcctcagt cctgca 56

<210> 139  
<211> 54  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
oligonucleotide

<400> 139  
ctaggactga ggccgcccgg gcgtcgggcg acctttggtc gcccggcctc agtc 54

<210> 140  
<211> 48  
<212> DNA  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 140  
ggactgaggc cgggcgacca aaggctgccc gacggcctca gtcctgca 48

<210> 141  
<211> 46  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 141  
ctaggactga ggccgtcggg cgacctttgg tcgcccggcc tcagtc 46

<210> 142  
<211> 67  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 142  
ggactgaggc ccgggcgacc aaaggctgcc cgacgcccgg gctttgcccg ggcgcctcag 60  
tcctgca 67

<210> 143  
<211> 47  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 143  
atacctaggc acgcgtgtta ctagttatta atagtaatca attacgg 47

<210> 144  
<211> 29  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 144  
atacctaggg gccgcacgcg tgttactag 29

<210> 145  
<211> 42  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 145  
atacactcag tgcctgcagg cacgtggtcc ggagatccag ac 42

<210> 146  
<211> 3754  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
polynucleotide

<400> 146  
cctaggtgag cgagcgagcg cgcagagagg gagtggccaa ctccatcact aggggttcct 60  
tgtagttaat gattaacccg ccatgctact tatcgcggcc gctcaatatt ggccattagc 120  
catattattc attggttata tagcataaat caatattggc tattggccat tgcatacgtt 180  
gtatctatat cataatatgt acatttatat tggctcatgt ccaatatgac cgccatgttg 240  
gcattgatta ttgactagtt attaatagta atcaattacg gggtcattag ttcatagccc 300  
atatatggag ttccgcgtta cataacttac ggtaaattggc ccgcctggct gaccgcccaa 360  
cgacccccgc ccattgacgt caataatgac gtatgttccc atagtaacgc caatagggac 420  
tttccattga cgtcaatggg tggagtatth acggtaaact gcccacttgg cagtacatca 480  
agtgtatcat atgccaagtc cccccctat tgacgtcaat gacggtaaht ggcccgcctg 540  
gcattatgcc cagtacatga ccttacggga ctttcctact tggcagtaca tctacgtatt 600  
agtcatcgct attaccatgg tgcaggtgag cccacgttc tgcttcactc tccccatctc 660  
ccccccctcc ccacccccaa ttttgtatth atthatttht taattattht gtgcagcgat 720  
gggggcgggg gggggggggg ggcgcgcgcc aggcggggcg gggcggggcg aggggcgggg 780  
cggggcgagg cggagaggtg cggcggcagc caatcagagc ggcgcgctcc gaaagtttcc 840  
ttttatggcg aggcggcggc ggcggcggcc ctataaaaag cgaagcgcgc ggcgggcggg 900  
agtgcgtgcg acgctgcctt cccccgtgc cccgctccgc cgcgcctcg cgcgcccgc 960  
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cgggctgtaa ttagcgcttg gtttaatgac ggcttgtht ttttctgtgg ctgcgtgaaa 1080  
gccttgaggg gctccgggag ggcctthtgt gcggggggga gcggctcggg ggggtgcgtgc 1140  
gtgtgtgtgt gcgtggggag gcgcgcgtgc ggcccgcgt gcccggggc tgtgagcgct 1200  
gcgggcgcgg gcgggggctt tgtgcgtcc gcagtgtgcg cgaggggagc gcggccgggg 1260  
gcggtgcccc gcggtgcggg gggggctgcg aggggaacaa aggctgcgtg cggggtgtgt 1320  
gcgtgggggg gtgagcaggg ggtgtgggcg cggcggtcgg gctgtaacct cccctgcac 1380  
ccccctccc gagttgtga gcacggccc gcttcgggtg cggggctccg tacggggcgt 1440  
ggcgcggggc tcgccgtgcc gggcgggggg tggcggcagg tgggggtgcc gggcggggcg 1500  
gggccgcctc gggccgggga gggctcgggg gaggggcgcg gcggccccg gagcgccggc 1560  
ggctgtcgag gcgcggcgag ccgcagccat tgccttht atgtaatcgt cgagagggcg 1620

|            |             |             |            |             |            |      |
|------------|-------------|-------------|------------|-------------|------------|------|
| cagggacttc | ctttgtccca  | aatctgtgcg  | gagccgaaat | ctgggaggcg  | ccgccgcacc | 1680 |
| ccctctagcg | ggcgcggggc  | gaagcgggtgc | ggcgccggca | ggaaggaaat  | gggcggggag | 1740 |
| ggccttcgtg | cgtcgccgcg  | ccgccgtccc  | cttctccctc | tccagcctcg  | gggctgtccg | 1800 |
| cggggggacg | gctgccttcg  | ggggggacgg  | ggcagggcgg | ggttcggcct  | ctggcgtgtg | 1860 |
| accggcggct | ctagagcctc  | tgctaaccat  | gttttagcct | tcttcttttt  | cctacagctc | 1920 |
| ctgggcaacg | tgctggttat  | tgtgctgtct  | catcatttgt | cgacagaatt  | cctcgaagat | 1980 |
| ccgaaggggt | tcaagcttgg  | cattccggta  | ctgttggtaa | agccagttta  | aacgccgcca | 2040 |
| ccatggtgag | caagggcgag  | gagctgttca  | ccgggggtgg | gcccatacctg | gtcgagctgg | 2100 |
| acggcgacgt | aaacggccac  | aagttcagcg  | tgtccggcga | gggcgagggc  | gatgccacct | 2160 |
| acggcaagct | gacctgaag   | ttcatctgca  | ccaccggcaa | gctgcccgtg  | ccctggccca | 2220 |
| ccctcgtgac | cacctgacc   | tacggcgtgc  | agtgtttcag | ccgctacccc  | gaccacatga | 2280 |
| agcagcacga | cttcttcaag  | tccgccatgc  | ccgaaggcta | cgtccaggag  | cgcaccatct | 2340 |
| tcttcaagga | cgacggcaac  | tacaagacct  | gcgccgaggt | gaagttcgag  | ggcgacaccc | 2400 |
| tggtgaaccg | catcgagctg  | aagggcatcg  | acttcaagga | ggacggcaac  | atcctggggc | 2460 |
| acaagctgga | gtacaactac  | aacagccaca  | acgtctatat | catggccgac  | aagcagaaga | 2520 |
| acggcatcaa | ggtgaacttc  | aagatccgcc  | acaacatcga | ggacggcagc  | gtgcagctcg | 2580 |
| ccgaccacta | ccagcagaac  | acccccatcg  | gcgacggccc | cgtgctgctg  | cccgacaacc | 2640 |
| actacctgag | cacccagtcc  | gccctgagca  | aagaccccaa | cgagaagcgc  | gatcacatgg | 2700 |
| tcctgctgga | gttcgtgacc  | gccgccggga  | tcactctcgg | catggacgag  | ctgtacaagt | 2760 |
| aattaattaa | gagcatctta  | ccgccattta  | ttcccatatt | tgttctgttt  | ttcttgattt | 2820 |
| gggtatacat | ttaaattgta  | ataaaacaaa  | atggtggggc | aatcatttac  | atttttaggg | 2880 |
| atatgtaatt | actagttcag  | gtgtattgcc  | acaagacaaa | catgttaaga  | aactttcccg | 2940 |
| ttattttacg | tctgttcctg  | ttaatcaacc  | tctggattac | aaaatttgtg  | aaagattgac | 3000 |
| tgatattctt | aactatgttg  | ctccttttac  | gctgtgtgga | tatgctgctt  | tatagcctct | 3060 |
| gtatctagct | attgcttccc  | gtacggcttt  | cgttttctcc | tccttgata   | aatcctgggt | 3120 |
| gctgtctctt | ttagaggagt  | tgtggcccgt  | tgtccgtcaa | cgtggcgtgg  | tgtgctctgt | 3180 |
| gtttgctgac | gcaaccccc   | ctggctgggg  | cattgccacc | acctgtcaac  | tcctttctgg | 3240 |
| gactttcgct | ttccccctcc  | cgatcgccac  | ggcagaactc | atcgccgcct  | gccttgcccc | 3300 |
| ctgctggaca | ggggctaggt  | tgctgggcac  | tgataattcc | gtggtgttgt  | ctgtgccttc | 3360 |
| tagttgccag | ccatctgttg  | tttgcccctc  | ccccgtgcct | tccttgacct  | tggaagggtc | 3420 |
| cactcccact | gtcctttcct  | aataaaatga  | ggaaattgca | tcgcattgtc  | tgagtaggtg | 3480 |
| tcattctatt | ctgggggggtg | gggtggggca  | ggacagcaag | ggggaggatt  | gggaagacaa | 3540 |
| tagcaggcat | gctggggatg  | cggtgggctc  | tatggctcta | gagcatggct  | acgtagataa | 3600 |
| gtagcatggc | gggttaatca  | ttaactacac  | ctgcagcagg | aaccctagtg  | gatggagtgt | 3660 |
| gccactccct | ctctgcgcgc  | tcgctcgctc  | cctgcaggac | tgaggccggg  | cgaccaaagg | 3720 |

