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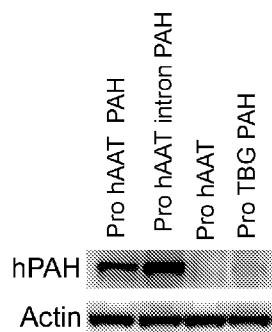


Figure 7

(57) Abstract: A lentiviral vector system for expressing a lentiviral particle is disclosed. The lentiviral vector system includes a therapeutic vector. The lentiviral vector system produces a lentiviral particle for upregulating PAH expression in the cells of a subject afflicted with phenylketonuria (PKU).

**VECTORS WITH PROMOTER AND ENHANCER COMBINATIONS FOR
TREATING PHENYLKETONURIA**

CROSS-REFERENCE TO RELATED APPLICATION

[1] This Application claims priority to U.S. Provisional Patent Application No.
5 62/566,979 filed on October 2, 2017, and entitled “VECTORS WITH PROMOTER AND
ENHANCER COMBINATIONS FOR TREATING PHENYLKETONURIA”, the disclosure
of which is incorporated herein by reference in its entirety.

FIELD

[2] Aspects of the disclosure relate to genetic medicines for treating
10 phenylketonuria (PKU). More specifically, aspects of the disclosure relate to lentiviral
vectors, including PAH-containing lentiviral vectors, whose expression is controlled by
various promoter and enhancer combinations.

BACKGROUND

[3] Phenylketonuria (PKU) refers to a heterogeneous group of disorders that can
15 lead to intellectual disability, seizures, behavioral problems, and impaired growth and
development in affected children if left untreated. The mechanisms by which
hyperphenylalaninemia results in intellectual impairment reflect the surprising toxicity of
high dose phenylalanine and involve hypomyelination or demyelination of nervous system
tissues. PKU has an average reported incidence rate of 1 in 12,000 in North America,
20 affecting males and females equally. The disorder is most common in people of European or
Native American Ancestry and reaches much higher levels in the eastern Mediterranean
region.

[4] Neurological changes in patients with PKU have been demonstrated within
one month of birth, and magnetic resonance imaging (MRI) in adult PKU patients has shown
25 white matter lesions in the brain. The size and number these lesions relate directly to blood
phenylalanine concentration. The cognitive profile of adolescents and adults with PKU

compared with control subjects can include significantly reduced IQ, processing speed, motor control and inhibitory abilities, and reduced performance on tests of attention.

[5] The majority of PKU is caused by a deficiency of hepatic phenylalanine hydroxylase (PAH). PAH is a multimeric hepatic enzyme that catalyzes the hydroxylation of phenylalanine (Phe) to tyrosine (Tyr) in the presence of molecular oxygen and catalytic amounts of tetrahydrobiopterin (BH₄), its nonprotein cofactor. In the absence of sufficient expression of PAH, phenylalanine levels in the blood increase leading to hyperphenylalaninemia and harmful side effects in PKU patients. Decreased or absent PAH activity can lead to a deficiency of tyrosine and its downstream products, including melanin, l-thyroxine and the catecholamine neurotransmitters including dopamine.

[6] PKU can be caused by mutations in PAH and/or a defect in the synthesis or regeneration of PAH cofactors (*i.e.*, BH₄). Notably, several PAH mutations have been shown to affect protein folding in the endoplasmic reticulum resulting in accelerated degradation and/or aggregation due to missense mutations (63%) and small deletions (13%) in protein structure that attenuate or largely abolish enzyme catalytic activity.

[7] In general, three major phenotypic groups are used to classify PKU based on blood plasma Phe levels, dietary tolerance to Phe and potential responsiveness to therapy. These groups include classical PKU (Phe > 1200 µM), atypical or mild PKU (Phe is 600 - 1200 µM), and permanent mild hyperphenylalaninemia (HPA, Phe 120 - 600 µM).

[8] Detection of PKU relies on universal newborn screening (NBS). A drop of blood collected from a heel stick is tested for phenylalanine levels in a screen that is mandatory in all 50 states of the USA.

[9] Currently, lifelong dietary restriction of Phe and BH₄ supplementation are the only two available treatment options for PKU, where early therapeutic intervention is critical to ensure optimal clinical outcomes in affected infants. However, costly medication and

special low-protein foods imposes a major burden on patients that can lead to malnutrition, psychosocial or neurocognitive complications notably when these products are not fully covered by private health insurance. Moreover, BH₄ therapy is primarily effective for treatment of mild hyperphenylalaninemia as related to defects in BH₄ biosynthesis, whereas
5 only 20-30% of patients with mild or classical PKU are responsive. Thus, there is an urgent need for new treatment modalities for PKU as an alternative to burdensome Phe-restriction diets. Thus, it would be desirable to develop an alternative method for the treatment of phenylketonuria.

[10] Genetic medicines have the potential to effectively treat PKU. Genetic
10 medicines may involve delivery and expression of genetic constructs for the purposes of disease therapy or prevention. Expression of genetic constructs may be modulated by various promoters, enhancers, and/or combinations thereof.

SUMMARY

[11] In an aspect of the disclosure, a viral vector comprises a therapeutic cargo
15 portion, wherein the therapeutic cargo portion comprises a PAH sequence or a variant thereof, a promoter, and a liver-specific enhancer, wherein the PAH sequence or the variant thereof is operatively controlled by both the promoter and the liver-specific enhancer.

[12] In embodiments, the liver-specific enhancer comprises a prothrombin
enhancer. In embodiments, the promoter is a liver-specific promoter. In embodiments, the
20 liver-specific promoter comprises a hAAT promoter. In embodiments, the therapeutic cargo portion further comprises a beta globin intron. In embodiments, the therapeutic cargo portion further comprises at least one hepatocyte nuclear factor binding site. In embodiments, the at least one hepatocyte nuclear factor binding site is disposed upstream of the prothrombin enhancer. In embodiments, the at least one hepatocyte nuclear factor binding site is disposed
25 downstream of the prothrombin enhancer.

[13] In embodiments, the PAH sequence or the variant thereof is truncated. In embodiments, the portion of the PAH sequence or the variant thereof is truncated is the 3' untranslated region (UTR) of the PAH sequence or the variant thereof.