tcgcccgcacg cccgggcggc ctcagtcctg cagg

3754

<210> 147

<211> 8418

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 147

|                                                                   |      |
|-------------------------------------------------------------------|------|
| ggcagctgcg cgctcgctcg ctcacctagg ccgcccgggc aaagcccggg cgtcgggcga | 60   |
| cctttggtcg cccggcctag gtgagcgagc gagcgcgag agagggagtg gccaaactcca | 120  |
| tcactagggg ttctttgtag ttaatgatta acccgccatg ctacttatcg cggccgctca | 180  |
| atattggcca ttagccatat tattcattgg ttatatagca taaatcaata ttggctattg | 240  |
| gccattgcat acgttgatc tatatcataa tatgtacatt tatattggct catgtccaat  | 300  |
| atgaccgcca tgttggcatt gattattgac tagttattaa tagtaatcaa ttacggggtc | 360  |
| attagttcat agcccatata tggagtccg cgttacataa cttacggtaa atggcccgcc  | 420  |
| tggctgaccg cccaacgacc cccgcccatt gacgtcaata atgacgatg ttcccatagt  | 480  |
| aacgccaata gggactttcc attgacgtca atgggtggag tatttacggt aaactgcca  | 540  |
| cttggcagta catcaagtgt atcatatgcc aagtcgccc cctattgacg tcaatgacgg  | 600  |
| taaatggccc gcctggcatt atgcccagta catgacctta cgggactttc ctacttggca | 660  |
| gtacatctac gtattagtca tcgctattac catggtcgag gtgagcccca cgttctgctt | 720  |
| cactctcccc atctcccccc cctccccacc cccaattttg tatttattta ttttttaatt | 780  |
| attttgtgca gcgatggggg cggggggggg gggggggcg gcgccaggcg gggcggggcg  | 840  |
| gggcgagggg cggggcgggg cgaggcggag aggtgcggcg gcagccaatc agagcggcg  | 900  |
| gctccgaaag tttcctttta tggcgaggcg gcggcggcgg cggccctata aaaagcgaag | 960  |
| cgcgcgggcg gcgggagtcg ctgcgacgct gccttcgccc cgtgccccgc tccgccgccg | 1020 |
| cctcgcgccg cccgccccgg ctctgactga ccgcgttact cccacagggt agcgggcggg | 1080 |
| acggcccttc tcctccgggc tgtaattagc gcttggttta atgacggctt gtttcttttc | 1140 |
| tgtggctgcg tgaagcctt gaggggctcc gggaggggcc tttgtgcggg ggggagcggc  | 1200 |
| tcggggggtg cgtgcgtgtg tgtgtgcgtg gggagcgccg cgtgcggccc gcgctgcccg | 1260 |
| gcggctgtga gcgctgcggg gcggcgcggg ggctttgtgc gctccgcagt gtgcgcgagg | 1320 |
| ggagcgcggc cggggcggtt gccccggtt gcgggggggg ctgcgagggg aacaaaggct  | 1380 |
| gcgtgcgggg tgtgtgcgtg ggggggtgag cagggggtgt gggcgcgcg gtcgggctgt  | 1440 |
| aacccccccc tgcaccccc tccccagatt gctgagcacg gcccggttc gggcgcgggg   | 1500 |
| ctccgtacgg ggcgtggcgc ggggctcgcc gtgccgggcg gggggtggcg gcaggtgggg | 1560 |
| gtgccgggcg gggcggggcc gcctcgggcc ggggagggct cgggggaggg gcgcggcg   | 1620 |
| ccccggagcg ccggcggctg tcgaggcgcg gcgagccga gccattgcct tttatggtaa  | 1680 |