[14] In embodiments, the PAH sequence or the variant thereof comprises a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with:

ATGTCCACTGCGGTCCTGGAAAACCCAGGCTTGGGCAGGAACTCT
CTGACTTTGGACAGGAAACAAGCTATATTGAAGACAAGTCAATCA
AAATGGTGCCATATCACTGATCTTCTCACTCAAAGAAGAAGTTGGT
10 GCATTGGCCAAAGTATTGCGCTTATTTGAGGAGAATGATGTAAACC
TGACCCACATTGAATCTAGACCTTCTCGTTTAAAGAAAGATGAGTA
TGAATTTTTCACCCATTTGGATAAACGTAGCCTGCCTGCTCTGACAA
ACATCATCAAGATCTTGAGGCATGACATTGGTGCCACTGTCCATGA
GCTTTCACGAGATAAGAAGAAAGACACAGTGCCCTGGTTCCCAAG
15 AACCATTCAAGAGCTGGACAGATTGCCAATCAGATTCTCAGCTAT
GGAGCGGAACTGGATGCTGACCACCCTGGTTTTAAAGATCCTGTGT
ACCGTGCAAGACGGAAGCAGTTTGCTGACATTGCCTACAACCTACCG
CCATGGGCAGCCCATCCCTCGAGTGGAATACATGGAGGAAGAAAA
GAAAACATGGGGCACAGTGTTCAAGACTCTGAAGTCCTTGTATAAA
20 ACCCATGCTTGCTATGAGTACAATCACATTTTCCACTTCTTGAAAA
GTACTGTGGCTTCCATGAAGATAACATTCCCCAGCTGGAAGACGTT
TCTCAATTCCTGCAGACTTGCACTGGTTTCCGCCTCCGACCTGTGGC
TGGCCTGCTTTCCTCTCGGGATTTCTTGGGTGGCCTGGCCTTCCGAG
TCTTCCACTGCACACAGTACATCAGACATGGATCCAAGCCCATGTA
25 TACCCCGAACCTGACATCTGCCATGAGCTGTTGGGACATGTGCCC
TTGTTTTAGATCGCAGCTTTGCCCAGTTTTCCAGGAAATTGGCCT
TGCCTCTCTGGGTGCACCTGATGAATACATTGAAAAGCTCGCCACA
ATTACTGGTTTACTGTGGAGTTTGGGCTCTGCAAACAAGGAGACT
CCATAAAGGCATATGGTGCTGGGCTCCTGTCATCCTTTGGTGAATT
30 ACAGTACTGCTTATCAGAGAAGCCAAAGCTTCTCCCCCTGGAGCTG
GAGAAGACAGCCATCCAAAATTACACTGTCACGGAGTTCCAGCCCC
TGTATTACGTGGCAGAGAGTTTTAATGATGCCAAGGAGAAAGTAA

GGAAC TTTGCTGCCACAATACCTCGGCCCTTCTCAGTTCGCTACGA
CCCATACACCCAAAGGATTGAGGTCTTGGACAATACCCAGCAGCTT
AAGATTTTGGCTGATTCCATTAACAGTGAAATTGGAATCCTTTGCA
GTGCCCTCCAGAAAATAAAGTAA (SEQ ID NO: 1);
5 ATGAGTACGGCTGTGCTCGAGAATCCAGGTTTGGGCCGAAAGCTGT
CTGATTTTGGACAGGAGACATCTTATATTGAAGACAACCTGCAACCA
GAATGGTGC GATATCCCTTATTTTTTCTCTGAAAGAAGAAGTAGGT
GCGCTGGCAAAGGTCTTGCGGCTGTTTGAAGAGAACGATGTTAATC
TTACTCATATTGAGTCCAGACCATCACGGCTGAAAAAAGACGAGTA
10 CGAATTTTTTACTCACTTGGACAAACGAAGCTTGCCGGCTCTTACTA
ATATCATTAAGATCCTCCGGCATGACATAGGGGCGACAGTGCATGA
GCTTTCAAGGGATAAAAAGAAAGATACCGTCCCCTGGTTTCCAAGG
ACCATACAAGAACTCGACCGATTTCGCGAACCAGATCCTTTCATATG
GTGCTGAGTTGGATGCTGACCACCCCGGCTTCAAAGACCCGGTCTA
15 CCGAGCGCGGCGGAAACAATTTGCTGACATCGCATACAATTACAG
GCATGGCCAGCCAATTCCTAGAGTAGAATACATGGAAGAAGAGAA
AAAAACCTGGGGTACCGTCTTCAAGACGCTGAAATCATTGTATAAA
ACTCATGCATGTTACGAATATAACCATATTTTTCCGTTGCTCGAGAA
ATATTGCGGGTTCCACGAAGATAACATCCCACAACCTCGAGGATGTA
20 TCTCAGTTCCTCCAGACCTGTACGGGGTTTCGACTTAGGCCTGTTCGC
GGGTTTGCTCAGTTCTCGAGACTTCCTGGGTGGATTGGCGTTTCGG
GTATTCCATTGCACGCAGTATATCCGACACGGAAGTAAGCCAATGT
ACACGCCAGAACCCGATATCTGTCACGAATTGCTTGGACACGTTCC
TCTGTTTTCTGATCGATCATTCGCTCAGTTTTACAGGAAATCGGCC
25 TGGCATCTTTGGGAGCGCCGGATGAATATATTGAGAAGCTCGCTAC
AATTTACTGGTTCACGGTAGAATTTGGGTGTGCAAGCAGGGTGAT
AGTATTAAAGCATACGGTGCGGGATTGCTGTCTCATTGCGGGGAGC
TTCAGTATTGCCTGTCCGAGAAACCCAAGCTGTTGCCGTTGGAATT
GGAAAAAACCGCTATCCAAAATTACACAGTAACGGAGTTCCAACC
30 TTTGTACTACGTAGCCGAGTCATTTAACGATGCAAAGGAGAAGGTC
AGAAATTTTGCTGCGACGATACCCAGACCGTTCTCAGTAAGGTACG
ATCCTTACACTCAGAGGATTGAAGTCCTGGATAATACGCAACAGCT
CAAGATCCTGGCAGACTCCATAAATTCTGAAATCGGCATCTTGTGT
TCAGCACTGCAAAAGATAAAATAA (SEQ ID NO: 2);

AGCCATGGACAGAATGTGGTCTGTCAGCTGTGAATCTGTTGATGGA
 GATCCAACCTATTTCTTTCATCAGAAAAAGTCCGAAAAGCAAACCTT
 AATTTGAAATAACAGCCTTAAATCCTTTACAAGATGGAGAAACAAC
 AAATAAGTCAAAATAATCTGAAATGACAGGATATGAGTACATACT
 5 CAAGAGCATAATGGTAAATCTTTTGGGGTCATCTTTGATTTAGAGA
 TGATAATCCCATACTCTCAATTGAGTTAAATCAGTAATCTGTCGCAT
 TTCATCAAGATTAATTAATAATTTGGGACCTGCTTCATTCAAGCTTCA
 TATATGCTTTGCAGAGAACTCATAAAGGAGCATATAAGGCTAAATG
 TAAAACCCAAGACTGTCATTAGAATTGAATTATTGGGCTTAATATA
 10 AATCGTAACCTATGAAGTTTATTTTTTATTTTAGTTAACTATGATTC
 CAATTACTACTTTGTTATTGTACCTAAGTAAATTTTCTTTAAGTCAG
 AAGCCCATTAATAATAGTTACAAGCATTGAACCTCTTTAGTATTATA
 TTAATATAAAAACATTTTTGTATGTTTTATTGTAATCATAAATACTG
 CTGTATAAGGTAATAAACTCTGCACCTAATCCCCATAACTTCCAG
 15 TATCATTTTTCCAATTAATTATCAAGTCTGTTTTGGGAAACACTTTGA
 GGACATTTATGATGCAGCAGATGTTGACTAAAGGCTTGTTGGTAG
 ATATTCAGGAAATGTTCACTGAATAAATAAGTAAATACATTATTGA
 AAAGCAAATCTGTATAAATGTGAAATTTTTATTTGTATTAGTAATA
 AAACATTAGTAGTTTAAACAAAAAAAAAAAAAAAAAAAAAAAAAAAA
 20 AAAAACTCGACTCTAGATT (SEQ ID NO: 3); or
 AGCCATGGACAGAATGTGGTCTGTCAGCTGTGAATCTGTTGATGGA
 GATCCAACCTATTTCTTTCATCAGAAAAAGTCCGAAAAGCAAACCTT
 AATTTGAAATAACAGCCTTAAATCCTTTACAAGATGGAGAAACAAC
 AAATAAGTCAAAATAATCTGAAATGACAGGATATGAGTACATACT
 25 CAAGAGCATAATGGTAAATCTTTTGGGGTCATCTTTGATTTAGAGA
 TGATAATCCCATACTCTCAATTGAGTTAAATCAGTAATCTGTCGCAT
 TTCATCAAGATTA (SEQ ID NO: 4).