|            |            |             |             |             |            |      |
|------------|------------|-------------|-------------|-------------|------------|------|
| tcgtgcgaga | gggcgaggg  | acttcctttg  | tcccaaactct | gtgcggagcc  | gaaatctggg | 1740 |
| aggcgccgcc | gcaccccctc | tagcgggcgc  | ggggcgaagc  | ggtgcggcgc  | cggcaggaag | 1800 |
| gaaatgggcg | gggagggcct | tcgtgcgtcg  | ccgcgccgcc  | gtccccttct  | ccctctccag | 1860 |
| cctcggggct | gtccgcgggg | ggacggctgc  | cttcgggggg  | gacggggcag  | ggcggggttc | 1920 |
| ggcttctggc | gtgtgaccgg | cggctctaga  | gcctctgcta  | accatgtttt  | agccttcttc | 1980 |
| tttttcctac | agctcctggg | caacgtgctg  | gttattgtgc  | tgtctcatca  | tttgtcgaca | 2040 |
| gaattcctcg | aagatccgaa | ggggttcaag  | cttggcattc  | cgggtactgtt | ggtaaagcca | 2100 |
| gtttaaacgc | cgccaccatg | gtgagcaagg  | gcgaggagct  | gttcaccggg  | gtggtgcca  | 2160 |
| tcctgggtcg | gctggacggc | gacgtaaacg  | gccacaagtt  | cagcgtgtcc  | ggcgagggcg | 2220 |
| agggcgatgc | cacctacggc | aagctgacct  | tgaagtctat  | ctgcaccacc  | ggcaagctgc | 2280 |
| ccgtgccctg | gcccaccctc | gtgaccaccc  | tgacctacgg  | cgtgcagtgc  | ttcagccgct | 2340 |
| accccgacca | catgaagcag | cacgacttct  | tcaagtccgc  | catgcccga   | ggctacgtcc | 2400 |
| aggagcgcac | catcttcttc | aaggacgacg  | gcaactacaa  | gacccgcgcc  | gaggtgaagt | 2460 |
| tcgagggcga | caccttggtg | aaccgcatcg  | agctgaaggg  | catcgacttc  | aaggaggacg | 2520 |
| gcaacatcct | ggggcacaag | ctggagtaca  | actacaacag  | ccacaacgtc  | tatatcatgg | 2580 |
| ccgacaagca | gaagaacggc | atcaaggtga  | acttcaagat  | ccgccacaac  | atcgaggacg | 2640 |
| gcagcgtgca | gctcgccgac | cactaccagc  | agaacacccc  | catcggcgac  | ggccccgtgc | 2700 |
| tgctgcccga | caaccactac | ctgagcaccc  | agtccgccct  | gagcaaagac  | cccaacgaga | 2760 |
| agcgcgatca | catggtcctg | ctggagttcg  | tgaccgccgc  | cgggatcact  | ctcggcatgg | 2820 |
| acgagctgta | caagtaatta | attaagagca  | tcttaccgcc  | atttattccc  | atatttgttc | 2880 |
| tgtttttctt | gatttggtga | tacatttaaa  | tgtaataaaa  | acaaaatggg  | ggggcaatca | 2940 |
| tttacatttt | tagggatatg | taattactag  | ttcaggtgta  | ttgccacaag  | acaaacatgt | 3000 |
| taagaaactt | tcccgttatt | tacgctctgt  | tcctgttaat  | caacctctgg  | attacaaaat | 3060 |
| ttgtgaaaga | ttgactgata | ttcttaacta  | tgttgctcct  | tttacgctgt  | gtggatatgc | 3120 |
| tgctttatag | cctctgtatc | tagctatttg  | ttcccgtacg  | gctttcgttt  | tctcctcctt | 3180 |
| gtataaatcc | tggttgctgt | ctctttttaga | ggagttgtgg  | cccgttgtcc  | gtcaacgtgg | 3240 |
| cgtgggtgtc | tctgtgtttg | ctgacgcaac  | ccccactggc  | tggggcattg  | ccaccactg  | 3300 |
| tcaactcctt | tctgggactt | tcgctttccc  | cctcccgatc  | gccacggcag  | aactcatcgc | 3360 |
| cgcctgcctt | gcccgtgct  | ggacaggggc  | taggttgctg  | ggcactgata  | attccgtggt | 3420 |
| gttgctgtg  | ccttctagtt | gccagccatc  | tgttgtttgc  | ccctcccccg  | tgcttctctt | 3480 |
| gacctggaa  | ggtgccactc | ccactgtcct  | ttcctaataa  | aatgaggaaa  | ttgcatcgca | 3540 |
| ttgtctgagt | aggtgtcatt | ctattctggg  | gggtgggggtg | gggcaggaca  | gcaaggggga | 3600 |
| ggattgggaa | gacaatagca | ggcatgctgg  | ggatgcggtg  | ggctctatgg  | ctctagagca | 3660 |
| tggctacgta | gataagtagc | atggcggggtt | aatcattaac  | tacacctgca  | gcaggaaccc | 3720 |

|                                                                   |      |
|-------------------------------------------------------------------|------|
| ctagtgatgg agttggccac tccctctctg cgcgctcgct cgctccctgc aggactgagg | 3780 |
| ccgggcgacc aaaggtcgcc cgacgcccgg gcggcctcag tcctgcaggg agcgagcgag | 3840 |
| cgcgagctg cctgcacggg cgcgccggta ccgggagatg ggggaggcta actgaaacac  | 3900 |
| ggaaggagac aataaccgaa ggaacccgcg ctatgacggc aataaaaaga cagaataaaa | 3960 |
| cgcacgggtg ttgggtcggt ttgtcataaa cgcgggggtt ggtcccaggg ctggcactct | 4020 |
| gtcgataccc caccgagacc ccattgggac caatacgccc gcgtttcttc cttttcccca | 4080 |
| ccccaacccc caagttcggg tgaaggccca gggctcgag ccaacgtcgg ggcggaagc   | 4140 |
| cctgccatag ccactacggg tacgtaggcc aaccactaga actatagcta gagtctggg  | 4200 |
| cgaacaaacg atgctcgctt tccagaaaac cgaggatgcg aaccacttca tccggggtca | 4260 |
| gcaccaccgg caagcgccgc gacggccgag gtctaccgat ctctgaagc cagggcagat  | 4320 |
| ccgtgcacag caccttgccg tagaagaaca gcaaggccgc caatgcctga cgatgcgtgg | 4380 |
| agaccgaaac cttgcgctcg ttgccagcc aggacagaaa tgcctcgact tcgctgctgc  | 4440 |
| ccaaggttgc cgggtgacgc acaccgtgga aacggatgaa ggcacgaacc cagttgacat | 4500 |
| aagcctgttc ggttcgtaaa ctgtaatgca agtagcgat gcgctcacgc aactggtcca  | 4560 |
| gaaccttgac cgaacgcagc ggtggtaacg gcgcagtggc ggttttcatg gcttgttatg | 4620 |
| actgtttttt tgtacagtct atgcctcggg catccaagca gcaagcgctg tacgccgtgg | 4680 |
| gtcgatgttt gatgttatgg agcagcaacg atgttacgca gcagcaacga tgttacgcag | 4740 |
| cagggcagtc gccctaaaac aaagttaggt ggctcaagta tgggcatcat tcgcacatgt | 4800 |
| aggctcggcc ctgaccaagt caaatccatg cgggctgctc ttgatctttt cggtcgtgag | 4860 |
| ttcggagacg tagccaccta ctcccaacat cagccggact ccgattacct cgggaacttg | 4920 |
| ctccgtagta agacattcat cgcgcttgct gccttcgacc aagaagcggg tggtggcgct | 4980 |
| ctcgcggctt acgttctgcc caggtttgag cagccgcgta gtgagatcta tatctatgat | 5040 |
| ctcgcagtct ccggcgagca ccggaggcag ggcattgcc aacgcgctcat caatctctc  | 5100 |
| aagcatgagg ccaacgcgct tggtgcttat gtgatctacg tgcaagcaga ttacggtgac | 5160 |
| gatcccgagc tggctctcta tacaagttag ggcataggg aagaagtgat gcactttgat  | 5220 |
| atcgacccaa gtaccgccac ctaacaattc gttcaagccg agatcggctt cccggccgcg | 5280 |
| gagttgttcg gtaaatgttc acaacgccg gaatatagtc tttaccatgc ctttgccac   | 5340 |
| gcccctcttt aatacgacgg gcaatttgca cttcagaaaa tgaagagttt gctttagcca | 5400 |
| taacaaaagt ccagtatgct ttttcacagc ataactggac tgatttcagt ttacaactat | 5460 |
| tctgtctagt ttaagacttt attgtcatag tttagatcta tttgttcag ttttaagactt | 5520 |
| tattgtccgc ccacaccgc ttacgcaggg catccattta ttactcaacc gtaaccgatt  | 5580 |
| ttgccagggt acgcggctgg tctgcggtgt gaaataccgc acagatgcgt aaggagaaaa | 5640 |
| taccgcatca ggcgctcttc cgcttcctcg ctactgact cgctgcgctc ggctcgttcgg | 5700 |
| ctgcggcgag cggtatcagc tcaactaaag gcggtaatac ggttatccac agaatacagg | 5760 |
| gataacgcag gaaagaacat gtgagcaaaa ggccagcaaa aggccaggaa ccgtaaaaa  | 5820 |