[15] In embodiments, the PAH sequence or the variant thereof comprises: SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3; or SEQ ID NO: 4.

30 [16] In embodiments, the prothrombin enhancer comprises a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with:

GCGAGAACTTGTGCCTCCCCGTGTTCTGCTCTTTGTCCCTCTGTCC
 TACTTAGACTAATATTTGCCTTGGGTACTGCAAACAGGAAATGGGG
 GAGGGACAGGAGTAGGGCGGAGGGTAG (SEQ ID NO: 5).

[17] In embodiments, the sequence of prothrombin enhancer comprises SEQ ID
 5 NO: 5.

[18] In embodiments, the sequence of the hAAT promoter comprises SEQ ID NO:
 6. In embodiments, the sequence of the beta globin intron comprises one of SEQ ID NOs: 7
 or 8. In embodiments, the sequence of the hepatocyte nuclear factor binding site comprises
 any one of SEQ ID NOs: 9-12.

10 [19] In embodiments, the therapeutic cargo portion further comprises at least one
 small RNA sequence that is capable of binding to at least one pre-determined complementary
 mRNA sequence. In embodiments, the at least one small RNA sequence targets a
 complementary mRNA sequence that contains a full-length UTR. In embodiments, the at
 least one pre-determined complementary mRNA sequence is a PAH mRNA sequence. In
 15 embodiments, the at least one small RNA sequence inhibits production of endogenous PAH.
 In embodiments, the at least one small RNA sequence comprises a shRNA. In embodiments,
 the at least one small RNA sequence is under the control of a first promoter and the PAH
 sequence or the variant thereof is under the control of a second promoter. In embodiments,
 the first promoter comprises a H1 promoter. In embodiments, the second promoter comprises
 20 a liver-specific promoter. In embodiments, the liver-specific promoter comprises a hAAT
 promoter. In embodiments, the at least one small RNA sequence comprises a sequence
 having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with:

TCGCATTTTCATCAAGATTAATCTCGAGATTAATCTTGATGAAATGC
GATTTTT (SEQ ID NO: 13); or

ACTCATAAAGGAGCATATAAGCTCGAGCTTATATGCTCCTTTATGA
GTTTTTT (SEQ ID NO: 14).

5 [20] In embodiments, the at least one small RNA sequence comprises SEQ ID NO: 13; or SEQ ID NO: 14.

[21] In embodiments, the viral vector is a lentiviral vector. In embodiments, the viral vector is an AAV vector.

[22] In an aspect of the disclosure, a lentiviral particle capable of infecting a target
10 cell comprises an envelope protein optimized for infecting the target cell, and the viral vector according to any of the embodiments of the disclosure. In embodiments, the target cell is a hepatic cell, a muscle cell, an epithelial cell, an endothelial cell, a neural cell, a neuroendocrine cell, an endocrine cell, a lymphocyte, a myeloid cell, a cell present within a solid organ, or cell of a hematopoietic lineage, a hematopoietic stem cell, or a precursor
15 hematopoietic stem cell.

[23] In an aspect of the disclosure, a method of treating PKU in a subject is disclosed. The method comprises administering to the subject a therapeutically effective amount of the lentiviral particle described herein. In an aspect of the disclosure, a method of preventing PKU in a subject comprises administering to the subject a therapeutically effective
20 amount of the lentiviral particle described herein. In embodiments, the method further comprises diagnosing a PKU genotype in the subject that correlates with a PKU phenotype. In embodiments, the subject is *in utero*. In embodiments, the diagnosing occurs during prenatal screening of the subject. In embodiments, the diagnosing occurs *in vitro*. In embodiments, the therapeutically effective amount of the lentiviral particle comprises a
25 plurality of single doses of the lentiviral particle. In embodiments, the therapeutically effective amount of the lentiviral particle comprises a single dose of the lentiviral particle.

BRIEF DESCRIPTION OF THE DRAWINGS

[24] **Figure 1** depicts an exemplary 3-vector lentiviral vector system in a circularized form.

[25] **Figure 2** depicts an exemplary 4-vector lentiviral vector system in a
5 circularized form.

[26] **Figure 3** depicts linear maps of eight exemplary lentiviral vectors containing variations of the prothrombin enhancer and hAAT promoter to regulate the expression of PAH.

[27] **Figures 4A and 4B** depict immunoblot data comparing levels of PAH with
10 different enhancer elements and with or without the 3' UTR in (Figure 4A) Hepal-6 and (Figure 4B) 293T cells.

[28] **Figures 5A-5C** depict immunoblot data comparing levels of PAH in Hepal-6 cells (Figure 5A) with or without a rabbit beta globin intron, (Figure 5B) a codon-optimized PAH sequence, and (Figure 5C) a prothrombin enhancer with a HNF1 or HNF1/4 binding site
15 either upstream or downstream.

[29] **Figure 6** depicts PAH RNA expression in Hepal-6 cells transduced with lentiviral vectors expressing PAH via variations in the prothrombin enhancer.

[30] **Figure 7** depicts immunoblot data comparing levels of PAH expression with either the anti-alpha 1 trypsin (hAAT) or thyroxin-binding globulin (TBG) promoter in
20 Hepal-6 cells.

[31] **Figures 8A and 8B** depict immunoblot data comparing levels of PAH with or without a rabbit or human beta globin intron in Hepal-6 cells (Figure 8A) or Hep3B cells (Figure 8B).

[32] **Figure 9** depicts immunoblot data of PAH expression in human primary
25 hepatocytes with lentiviral vectors expressing PAH.

[33] **Figures 10A-10C** depict PAH activity by detection of phenylalanine levels in (Figures 10A and 10C) cell media or (Figure 10B) lysate of Hepal-6 cells that were transduced with lentiviral vectors expressing PAH and treatment with sepiapterin, a BH4 cofactor precursor.

5 [34] **Figure 11** depicts decreased levels of Phe in blood of Pah^{enu2} mice after treatment with a lentiviral vector containing PAH.

[35] **Figure 12** depicts blood phenylalanine suppression by LV-Pro-hAAT-PAH.

[36] **Figures 13A-13D** depict PAH protein expression (Figure 13A) and PAH RNA expression (Figure 13D) after AAV-delivered expression of PAH in 293 cells with various
10 DJ or AAV/2 serotype vectors; fold changes of PAH protein expression were also analyzed after delivery of the AAV/DJ vectors (Figure 13B) and the AAV/2 vectors (Figure 13C).

[37] **Figure 14** depicts decreased levels of Phe in neonatal $enu2/enu2$ mice treated with LV-Pro-hAAT-PAH lentivirus vector therapy for PKU.

[38] **Figure 15** depicts data from Hep3B cells showing PAH expression following
15 treatment with lentiviral vectors encoding prothrombin-hAAT-PAH-PAH shRNA sequence #1 (SEQ ID NO: 13) or prothrombin-hAAT-PAH-PAH shRNA sequence #2 (SEQ ID NO: 14), each of which target the 3'UTR of the mRNA expressed by the endogenous Pah gene and inhibit expression of the PAH protein.

DETAILED DESCRIPTION

20 Overview of the Disclosure

[39] This disclosure relates to therapeutic vectors and delivery of the same to cells. In embodiments, the therapeutic vectors include PAH sequences or variants thereof, and a liver-specific enhancer. In embodiments, the therapeutic vectors also include a small RNA that regulates host (*i.e.*, endogenous) PAH protein expression.

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Definitions and Interpretation

[40] Unless otherwise defined herein, scientific and technical terms used in connection with this disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclature used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art. The methods and techniques of the disclosure are generally performed according to conventional methods well-known in the art and as described in various general and more specific references that are cited and discussed throughout the specification unless otherwise indicated. See, *e.g.*: Sambrook J. & Russell D. Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2000); Ausubel et al., Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology, Wiley, John & Sons, Inc. (2002); Harlow and Lane Using Antibodies: A Laboratory Manual; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1998); and Coligan et al., Short Protocols in Protein Science, Wiley, John & Sons, Inc. (2003). Any enzymatic reactions or purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The nomenclature used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art.

[41] As used in the description and the appended claims, the singular forms “a”, “an” and “the” are used interchangeably and intended to include the plural forms as well and fall within each meaning, unless the context clearly indicates otherwise. Also, as used herein,

“and/or” refers to and encompasses any and all possible combinations of one or more of the listed items, as well as the lack of combinations when interpreted in the alternative (“or”).

[42] All numerical designations, *e.g.*, pH, temperature, time, concentration, and molecular weight, including ranges, are approximations which are varied (+) or (-) by increments of 0.1. It is to be understood, although not always explicitly stated that all numerical designations are preceded by the term “about”. The term “about” also includes the exact value “X” in addition to minor increments of “X” such as “X + 0.1” or “X – 0.1.” It also is to be understood, although not always explicitly stated, that the reagents described herein are merely exemplary and that equivalents of such are known in the art.

10 [43] As used herein, the term “about” will be understood by persons of ordinary skill in the art and will vary to some extent depending upon the context in which it is used. If there are uses of the term which are not clear to persons of ordinary skill in the art given the context in which it is used, “about” will mean up to plus or minus 10% of the particular term.

[44] The terms “administration of” or “administering” an active agent should be understood to mean providing an active agent to the subject in need of treatment in a form that can be introduced into that individual's body in a therapeutically useful form and therapeutically effective amount.

[45] As used herein, the term “comprising” is intended to mean that the compositions and methods include the recited elements, but not excluding others. “Consisting essentially of” when used to define compositions and methods, shall mean excluding other elements of any essential significance to the composition or method. “Consisting of” shall mean excluding more than trace elements of other ingredients for claimed compositions and substantial method steps. Embodiments defined by each of these transition terms are within the scope of this disclosure. Accordingly, it is intended that the methods and compositions can include additional steps and components (comprising) or alternatively including steps and

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compositions of no significance (consisting essentially of) or alternatively, intending only the stated method steps or compositions (consisting of).

[46] As used herein, “expression”, “expressed”, or “encodes” refers to the process by which polynucleotides are transcribed into mRNA and/or the process by which the transcribed mRNA is subsequently being translated into peptides, polypeptides, or proteins. Expression may include splicing of the mRNA in a eukaryotic cell or other forms of post-transcriptional modification or post-translational modification.

[47] As used herein, the term “adeno-associated viral vector,” refers to a carrier or transporter of that adeno-associated virus. The term “adeno-associated viral vector” may also be referred to herein as an “AAV vector”.

[48] As used herein, the term “adeno-associated virus,” refers to a small virus that generates a mild immune response, is capable of integrating into a host cell genome, and is not pathogenic.

[49] As used herein, the term “AAV/DJ” (also referred to herein as “AAV-DJ”) is a serotype of an AAV vector engineered from different AAV serotypes, which mediates higher transduction and infectivity rates than wild-type AAV serotypes.

[50] As used herein, the term “AAV2” (also referred to herein as “AAV/2” or “AAV-2”) is a naturally occurring AAV serotype.

[51] As used herein, the term “AAV-Pro-hAAT-PAH” refers to an AAV vector comprising a prothrombin enhancer, a hAAT promoter, and a PAH sequence.

[52] As used herein, the abbreviation “ApoE enhancer” refers to an Apolipoprotein E enhancer.

[53] As used herein, the term “genetic medicine” or “genetic medicines” refers generally to therapeutics and therapeutic strategies that focus on genetic targets to treat a

clinical disease or manifestation. The term “genetic medicine” encompasses gene therapy and the like.

[54] As used herein, the abbreviation “hAAT” refers to a hAAT promoter.

[55] As used herein, the term “hAAT-hPAH-3’UTR₂₈₉” may also be referred to
5 herein as U₂₈₉, or generally as transgene-expressed truncated hPAH 3’UTR, or generally a truncated 3’ UTR.

[56] As used herein, the term “hepatocyte nuclear factors” refers to transcription factors that are predominantly expressed in the liver. Types of hepatocyte nuclear factors include, but are not limited to, hepatocyte nuclear factor 1, hepatocyte nuclear factor 2,
10 hepatocyte nuclear factor 3, and hepatocyte nuclear factor 4.

[57] As used herein, the abbreviation “HNF” refers to hepatocyte nuclear factor. Accordingly HNF1 refers to hepatocyte nuclear factor 1, HNF2 refers to hepatocyte nuclear factor 2, HNF3 refers to hepatocyte nuclear factor 3, and HNF4 refers to hepatocyte nuclear factor 4.

15 [58] As used herein, the phrase “HNF binding site,” refers to a region of DNA to which a HNF transcription factor can bind. Accordingly, a HNF1 binding site is a region of DNA to which HNF1 can bind, and a HNF4 binding site is a region of DNA to which HNF4 can bind.

[59] As used herein, the terms “individual,” “subject,” and “patient” are used
20 interchangeably herein, and refer to any individual mammal subject, *e.g.*, murine, porcine, bovine, canine, feline, equine, nonhuman primate or human primate.

[60] As used herein, the phrase “rabbit beta globin intron” refers to a nucleic acid segment within the rabbit beta globin gene that is spliced out during RNA maturation, and does not code for a protein.

[61] As used herein, the phrase “human beta globin intron” refers to a nucleic acid segment within the human beta globin gene that is spliced out during RNA maturation, and does not code for a protein.

[62] As used herein, the term “LV” refers generally to “lentivirus.” As a non-
5 limiting example, reference to “LV-PAH” is reference to a lentivirus that contains a PAH sequence and expresses PAH.

[63] As used herein, the term “LV-Pro-hAAT-PAH” refers to a lentivirus comprising a prothrombin enhancer, a hAAT promoter, and a PAH sequence. The LV-Pro-hAAT-PAH vector is also referred to as the AGT323 vector.