|                                                                      |      |
|----------------------------------------------------------------------|------|
| gccgcgttgc tggcgttttt ccataggctc cgccccctg acgagcatca caaaaatcga     | 5880 |
| cgctcaagtc agagggtggcg aaacccgaca ggactataaa gataccaggc gtttccccct   | 5940 |
| ggaagctccc tcgtgcgctc tcctgttccg accctgccgc ttaccggata cctgtccgcc    | 6000 |
| tttctccctt cggggaagcgt ggcgctttct caatgctcac gctgtaggta tctcagttcg   | 6060 |
| gtgtaggtcg ttcgctccaa gctgggctgt gtgcacgaac cccccgttca gcccgaccgc    | 6120 |
| tgcgcccttat ccggtaaacta tcgtcttagag tccaacccgg taagacacga cttatcgcca | 6180 |
| ctggcagcag ccactggtaa caggattagc agagcgaggc atgtaggcgg tgctacagag    | 6240 |
| ttcttgaagt ggtggcctaa ctacggctac actagaagga cagtatttgg tatctgcgct    | 6300 |
| ctgctgaagc cagttacctt cggaaaaaga gttggtagct cttgatccgg caaacaacc     | 6360 |
| accgctggta gcggtggttt ttttgtttgc aagcagcaga ttacgcgcag aaaaaagga     | 6420 |
| tctcaagaag atcctttgat cttttctacg gggcttgacg ctacgtggaa cgaaaactca    | 6480 |
| cgtaaggga ttttggctcat gagattatca aaaaggatct tcacctagat ctttttaaat    | 6540 |
| taaaaatgaa gttttaaatc aatctaaagt atatatgagt aaacttggtc tgacagttac    | 6600 |
| caatgcttaa tcagtgaggc acctatctca gcgatctgtc tatttcgttc atccatagtt    | 6660 |
| gcctgactcc ccgtcgtgta gataactacg atacgggagg gcttaccatc tggccccagt    | 6720 |
| gctgcaatga taccgcgaga ccacgcctca ccggctccag atttatcagc aataaaccag    | 6780 |
| ccagccggaa gggccgagcg cagaagtggc cctgcaactt tatccgcctc catccagtct    | 6840 |
| attaattggt gccgggaagc tagagtaagt agttcgccag ttaatagttt gcgcaacgtt    | 6900 |
| gttgccattg ctacaggcat cgtgggtgtca cgctcgtcgt ttggtatggc ttcattcagc   | 6960 |
| tccggttccc aacgatcaag gcgagttaca tgatccccc tgttgtgcaa aaaagcggtt     | 7020 |
| agctccttcg gtcctccgat cgttgtcaga agtaagtggc ccgcagtgtt atcactcatg    | 7080 |
| gttatggcag cactgcataa ttctcttact gtcatgccat ccgtaagatg cttttctgtg    | 7140 |
| actggtgagt actcaaccaa gtcattctga gaatagtgtg tgcggcgacc gagttgctct    | 7200 |
| tgcccgcgct caatacggga taataccgcg ccacatagca gaactttaaa agtgctcatc    | 7260 |
| attggaaaac gttcttcggg gcgaaaactc tcaaggatct taccgctgtt gagatccagt    | 7320 |
| tcgatgtaac ccactcgtgc acccaactga tcttcagcat cttttacttt caccagcgtt    | 7380 |
| tctgggtgag caaaaacagg aaggcaaaat gccgcaaaaa aggggaataag ggcgacacgg   | 7440 |
| aaatgttgaa tactcatact cttccttttt caatattatt gaagcattta tcagggttat    | 7500 |
| tgtctcatga gcggatacat atttgaatgt atttagaaaa ataaacaaat aggggttccg    | 7560 |
| cgcacatttc cccgaaaagt gccacctgaa attgtaaacg ttaatatattt gttaaaattc   | 7620 |
| gcgttaaatt tttgttaaat cagctcattt ttttaaccaat aggccgaaat cggcaaaatc   | 7680 |
| ccttataaat caaaagaata gaccgagata gggttgagtg ttgttccagt ttggaacaag    | 7740 |
| agtccactat taaagaacgt ggactccaac gtcaaagggc gaaaaaccgt ctatcagggc    | 7800 |
| gatggcccac tacgtgaacc atcacccctaa tcaagttttt tggggtcgag gtgccgtaaa   | 7860 |

|                                                                    |      |
|--------------------------------------------------------------------|------|
| gcactaaatc ggaaccctaa agggagcccc cgatttagag cttgacgggg aaagccggcg  | 7920 |
| aacgtggcga gaaaggaagg gaagaaagcg aaaggagcgg gcgctagggc gctggcaagt  | 7980 |
| gtagcgggtca cgctgcgcgt aaccaccaca cccgccgcgc ttaatgcgcc gctacagggc | 8040 |
| gcgtcccatt cgccattcag gctgcaaata agcgttgata ttcagtcaat tacaaacatt  | 8100 |
| aataacgaag agatgacaga aaaattttca ttctgtgaca gagaaaaagt agccgaagat  | 8160 |
| gacggtttgt cacatggagt tggcaggatg tttgattaaa aacataacag gaagaaaaat  | 8220 |
| gccccgctgt gggcggacaa aatagttggg aactgggagg ggtggaaatg gagtttttaa  | 8280 |
| ggattattta gggaagagtg acaaaataga tgggaactgg gtgtagcgtc gtaagctaat  | 8340 |
| acgaaaatta aaaatgacaa aatagtttgg aactagattt cacttatctg gttcggatct  | 8400 |
| cctagtgagc tccctgca                                                | 8418 |