10 [64] As used herein, the term “LV-HNF-Pro-hAAT-PAH” refers to a lentivirus comprising a HNF binding site, a prothrombin enhancer, a hAAT promoter, and a PAH sequence.

[65] As used herein, the term “LV-Pro-intron-PAH” refers to a lentivirus comprising a prothrombin enhancer, an intron, and a PAH sequence, wherein the intron is the
15 human beta globin intron.

[66] As used herein, the term “LV-Pro-hAAT” refers to a lentivirus comprising a prothrombin enhancer and a hAAT promoter.

[67] As used herein, the term “LV-Pro-TBG-PAH” refers to a lentivirus comprising a prothrombin enhancer, a thyroxin binding globulin, and a PAH sequence.

20 [68] As used herein, the term “LV-ApoE-hAAT-PAH-UTR” refers to a lentivirus comprising an apolipoprotein E enhancer, a hAAT promoter, a PAH sequence, and an untranslated region of a gene, wherein the untranslated region is the 3’UTR of the PAH gene.

[69] As used herein, the term “LV-Pro-hAAT-PAH-shPAH” refers to a lentivirus comprising a prothrombin enhancer, a hAAT promoter, a PAH sequence and a shPAH
25 sequence.

[70] As used herein, the term “packaging cell line” refers to any cell line that can be used to express a lentiviral particle.

[71] As used herein, the term “percent identity”, in the context of two or more nucleic acid or polypeptide sequences, refer to two or more sequences or subsequences that
5 have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned for maximum correspondence, as measured using one of the sequence comparison algorithms described below (*e.g.*, BLASTP and BLASTN or other algorithms available to persons of skill) or by visual inspection. Depending on the application, the “percent identity” can exist over a region of the sequence being compared, *e.g.*, over a
10 functional domain, or, alternatively, exist over the full length of the two sequences to be compared. For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence
15 comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

[72] As used herein, “pharmaceutically acceptable” refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues, organs, and/or bodily fluids of human
20 beings and animals without excessive toxicity, irritation, allergic response, or other problems or complications commensurate with a reasonable benefit/risk ratio.

[73] As used herein, a “pharmaceutically acceptable carrier” refers to, and includes, any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. The

compositions can include a pharmaceutically acceptable salt, e.g., an acid addition salt or a base addition salt (see, e.g., Berge *et al.* (1977) *J. Pharm. Sci.* 66:1-19).

[74] The term “phenylalanine hydroxylase” may also be referred to herein as PAH. The term phenylalanine hydroxylase includes all wild-type and variant PAH sequences, including both nucleotide and peptide sequences. Without limitation, the term phenylalanine hydroxylase includes reference to SEQ ID NOs: 1-4, and further includes variants having at least about 80% identity therewith. Human PAH may also be referred to herein as hPAH. Human PAH may also be referred to herein as hPAH.

[75] As used herein, the term “wild-type hPAH” may also be referred to herein as endogenous PAH or “full-length PAH”.

[76] As used herein, the term “phenylketonuria”, which is also referred to herein as “PKU”, refers to the chronic deficiency of phenylalanine hydroxylase, as well as all symptoms related thereto including mild and classical forms of disease. Treatment of “phenylketonuria”, therefore, may relate to treatment for all or some of the symptoms associated with PKU.

[77] As used herein, the term “prothrombin enhancer” is a region on the prothrombin gene that can be bound by proteins, which results in transcription of the prothrombin gene.

[78] As used herein, the abbreviation “Pro” refers to a prothrombin enhancer.

[79] As used herein, “small RNA” refers to non-coding RNA that are generally about 200 nucleotides or less in length and possess a silencing or interference function. In other embodiments, the small RNA is about 175 nucleotides or less, about 150 nucleotides or less, about 125 nucleotides or less, about 100 nucleotides or less, or about 75 nucleotides or less in length. Such RNAs include microRNA (miRNA), small interfering RNA (siRNA), double stranded RNA (dsRNA), and short hairpin RNA (shRNA). “Small RNA” of the

disclosure should be capable of inhibiting or knocking-down gene expression of a target gene, generally through pathways that result in the destruction of the target gene mRNA.

[80] As used herein, the term “shPAH” refers to a small hairpin RNA that targets PAH.

5 [81] As used herein, the abbreviation “lncRNA” refers to a long non-coding RNA.

[82] As used herein, the term “SEQ ID NO” is synonymous with the term “Sequence ID No.”

[83] As used herein, the term “thyroxin binding globulin,” is a transport protein responsible for carrying thyroid hormones in the bloodstream. As used herein, the
10 abbreviation “TBG” refers to thyroxin binding globulin.

[84] As used herein, the term “therapeutically effective amount” refers to a sufficient quantity of the active agents of the present disclosure, in a suitable composition, and in a suitable dosage form to treat or prevent the symptoms, progression, or onset of the complications seen in patients suffering from a given ailment, injury, disease, or condition.

15 The therapeutically effective amount will vary depending on the state of the patient’s condition or its severity, and the age, weight, *etc.*, of the subject to be treated. A therapeutically effective amount can vary, depending on any of a number of factors, including, *e.g.*, the route of administration, the condition of the subject, as well as other factors understood by those in the art.

20 [85] As used herein, the term “therapeutic vector” includes, without limitation, reference to a lentiviral vector or an adeno-associated viral (AAV) vector. Additionally, as used herein with reference to the lentiviral vector system, the term “vector” is synonymous with the term “plasmid”. For example, the 3-vector and 4-vector systems, which include the 2-vector and 3-vector packaging systems, can also be referred to as 3-plasmid and 4-plasmid
25 systems.

[86] As used herein, the term “treatment” or “treating” generally refers to an intervention in an attempt to alter the natural course of the subject being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects include, but are not limited to, preventing occurrence or recurrence of disease, alleviating
5 symptoms, suppressing, diminishing or inhibiting any direct or indirect pathological consequences of the disease, ameliorating or palliating the disease state, and causing remission or improved prognosis.

[87] “A treatment” is intended to target the disease state and combat it, *i.e.*, ameliorate or prevent the disease state. The particular treatment thus will depend on the
10 disease state to be targeted and the current or future state of medicinal therapies and therapeutic approaches. A treatment may have associated toxicities.

[88] As used herein, the term “truncated” may also be referred to herein as “shortened” or “without”.

[89] As used herein, the term “UTR” is in reference to a region of a gene that is
15 either 5’ or 3’ of the coding region of a gene.

[90] As used herein, the term “3’ UTR” is the “UTR” that is 3’ of the coding region of a gene.

[91] As used herein, the term “variant” may also be referred to herein as analog or variation. A variant refers to any substitution, deletion, or addition to a nucleotide sequence.

20 [92] As considered herein, optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson & Lipman, Proc. Nat’l. Acad. Sci. USA 85:2444 (1988), by computerized implementations of these algorithms (GAP,
25 BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics

Computer Group, 575 Science Dr., Madison, Wis.), or by visual inspection (see generally Ausubel et al., *infra*).

[93] One example of an algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul *et al.*, J. Mol. Biol. 215:403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information website.