<210> 148  
 <211> 225  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic polynucleotide

|                                                                   |     |
|-------------------------------------------------------------------|-----|
| <400> 148                                                         |     |
| tgtgccttct agttgccagc catctgttgt ttgcccctcc cccgtgcctt ccttgaccct | 60  |
| ggaagggtgcc actcccactg tcctttccta ataaaatgag gaaattgcat cgcatgtct | 120 |
| gagtaggtgt cattctattc tggggggtgg ggtggggcag gacagcaagg gggaggattg | 180 |
| ggaagacaat agcaggcatg ctggggatgc ggtgggctct atggc                 | 225 |

<210> 149  
 <211> 1177  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic polynucleotide

|                                                                    |     |
|--------------------------------------------------------------------|-----|
| <400> 149                                                          |     |
| ggctcagagg ctgagaggca cacaggagtt tctgggctca ccctgcccc ttccaacccc   | 60  |
| tcagttccca tcctccagca gctgtttgtg tgctgcctct gaagtccaca ctgaacaaac  | 120 |
| ttcagcctac tcatgtccct aaaatgggca aacattgcaa gcagcaaaca gcaaacacac  | 180 |
| agccctccct gcctgctgac cttggagctg gggcagaggt cagagacctc tctgggcca   | 240 |
| tgccacctcc aacatccact cgacccttg gaatttcggt ggagaggagc agaggttgtc   | 300 |
| ctggcgtggt ttaggtagtg tgagagggtc cgggttcaaa accacttgct ggggtggggag | 360 |
| tcgtcagtaa gtggctatgc ccgaccccg aagcctgttt cccatctgt acaatggaaa    | 420 |
| tgataaagac gcccatctga tagggttttt gtggcaaata aacatttggt tttttgttt   | 480 |
| tgttttgttt tgttttttga gatggaggtt tgctctgtcg cccaggctgg agtgagtga   | 540 |
| cacaatctca tctcaccaca accttcccct gcctcagcct cccaagtagc tgggattaca  | 600 |

|                                                                    |      |
|--------------------------------------------------------------------|------|
| agcatgtgcc accacacctg gctaattttc tatttttagt agagacgggt ttctccatgt  | 660  |
| tggtcagcct cagcctccca agtaactggg attacaggcc tgtgccacca caccgggcta  | 720  |
| atTTTTtcta tttttgacag ggacgggggtt tcaccatgtt ggtcaggctg gtctagaggt | 780  |
| accggatcct gctaccagtg gaacagccac taaggattct gcagtgagag cagagggcca  | 840  |
| gctaagtggg actctcccag agactgtctg actcacgcca cccctccac cttggacaca   | 900  |
| ggacgctgtg gtttctgagc caggtacaat gactcctttc ggtaagtgca gtggaagctg  | 960  |
| tacactgccc aggcaaagcg tccgggcagc gtaggcgggc gactcagatc ccagccagtg  | 1020 |
| gacttagccc ctgtttgctc ctccgataac tggggtgacc ttggttaata ttcaccagca  | 1080 |
| gcctcccccg ttgcccctct ggatccactg cttaaatacg gacgaggaca gggccctgtc  | 1140 |
| tcctcagctt caggcaccac cactgacctg ggacagt                           | 1177 |

<210> 150

<211> 1326

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic polynucleotide

<400> 150

|                                                                   |      |
|-------------------------------------------------------------------|------|
| ctgcagggcc cactagtgga gccgagagta attcatacaa aaggagggat cgccttcgca | 60   |
| aggggagagc ccagggaccg tccctaaatt ctcacagacc caaatccctg tagccgcccc | 120  |
| acgacagcgc gaggagcatg cgcccagggc tgagcgcggg tagatcagag cacacaagct | 180  |
| cacagtcccc ggcggtgggg ggagggggcg gctgagcggg ggccaggag ctggcgcggg  | 240  |
| gcaaactggg aaagtgggtg cgtgtgctgg ctccgccctc ttcccagggg tgggggagaa | 300  |
| cggtatataa gtgcggtagt cgccttgac gttctttttc gcaacgggtt tgccgtcaga  | 360  |
| acgcaggatga gtggcgggtg tggcttcgc gggccccgga gctggagccc tgctctgagc | 420  |
| gggcccgggt gatatgcgag tgcgtccgc agggtttagc tgtgagcatt cccacttcga  | 480  |
| gtggcgggcg gtgcgggggt gagagtgcga ggcctagcgg caaccccgta gcctcgcctc | 540  |
| gtgtccggct tgaggcctag cgtggtgtcc gccgccgct gccactccgg ccgcactatg  | 600  |
| cgttttttgt ccttgctgcc ctcgattgcc ttccagcagc atgggctaac aaaggagggg | 660  |
| tgtggggctc actcttaagg agcccatgaa gcttacgttg gataggaatg gaagggcagg | 720  |
| aggggcgact ggggccccgc cgccttcgga gcacatgtcc gacgccacct ggatggggcg | 780  |
| aggcctgtgg ctttccgaag caatcgggcg tgagtttagc ctacctgggc catgtggccc | 840  |
| tagcactggg cacggtctgg cctggcggtg ccgcgttccc ttgcctcca acaagggtga  | 900  |
| ggccgtcccg cccggcacca gttgcttgcg cggaagatg gccgctccc gggccctgtt   | 960  |
| gcaaggagct caaaatggag gacgcggcag cccggtggag cgggcgggtg agtcaccac  | 1020 |
| acaaaggaag agggccttgc ccctcgccgg ccgctgcttc ctgtgacccc gtggtctatc | 1080 |
| ggccgcatag tcacctcggg cttctcttga gcaccgctcg tcgcggcggg gggaggggat | 1140 |

|                                                                     |      |
|---------------------------------------------------------------------|------|
| ctaattggcgt tggagtttgt tcacatttgg tgggtggaga ctagtcaggc cagcctggcg  | 1200 |
| ctggaagtca ttcttgggaat ttgccccctt gagtttggag cgaggctaatt tctcaagcct | 1260 |
| cttagcgggtt caaaggtatt ttctaaaccc gtttccagggt gttgtgaaag ccaccgctaa | 1320 |
| ttcaaa                                                              | 1326 |

<210> 151  
 <211> 573  
 <212> DNA  
 <213> Mus musculus

|                                                                                 |     |
|---------------------------------------------------------------------------------|-----|
| <400> 151<br>gtaagagttt tatgtttttt catctctgct tgtatttttc tagtaatgga agcctgggtat | 60  |
| tttaaaatag ttaaattttc ctttagtgct gatttctaga ttattattac tgttggtgtt               | 120 |
| gttattattg tcattatttg catctgagaa cccttaggtg gttatattat tgatatattt               | 180 |
| ttggtatctt tgatgacaat aatgggggat tttgaaagct tagcttttaa tttcttttaa               | 240 |
| ttaaaaaaaa atgctaggca gaatgactca aattacgttg gatacagttg aatttattac               | 300 |
| ggtctcatag ggctgcctg ctcgaccatg ctatactaaa aattaaaagt gtgtgttact                | 360 |
| aattttataa atggagtttc catttatatt tacctttatt tcttatttac cattgtctta               | 420 |
| gtagatatatt acaaacatga cagaaacact aaatcttgag tttgaatgca cagatataaa              | 480 |
| cacttaacgg gttttaaaaa taataatgtt ggtgaaaaaa tataactttg agtgtagcag               | 540 |
| agaggaacca ttgccacctt cagattttcc tgt                                            | 573 |