[94] The nucleic acid and protein sequences of the present disclosure can further be used as a “query sequence” to perform a search against public databases to, for example, identify related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, word length = 12 to obtain nucleotide sequences homologous to the nucleic acid molecules provided in the disclosure. BLAST protein searches can be performed with the XBLAST program, score = 50, word length = 3 to obtain amino acid sequences homologous to the protein molecules of the disclosure. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>.

Description of Aspects and Embodiments of the Disclosure

[95] In an aspect of the disclosure, a viral vector comprises a therapeutic cargo portion, wherein the therapeutic cargo portion comprises a PAH sequence or a variant thereof, a promoter, and a liver-specific enhancer, wherein the PAH sequence or the variant thereof is operatively controlled by both the promoter and the liver-specific enhancer.

[96] In embodiments, the liver-specific enhancer comprises a prothrombin enhancer. In embodiments, the promoter is a liver-specific promoter. In embodiments, the liver-specific promoter comprises a hAAT promoter. In embodiments, the therapeutic cargo portion further comprises a beta globin intron. In embodiments, the therapeutic cargo portion further comprises at least one hepatocyte nuclear factor binding site. In embodiments, the at least one hepatocyte nuclear factor binding site is disposed upstream of the prothrombin enhancer. In embodiments, the at least one hepatocyte nuclear factor binding site is disposed downstream of the prothrombin enhancer.

[97] In embodiments, a lentiviral vector is provided comprising a prothrombin enhancer, a hAAT promoter, and a PAH sequence (LV-Pro-hAAT-PAH). In embodiments, a lentiviral vector is provided comprising a HNF binding site, a prothrombin enhancer, a hAAT promoter, and a PAH sequence (LV-HNF-Pro-hAAT-PAH). In embodiments, the HNF binding site is a HNF1 or HNF1/4 binding site. In embodiments, a lentiviral vector is provided comprising a prothrombin enhancer, a hAAT promoter, an intron, and a PAH sequence (LV-Pro-intron-PAH). In embodiments, the intron is a rabbit globin intron. In embodiments, the intron is a human globin intron. In embodiments, a lentiviral vector is provided comprising a prothrombin enhancer and a hAAT promoter (LV-Pro-hAAT). In embodiments, a lentiviral vector is provided comprising a prothrombin enhancer, a thyroxin binding globulin, and a PAH sequence (LV-Pro-TBG-PAH). In embodiments, a lentiviral vector is provided comprising a ApoE enhancer, a hAAT promoter, a PAH sequence, and the 3'UTR of PAH (LV-ApoE-hAAT-PAH-UTR).

[98] In embodiments, the PAH sequence or the variant thereof is truncated. In embodiments, the portion of the PAH sequence or the variant thereof that is truncated is the 3' untranslated region (UTR) of the PAH sequence or the variant thereof.

- [99] In embodiments, the PAH truncation at the 3'UTR prevents binding of certain regulatory RNA to the 3'UTR. In embodiments, the regulatory RNA is a lncRNA. In embodiments, the regulatory RNA is a microRNA. In embodiments, the regulatory RNA is a piRNA. In embodiments, the regulatory RNA is a shRNA. In embodiments the regulatory RNA is a siRNA between 19 and 25 nucleotides in length. In embodiments, the regulatory RNA is a small RNA sequence comprising a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95% or more percent identity with SEQ ID NOs: 13 or 14.
- 10 [100] In embodiments, the PAH sequence comprises SEQ ID NO: 1. In embodiments, the PAH sequence comprises a codon optimized PAH sequence (SEQ ID NO: 2). In embodiments, the PAH sequence or the variant thereof comprises a truncated 3' UTR (289 nucleotides) (SEQ ID NO: 4). In embodiments, the PAH sequence or the variant thereof comprises a 5' UTR (897 nucleotides) (SEQ ID NO: 3).
- 15 [101] In embodiments, the PAH sequence or the variant thereof comprises a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95% or more percent identity with SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3; or SEQ ID NO: 4.
- 20 [102] In embodiments, variants can be made to any of the above-described sequences. In embodiments, the PAH sequence or the variant thereof comprises SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3; or SEQ ID NO: 4.
- [103] In embodiments, the prothrombin enhancer comprises a sequence having at least 80%, or at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%,

at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%,
at least 94%, at least 95% or more percent identity with SEQ ID NO: 5

[104] In embodiments, variants can be made to the above-described sequences. In
embodiments, the sequence of prothrombin enhancer comprises SEQ ID NO: 5.

5 [105] In embodiments, the sequence of the hAAT promoter comprises SEQ ID NO:
6. In embodiments, the sequence of the beta globin intron comprises one of SEQ ID NOs: 7
or 8. In embodiments, the sequence of the hepatocyte nuclear factor binding site comprises
any one of SEQ ID NOs: 9-12.

[106] In embodiments, the therapeutic cargo portion further comprises at least one
10 small RNA sequence that is capable of binding to at least one pre-determined complementary
mRNA sequence. In embodiments, the at least one small RNA sequence targets a
complementary mRNA sequence that contains a full-length UTR. In embodiments, the at
least one pre-determined complementary mRNA sequence is a PAH mRNA sequence. In
embodiments, the at least one small RNA sequence comprises a shRNA. In embodiments, the
15 at least one small RNA sequence is under the control of a first promoter and the PAH
sequence or the variant thereof is under the control of a second promoter. In embodiments,
the first promoter comprises a H1 promoter. In embodiments, the second promoter comprises
a liver-specific promoter. In embodiments, the liver-specific promoter comprises a hAAT
promoter. In embodiments, the at least one small RNA sequence comprises a sequence
20 having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at
least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at
least 93%, at least 94%, at least 95% or more percent identity with SEQ ID NO: 13; or SEQ
ID NO: 14.

[107] In embodiments, variants can be made to any of the above-described
25 sequences. In embodiments, the at least one small RNA sequence comprises SEQ ID NO: 13;

or SEQ ID NO: 14.

[108] In embodiments, a lentiviral vector is provided comprising a prothrombin enhancer, a hAAT promoter, a PAH sequence, and a shRNA that targets endogenous PAH (LV-Pro-hAAT-PAH-shPAH). In embodiments, the shRNA targets the 3'UTR of
5 endogenous PAH. In embodiments, the shPAH comprises SEQ ID NO: 13. In embodiments, the shPAH comprises SEQ ID NO: 14.

[109] In an aspect of the disclosure, a lentiviral particle capable of infecting a target cell comprises an envelope protein optimized for infecting the target cell, and any of the viral vectors disclosed herein. In embodiments, the target cell is a hepatic cell, a muscle cell, an
10 epithelial cell, an endothelial cell, a neural cell, a neuroendocrine cell, an endocrine cell, a lymphocyte, a myeloid cell, a cell present within a solid organ, or cell of a hematopoietic lineage, a hematopoietic stem cell, or a precursor hematopoietic stem cell.

[110] In an aspect of the disclosure, a method of treating PKU in a subject comprises administering to the subject a therapeutically effective amount of any of the lentiviral
15 particles disclosed herein. In an aspect of the disclosure, a method of preventing PKU in a subject comprises administering to the subject a therapeutically effective amount of any of the lentiviral particles disclosed herein. In another aspect of the disclosure, use of a therapeutically effective amount of any of the lentiviral particles disclosed herein for treating PKU in a subject is disclosed. In embodiments, the method further comprises diagnosing a
20 PKU genotype in the subject that correlates with a PKU phenotype. In embodiments, the subject is *in utero*. In embodiments, the diagnosing occurs during prenatal screening of the subject or after genetic screening of the parents. In embodiments, the diagnosing occurs *in vitro*. In embodiments, the therapeutically effective amount of the lentiviral particle comprises a plurality of single doses of the lentiviral particle. In embodiments, the
25 therapeutically effective amount of the lentiviral particle comprises a single dose of the

lentiviral particle.

[111] In an aspect of the disclosure, a method of treating PKU in a subject is provided which comprises treating a subject that has a mutant form of PAH with a therapeutically effective amount of a lentiviral vector comprising exogenous PAH. In 5 embodiments, the subject is a mammal. In embodiments, the mammal is a human. In embodiments, the mammal is a rodent. In embodiments, the rodent is a mouse or a rat. In embodiments, the mammal is porcine.

[112] In embodiments, the subject is treated with a lentiviral vector. In embodiments the lentiviral vector comprises a PAH sequence or a variant thereof. In 10 embodiments, the PAH sequence is any of the PAH sequences or variants described herein.

[113] In embodiments, the lentiviral vector is any of the lentiviral vectors comprising PAH or variants described herein. In embodiments, the lentiviral vector comprising PAH is a lentiviral vector expressing PAH as depicted in Figures 1 and 2. In 15 embodiments, the lentiviral vector comprising PAH is the lentiviral vector expressing PAH depicted in Figure 3.

[114] In embodiments, the viral vector comprises a prothrombin enhancer, a hAAT promoter, and a PAH sequence (also referred to herein as LV-Pro-hAAT-PAH or AGT323). In embodiments, the prothrombin enhancer sequence is any of the prothrombin sequences or 20 variants described herein. In embodiments, the hAAT promoter is any of the hAAT promoter sequences or variants described herein. In embodiments, the PAH sequences are any of the PAH sequences or variants described herein.

[115] In embodiments, the lentiviral vector is comprised of an integrated lentiviral vector. In embodiments, the integrated lentiviral vector is derived from a lentiviral vector system. In embodiments, the lentiviral vector system comprises separate plasmids encoding 25 a rev gene and an env gene. In embodiments, the integrated lentiviral vector is derived from

a 3-vector lentiviral system. In embodiments, the 3-vector lentiviral system is illustrated in Figure 1. In embodiments, the integrated lentiviral vector is derived from a 4-vector lentiviral system. In embodiments, the 4-vector lentiviral vector system is illustrated in Figure 2.

5 [116] In embodiments, the subject is treated with adeno-associated viral (AAV) vector. In embodiments, the AAV vector comprises any of the AAV vectors disclosed herein. In embodiments, the AAV vector comprises a PAH sequence or variants thereof. In embodiments, the PAH sequence is any of the PAH sequences or variants described herein.

[117] In embodiments, the injection is an intradermal injection. In embodiments,
10 the injection is an intramuscular injection. In embodiments, the injection is a subcutaneous injection. In embodiments, the injection is an intravenous injection.

[118] In embodiments, the methods described herein further comprise producing a specific titer of an integrated lentiviral vector prior to treating the subject with the lentiviral particle. The specific titer is determined in a test system utilizing a cell target and lentiviral
15 vector transduction *in vitro*, followed by quantitative PCR analysis of chromosomal DNA from transduced cells to measure the frequency of transduced cells and the number of integrated vector copy numbers per cell. Titer is expressed as the number of integrated copy numbers that will result from transduction with an appropriate column of lentiviral vector into an appropriate number of cells. In embodiments, the titer is between 1×10^5 and 1×10^{15}
20 integrated vector copies, for example, between 1×10^7 and 1×10^{13} integrated vector copies, or between 1×10^9 and 1×10^{11} integrated vector copies. In embodiments, the titer is 1×10^{10} integrated vector copies.

[119] In embodiments, producing a specific titer of an integrated lentiviral vector comprises adding a vector system to one or more cells. In embodiments, the one or more
25 cells is a cell line. In embodiments, the cell line is a 293T cell line. In embodiments, the cell

line is a HeLa cell line. In embodiments, the cell line is a CHO cell line. In embodiments, the cell line is a Hep3B cell line. Persons skilled in the art will also appreciate that in other embodiments, the cell line may be any suitable cell line known in the art.

[120] In embodiments, the method further comprises measuring the levels of Phe in the blood following injection of the lentiviral vector comprising PAH.

[121] In an aspect of the disclosure, human PAH is expressed in cells using an AAV-delivered expression system. In embodiments a AAV-2 serotype is used. In embodiments, a AAV-DJ serotype is used. In embodiments, the AAV vector contains GFP. In embodiments, the AAV vector may represent any serotype or may be generated by recombinant DNA or other synthetic approaches designed to improve transduction of human hepatocytes.

[122] In embodiments, a human PAH is introduced into an AAV vector. In embodiments, a prothrombin enhancer is introduced into an AAV vector. In embodiments, a hAAT promoter is introduced into a AAV vector. In embodiments, a rabbit globin intron is introduced into a AAV vector. In embodiments, any one or more of a human PAH, a prothrombin enhancer, a hAAT promoter, and rabbit globin intron are introduced into an AAV vector. In embodiments the viral vector comprises a prothrombin enhancer, a hAAT promoter, and a PAH sequence (AAV-Pro-hAAT-PAH; AGT323).

[123] In embodiments, the prothrombin enhancer sequence is any of the prothrombin sequences or variants disclosed herein. In embodiments, the PAH sequence is any of the PAH sequences or variants described herein. In embodiments, the hAAT sequence is any of the hAAT sequences or variants disclosed herein. In embodiments, the intron sequence is any of the intron sequences or variants disclosed herein.

[124] In an aspect of the disclosure, lentiviral vector therapy is used in treatment of a subject that has a mutant PAH gene. In embodiments, the subject is a human. In other

embodiments, as shown experimentally herein, the subject is a neonatal mouse derived from a Pah mutant mouse line. In embodiments, the mutant mouse line is Pah^{enu1}. In embodiments, the mutant mouse line is Pah^{enu2}. In embodiments, the mutant mouse line is Pah^{enu3}.

5 [125] In embodiments, the PAH sequence in the lentiviral vector is any of the PAH sequences or variants described herein and including those listed in the PAHvdb, BLODEF, BIOPKU, JAKE or PNDdb databases found at www.biopku.org.

[126] In embodiments, the lentiviral vector is comprised of an integrated lentiviral vector. In embodiments, the integrated lentiviral vector is derived from a lentiviral vector system. In embodiments, the lentiviral vector system comprises separate plasmids encoding a rev gene and a envelope gene. In embodiments, the integrated lentiviral vector is derived from a 3-vector lentiviral system. In embodiments, the 3-vector lentiviral system is illustrated in Figure 1. In embodiments, the integrated lentiviral vector is derived from a 4-vector lentiviral system. In embodiments, the 4-vector lentiviral vector system is illustrated in Figure 2.