<210> 152  
 <211> 1993  
 <212> DNA  
 <213> Mus musculus

|                                                                               |     |
|-------------------------------------------------------------------------------|-----|
| <400> 152<br>acgatcggga actggcatct tcaggagta gcttaggtca gtgaagagaa gaacaaaaag | 60  |
| cagcatatta cagttagttg tcttcatcaa tctttaaata tgttgtgtgg tttttctctc             | 120 |
| cctgtttcca cagacaagag tgagatcgcc catcgggtata atgatttggg agaacaacat            | 180 |
| ttcaaaggcc tgtaagttat aatgctgaaa gcccaactaa tatttctggt agtattagtt             | 240 |
| aaagttttta aacacctttt tccaccttga gtgtgagaat tgtagagcag tgctgtccag             | 300 |
| tagaaatgtg tgcattgaca gaaagactgt ggatctgtgc tgagcaatgt ggcagccaga             | 360 |
| gatcacaagg ctatcaagca ctttgcacat ggcaagtgtg actgagaagc acacattcaa             | 420 |
| ataatagtta attttaattg aatgtatcta gccatgtgtg gctagtagct cttttcctgg             | 480 |
| agagagaatc tggagcccac atctaacttg ttaagtctgg aatcttattt tttatttctg             | 540 |
| gaaagggtcta tgaactatag ttttgggggc agctcactta ctaactttta atgcaataag            | 600 |
| atctcatggg atcttgagaa cattattttg tctctttgta gtactgaaac cttatacatg             | 660 |
| tgaagtaagg ggtctatact taagtcacat ctccaacctt agtaatgttt taatgtagta             | 720 |
| aaaaaatgag taattaattt atttttagaa ggtcaatagt atcatgtatt ccaaataaca             | 780 |

|                                                                    |      |
|--------------------------------------------------------------------|------|
| gaggtatatg gttagaaaag aaacaattca aaggacttat ataatatcta gccttgacaa  | 840  |
| tgaataaatt tagagagtag tttgcctggt tgccatcatgt tcataaatct attgacacat | 900  |
| atgtgcatct gcacttcagc atggtagaag tccatattcc tttgcttgga aaggcagggtg | 960  |
| ttcccattac gcctcagaga atagctgacg ggaagaggct ttctagatag ttgtatgaaa  | 1020 |
| gatatacaaa atctcgcagg tatacacagg catgatttgc tggttgggag agccacttgc  | 1080 |
| ctcatactga ggTTTTTgtg tctgcttttc agagtcctga ttgccttttc ccagtatctc  | 1140 |
| cagaaatgct catacgatga gcatgccaaa ttagtgcagg aagtaacaga ctttgcaaag  | 1200 |
| acgtgtgttg ccgatgagtc tgccgccaac tgtgacaaat cccttgtgag taccttctga  | 1260 |
| ttttgtggat ctactttcct gctttctgga actctgtttc aaagccaatc atgactccat  | 1320 |
| cacttaaggc cccgggaaca ctgtggcaga gggcagcaga gagattgata aagccagggt  | 1380 |
| gatgggaatt ttctgtggga ctccatttca tagtaattgc agaagctaca atacactcaa  | 1440 |
| aaagtctcac cacatgactg cccaaatggg agcttgacag tgacagtgc agtagatatg   | 1500 |
| ccaaagtgga tgagggaag accacaagag ctaaaccctg taaaaagaac tgtaggcaac   | 1560 |
| taaggaatgc agagagaaga agttgccttg gaagagcata ccaactgcct ctccaatacc  | 1620 |
| aatggtcac cctaaaacat acgtatgaat aacatgcaga ctaagcaggc tacatttagg   | 1680 |
| aatatacatg tatttacata aatgtatatg catgtaacaa caatgaatga aaactgaggt  | 1740 |
| catggatctg aaagagagca agggggctta catgagaggg tttggaggga ggggttgag   | 1800 |
| ggagggaggt attattcttt agttttacag ggaacgtagt aaaaacatag gcttctcca   | 1860 |
| aaggagcaga gccatgagg agctgtgcaa ggttccccag cttgatttta cctgctcctc   | 1920 |
| aaattccctt gatttgtttt tattataatg actttactcc tagcttttag tgtcagatag  | 1980 |
| aaaacatgga agg                                                     | 1993 |

<210> 153  
 <211> 1350  
 <212> DNA  
 <213> Homo sapiens

|                                                                    |     |
|--------------------------------------------------------------------|-----|
| <400> 153                                                          |     |
| taggaggctg aggcaggagg atcgcttgag cccaggagtt cgagaccagc ctgggcaaca  | 60  |
| tagtgtgatc ttgtatctat aaaaataaac aaaattagct tgggtgtggtg gcgcctgtag | 120 |
| tccccagcca cttggagggg tgaggtgaga ggattgcttg agcccgggat ggtccaggct  | 180 |
| gcagtgagcc atgatcgtgc cactgcactc cagcctgggc gacagagtga gaccctgtct  | 240 |
| cacaacaaca acaacaacaa caaaaaggct gagctgcacc atgcttgacc cagtttctta  | 300 |
| aaattgttgt caaagcttca ttactccat ggtgctatag agcacaagat tttatttggt   | 360 |
| gagatggtgc tttcatgaat tcccccaaca gagccaagct ctccatctag tggacaggga  | 420 |
| agctagcagc aaaccttccc ttactacaa aacttcattg cttggccaaa aagagagtta   | 480 |
| attcaatgta gacatctatg taggcaatta aaaacctatt gatgtataaa acagtttgca  | 540 |
| ttcatggagg gcaactaaat acattctagg actttataaa agatcacttt ttatttatgc  | 600 |

|                                                                     |      |
|---------------------------------------------------------------------|------|
| acagggtgga acaagatgga ttatcaagtg tcaagtcгаа tctatgacat caattattat   | 660  |
| acatcggagc cctgccaaaa aatcaatgtg aagcaaatcg cagcccgct cctgcctccg    | 720  |
| ctctactcac tgggtgttcat ctttggtttt gtgggcaaca tgctgggtcat cctcatcctg | 780  |
| ataaactgca aaaggctgaa gagcatgact gacatctacc tgctcaacct ggccatctct   | 840  |
| gacctgtttt tccttcttac tgtcccttc tgggctcact atgctgccgc ccagtgggac    | 900  |
| tttggaaata caatgtgtca actcttgaca gggctctatt ttataggctt cttctctgga   | 960  |
| atcttcttca tcatcctcct gacaatcgat aggtacctgg ctgtcgtcca tgctgtgttt   | 1020 |
| gctttaaааg ccaggacggt cacctttggg gtggtgacaa gtgtgatcac ttgggtggtg   | 1080 |
| gctgtgtttg cgtctctccc aggaatcatc tttaccagat ctcaaaaaga aggtcttcat   | 1140 |
| tacacctgca gctctcattt tccatacagt cagtatcaat tctggaagaa tttccagaca   | 1200 |
| ttaaagatag tcatcttggg gctggtcctg ccgctgcttg tcatgggtcat ctgctactcg  | 1260 |
| ggaatcctaa aaactctgct tcgggtgtcga aatgagaaga agaggcacag ggctgtgagg  | 1320 |
| cttatcttca ccatcatgat tgtttatttt                                    | 1350 |

<210> 154  
 <211> 1223  
 <212> DNA  
 <213> Homo sapiens

|                                                                    |      |
|--------------------------------------------------------------------|------|
| <400> 154                                                          |      |
| tgacagagac tcttgggatg acgcactgct gcatcaacct catcatctat gcctttgtcg  | 60   |
| gggagaagtt cagaaactac ctcttagtct tcttccaaaa gcacattgcc aaacgcttct  | 120  |
| gcaaatgctg ttctattttc cagcaagagg ctcccagcg agcaagctca gtttacaccc   | 180  |
| gatccactgg ggagcaggaa atatctgtgg gcttgtgaca cggactcaag tgggctggtg  | 240  |
| accagtcag agttgtgcac atggcttagt tttcatacac agcctgggct gggggtgggg   | 300  |
| tgggagaggt cttttttaaаа aggaagttac tgttatagag ggtctaagat tcatccattt | 360  |
| atttggcatc tgtttaaаgt agattagatc ttttaagccc atcaattata gaaagccaaa  | 420  |
| tcaaaatatg ttgatgaaaa atagcaacct ttttatctcc cttcacatg catcaagtta   | 480  |
| ttgacaaact ctcccttcac tccgaaagtt cttatgtat atttaaaaga aagcctcaga   | 540  |
| gaattgtga ttcttgagtt tagtgatctg aacagaaata ccaaaattat ttcagaaatg   | 600  |
| tacaactttt tacctagtac aaggcaacat ataggttgta aatgtgttta aaacaggtct  | 660  |
| ttgtcttgct atggggagaa aagacatgaa tatgattagt aaagaaatga cacttttcat  | 720  |
| gtgtgatttc ccctccaagg tatggttaat aagtttact gacttagaac caggcgagag   | 780  |
| acttgtggcc tgggagagct ggggaagctt cttaaаtgag aaggaaattg agttggatca  | 840  |
| tctattgctg gcaaagacag aagcctcact gcaagcactg catgggcaag cttggctgta  | 900  |
| gaaggagaca gagctggttg ggaagacatg gggaggaagg acaaggctag atcatgaaga  | 960  |
| accttgacgg cattgctccg tctaagtcат gagctgagca gggagatcct ggttgggtgtt | 1020 |
| gcagaaggtt tactctgtgg ccaaaggagg gtcaggaagg atgagcattt agggcaagga  | 1080 |

|                                                                    |      |
|--------------------------------------------------------------------|------|
| gaccaccaac agccctcagg tcagggtgag gatggcctct gctaagctca aggcgtgagg  | 1140 |
| atgggaagga gggagggtatt cgtaaggatg ggaaggaggg aggtattcgt gcagcatatg | 1200 |
| aggatgcaga gtcagcagaa ctg                                          | 1223 |

<210> 155  
 <211> 215  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic polynucleotide

|                                                                                |     |
|--------------------------------------------------------------------------------|-----|
| <400> 155<br>gaacgctgac gtcatcaacc cgctccaagg aatcgcgggc ccagtgtcac taggcgggaa | 60  |
| cacccagcgc gcgtgcgccc tggcaggaag atggctgtga gggacagggg agtggcgccc              | 120 |
| tgcaatattt gcatgtcgct atgtgttctg ggaaatcacc ataaacgtga aatgtctttg              | 180 |
| gatttgggaa tcttataagt tctgtatgag accac                                         | 215 |

<210> 156  
 <211> 141  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic polynucleotide

|                                                                                |     |
|--------------------------------------------------------------------------------|-----|
| <400> 156<br>cctgcaggca gctgcgcgct cgctcgctca cctaggccgc ccgggcaaag cccgggcgtc | 60  |
| gggcgacctt tggtcgcccg gcctaggtga gcgagcgagc gcgcagagag ggagtggcca              | 120 |
| actccatcac taggggttcc t                                                        | 141 |

<210> 157  
 <211> 19  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic oligonucleotide

|                                   |    |
|-----------------------------------|----|
| <400> 157<br>gcgcgctcgc tcgctcacc | 19 |
|-----------------------------------|----|

<210> 158  
 <211> 22  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic oligonucleotide

|                                      |    |
|--------------------------------------|----|
| <400> 158<br>ctagtgagc gagcgagcgc gc | 22 |
|--------------------------------------|----|

<210> 159  
<211> 75  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
oligonucleotide

<400> 159  
cctgcaggac tgaggccgcc cgggcaaagc ccgggcgtcg ggcgacctt ggtcgcccgg 60  
  
cctcagtcct gcagg 75

<210> 160  
<211> 130  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
polynucleotide

<400> 160  
aggaaccctt agtgatggag ttggccactc cctctctgcg cgctcgctcg ctactgagt 60  
  
gcgggcgacc aaaggctgcc cgacgcccgg gcgcactcag tgagcgagcg agcgcgcagc 120  
  
tgcttcgagg 130

<210> 161  
<211> 142  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
polynucleotide

<400> 161  
cctgcaggca gctgcgcgt cgctcgctcc ctaggactga ggccgcccgg gcgtcgggcg 60  
  
acctttggtc gcccggcctc agtcctaggg agcgagcgag cgcgagaga gggagtggcc 120  
  
aactccatca ctaggggttc ct 142

<210> 162  
<211> 80  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic  
oligonucleotide

<400> 162  
gcgcgctcgc tcgctcactg agtgccggcg accaaaggct gcccgacgcc cgggcgcact 60  
  
cagtgagcga gcgagcgcgc 80

<210> 163  
<211> 21  
<212> DNA  
<213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 163  
 gcgcgctcgc tcgctcactg a 21

<210> 164  
 <211> 18  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 164  
 gtgagcgagc gagcgcg 18

<210> 165  
 <211> 89  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 165  
 gcgcgctcgc tcgctcactg aggccgccc ggcaaagccc gggcgtcggg cgactttgtc 60  
 gcccggcctc agtgagcgag cgagcgcg 89

<210> 166  
 <211> 89  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 166  
 gcgcgctcgc tcgctcactg aggccgggcg acaaagtcgc ccgacgccc ggctttgccc 60  
 gggcggcctc agtgagcgag cgagcgcg 89

<210> 167  
 <211> 87  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 167  
 gcgcgctcgc tcgctcactg aggccgccc ggcaaagccc gggcgtcggg cgattttcgc 60  
 ccggcctcag tgagcgagcg agcgcg 87

<210> 168  
 <211> 87  
 <212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
oligonucleotide

<400> 168

gcgcgctcgc tcgctcactg aggccgggcg aaaatcgccc gacgcccggg ctttggcccg 60

gcggcctcag tgagcgagcg agcgcgc 87

<210> 169

<211> 85

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
oligonucleotide

<400> 169

gcgcgctcgc tcgctcactg aggccgccc ggcaaagccc gggcgtcggg cgtttcgccc 60

ggcctcagt agcgagcgag cgcgc 85

<210> 170

<211> 85

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
oligonucleotide

<400> 170

gcgcgctcgc tcgctcactg aggccgggcg aaacgcccga cggccgggct ttgcccgggc 60

ggcctcagt agcgagcgag cgcgc 85

<210> 171

<211> 89

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
oligonucleotide

<400> 171

gcgcgctcgc tcgctcactg aggccgccc ggaaaccg gcgtcgggcg acctttggtc 60

gcccggcctc agtgagcgag cgagcgcgc 89

<210> 172

<211> 89

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
oligonucleotide

<400> 172

gcgcgctcgc tcgctcactg aggccgggcg accaaaggtc gcccgcgcc cgggtttccc 60

gggcggcctc agtgagcgag cgagcgcgc

89

<210> 173

<211> 87

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
oligonucleotide

<400> 173

gcgcgctcgc tcgctcactg aggccgcccg gaaaccgggc gtcgggacac ctttggtcgc

60

ccggcctcag tgagcgagcg agcgcgc

87

<210> 174

<211> 87

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
oligonucleotide

<400> 174

gcgcgctcgc tcgctcactg aggccgggac accaaaggtc gcccagacgc cggtttccgg

60

gcggcctcag tgagcgagcg agcgcgc

87

<210> 175

<211> 85

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
oligonucleotide

<400> 175

gcgcgctcgc tcgctcactg aggccgcccg aaacgggagt cgggacacgt ttggtcggcc

60

ggcctcagtg agcgagcgag cgcgc

85

<210> 176

<211> 85

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic  
oligonucleotide

<400> 176

gcgcgctcgc tcgctcactg aggccgggac accaaaggtc gcccagacgc cggtttcggc

60

ggcctcagtg agcgagcgag cgcgc

85

<210> 177

<211> 83

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 177  
gcgcgctcgc tcgctcactg aggccgcccc aaggcgctcg ggcgaccttt ggtcgcccgg 60  
cctcagttag cgagcgagcg cgc 83

<210> 178  
<211> 83  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 178  
gcgcgctcgc tcgctcactg aggccgggcg accaaaggct gcccgacgcc ctttgggcgg 60  
cctcagttag cgagcgagcg cgc 83

<210> 179  
<211> 81  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 179  
gcgcgctcgc tcgctcactg aggccgcccc aggcgtcggg cgacctttgg tcgcccggcc 60  
tcagttagcg agcgagcgcg c 81

<210> 180  
<211> 81  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 180  
gcgcgctcgc tcgctcactg aggccgggcg accaaaggct gcccgacgcc tttggcggcc 60  
tcagttagcg agcgagcgcg c 81

<210> 181  
<211> 79  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 181  
gcgcgctcgc tcgctcactg aggccgcaaa gcgtcgggcg acctttggct gcccggcctc 60  
agtgagcgag cgagcgcgcg 79

<210> 182  
 <211> 79  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence: Synthetic  
         oligonucleotide  
  
 <400> 182  
 ggcgcgctcgc tcgctcactg aggccgggcg accaaaggtc gcccgacgct ttgcggcctc 60  
  
 agtgagcgag cgagcgcg 79  
  
 <210> 183  
 <211> 81  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence: Synthetic  
         oligonucleotide  
  
 <400> 183  
 ctgcgcgctc gctcgctcac tgaggccgaa acgtcgggcg acctttggtc gcccggcctc 60  
  
 agtgagcgag cgagcgcgca g 81  
  
 <210> 184  
 <211> 81  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence: Synthetic  
         oligonucleotide  
  
 <400> 184  
 ctgcgcgctc gctcgctcac tgaggccggg cgaccaaagg tcgcccgcac tttcggcctc 60  
  
 agtgagcgag cgagcgcgca g 81  
  
 <210> 185  
 <211> 72  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence: Synthetic  
         oligonucleotide  
  
 <400> 185  
 ggcgcgctcgc tcgctcactg aggccgggcg accaaaggtc gcccgacggc ctcagtgagc 60  
  
 gagcgagcgc gc 72  
  
 <210> 186  
 <211> 80  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence: Synthetic  
         oligonucleotide

<400> 186  
 gcgcgctcgc tcgctcactg aggccgggcg accaaaggtc gcccgcgccc cgggcggcct 60  
 cagtgcgcga gcgagcgcg 80

<210> 187  
 <211> 79  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic  
 oligonucleotide

<400> 187  
 gcgcgctcgc tcgctcactg aggcgcccgg gcgtcgggcg acccttggtc gcccggcctc 60  
 agtgagcgag cgagcgcg 79

<210> 188  
 <211> 48  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic  
 oligonucleotide

<400> 188  
 ggagtcaaag ttctgtttgc cctgatctgc atcgtgtgg ccgaggcc 48

<210> 189  
 <211> 99  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic  
 oligonucleotide

<400> 189  
 attcatacca acttgaagaa aaagttcagc ctcttcatcc tggcttttct cctgttcgca 60  
 gtcacatctgtg ttggaagaa agggagcgac tatgaggcc 99

<210> 190  
 <211> 588  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Synthetic  
 polynucleotide

<400> 190  
 gcccctctcc ctccccccc cctaacttta ctggccgaag ccgcttgga taaggccggt 60  
 gtgcgtttgt ctatatgtta tttccacca tattgccgtc ttttggcaat gtgagggcc 120  
 ggaaacctgg ccctgtcttc ttgacgagca ttcctagggg tctttcccct ctgcctaaag 180  
 gaatgcaagg tctgttgaat gtcgtgaagg aagcagttcc tctggaagct tcttgaagac 240  
 aaacaacgtc tgtagcgacc ctttgcaggc agcgaaccc cccacctggc gacaggtgcc 300

|                                                                    |     |
|--------------------------------------------------------------------|-----|
| tctgcggcca aaagccacgt gtataagata cacctgcaaa ggcggcaciaa ccccagtgcc | 360 |
| acgttgtgag ttggatagtt gtggaaagag tcaaatggct ctcctcaagc gtattcaaca  | 420 |
| aggggctgaa ggatgcccag aaggtacccc attgtatggg atctgatctg gggcctcgg   | 480 |
| gcacatgctt tacatgtgtt tagtcgaggt taaaaaacg tctaggcccc ccgaaccacg   | 540 |
| gggacgtggg tttcctttga aaaacacgat gataatatgg ccacaacc               | 588 |