[127] In an aspect of the disclosure, expression of a lentivirus in cells containing a shRNA and PAH suppresses expression of endogenous PAH, but does not suppress expression of exogenous PAH that is expressed from the lentiviral vector.

[128] In embodiments, a lentivirus containing shRNA and PAH is expressed in a subject *in vivo* as described herein. In embodiments, the subject is a mammal. In embodiments, the mammal is a human.

[129] In embodiments, a lentivirus containing shRNA and PAH is expressed *in vitro* or *ex vivo*. In embodiments, the lentivirus is expressed *in vitro*, for example in a cell line. In embodiments, the cell line is any of the cell lines described herein or those known to those persons skilled in the relevant art. In embodiments, the cell line is a Hep3B cell line.

[130] In embodiments, a lentiviral vector is provided comprising a prothrombin enhancer, a hAAT promoter, a PAH sequence, and a shRNA that targets endogenous PAH (optionally referred to herein as: LV-Pro-hAAT-PAH-shPAH). In embodiments, the shRNA targets the 3'UTR of endogenous PAH.

5 [131] In embodiments, the prothrombin enhancer sequence comprises any of the prothrombin sequences or variants disclosed herein. In embodiments, the hAAT promoter comprises any of the hAAT promoter sequences or variants disclosed herein. In embodiments, the PAH sequence comprises any of the PAH sequences or variants described herein. In embodiments, the shRNA sequence in the lentiviral vector comprises SEQ ID NO:
10 13. In embodiments, the shRNA sequence in the lentiviral vector comprises SEQ ID NO: 14.

[132] Other aspects and advantages of the inventions described herein will become apparent from the following detailed description, taken in conjunction with the accompanying drawings, which illustrate by way of example the aspects of the inventions.

Phenylketonuria

15 [133] PKU is believed to be caused by mutations of PAH and/or a defect in the synthesis or regeneration of PAH cofactors (*i.e.*, BH₄). Notably, several PAH mutations have been shown to affect protein folding in the endoplasmic reticulum resulting in accelerated degradation and/or aggregation due to missense mutations (63%) and small deletions (13%) in protein structure that attenuates or largely abolishes enzyme catalytic activity. As there are
20 numerous mutations that can affect the functionality of PAH, an effective therapeutic approach for treating PKU will need to address the aberrant PAH and a mode by which replacement PAH can be administered.

[134] In general, three major phenotypic groups are classified in PKU based on Phe levels measured at diagnosis, dietary tolerance to Phe and potential responsiveness to therapy.

These groups include classical PKU (Phe > 1200 μ M), atypical or mild PKU (Phe is 600 - 1200 μ M), and permanent mild hyperphenylalaninemia (HPA, Phe 120 - 600 μ M).

[135] Detection of PKU relies on universal newborn screening (NBS). A drop of blood collected from a heel stick is tested for phenylalanine levels in a screen that is
5 mandatory in all 50 states of the USA and used routinely in most developed countries.

Genetic Medicines

[136] Genetic medicine includes reference to viral vectors that are used to deliver genetic constructs to host cells for the purposes of disease therapy or prevention.

[137] Genetic constructs can include, but are not limited to, functional genes or
10 portions of genes to correct or complement existing defects, DNA sequences encoding regulatory proteins, DNA sequences encoding regulatory RNA molecules including antisense, short hairpin RNA, short homology RNA, long non-coding RNA, small interfering RNA or others, and decoy sequences encoding either RNA or proteins designed to compete for critical cellular factors to alter a disease state. Genetic medicine involves delivering these
15 therapeutic genetic constructs to target cells to provide treatment or alleviation of a particular disease.

[138] By delivering a functional PAH gene to the liver *in vivo*, PAH activity should be reconstituted leading to normal clearance of Phe in the blood therefore eliminating the need for dietary restrictions or frequent enzyme replacement therapies. The effect of this
20 therapeutic approach should be improved by the targeting of a shRNA against endogenous PAH. In an aspect of the disclosure, a functional PAH gene or a variant thereof can also be delivered *in utero* if a fetus has been identified as being at risk to a PKU genotype. In embodiments, the diagnostic step can be carried out to determine whether the fetus is at risk for a PKU phenotype. If the diagnostic step determines that the fetus is at risk for a PKU

phenotype, then the fetus can be treated with the genetic medicines detailed herein. Treatment can occur *in utero* or *in vitro*.

Therapeutic Vectors

[139] A lentiviral virion (particle) in accordance with various aspects and
5 embodiments herein is expressed by a vector system encoding the necessary viral proteins to produce a virion (viral particle). In various embodiments, one vector containing a nucleic acid sequence encoding the lentiviral Pol proteins is provided for reverse transcription and integration, operably linked to a promoter. In another embodiment, the Pol proteins are expressed by multiple vectors. In other embodiments, vectors containing a nucleic acid
10 sequence encoding the lentiviral Gag proteins for forming a viral capsid, operably linked to a promoter, are provided. In embodiments, this gag nucleic acid sequence is on a separate vector than at least some of the pol nucleic acid sequence.

[140] Numerous modifications can be made to the vectors herein, which are used to create the particles to further minimize the chance of obtaining wild type revertants. These
15 include, but are not limited to deletions of the U3 region of the LTR, tat deletions and matrix (MA) deletions. In embodiments, the gag, pol and env vector(s) do not contain nucleotides from the lentiviral genome that package lentiviral RNA, referred to as the lentiviral packaging sequence.

[141] The vector(s) forming the particle preferably do not contain a nucleic acid
20 sequence from the lentiviral genome that expresses an envelope protein. Preferably, a separate vector that contains a nucleic acid sequence encoding an envelope protein operably linked to a promoter is used. This env vector also does not contain a lentiviral packaging sequence. In one embodiment the env nucleic acid sequence encodes a lentiviral envelope protein.

[142] In another embodiment the envelope protein is not from the lentivirus, but from a different virus. The resultant particle is referred to as a pseudotyped particle. By appropriate selection of envelope proteins one can infect virtually any cell. For example, one can use an env gene that encodes an envelope protein that targets an endocytic compartment
5 such as that of the influenza virus, VSV-G or similar envelope proteins from human and non-human rhabdovirus isolates, alpha viruses (Semliki forest virus, Sindbis virus), arenaviruses (lymphocytic choriomeningitis virus), flaviviruses (tick-borne encephalitis virus, Dengue virus, hepatitis C virus, GB virus), rhabdoviruses (vesicular stomatitis virus, rabies virus), paramyxoviruses (mumps or measles) and orthomyxoviruses (influenza virus). Other
10 envelope proteins that can preferably be used include those from Feline Leukemia Virus and feline endogenous retroviruses, Moloney Leukemia Virus such as MLV-E, MLV- A, Gibbon Ape Leukemia Virus GALV, and Baboon Endogenous Retrovirus. These latter envelope proteins are particularly preferred where the host cell is a primary cell. Other envelope proteins can be selected depending upon the desired host cell.

15 [143] Lentiviral vector systems as provided herein typically include at least one helper plasmid comprising at least one of a gag, pol, or rev gene. Each of the gag, pol and rev genes may be provided on individual plasmids, or one or more genes may be provided together on the same plasmid. In one embodiment, the gag, pol, and rev genes are provided on the same plasmid (*e.g.*, Figure 1). In another embodiment, the gag and pol genes are
20 provided on a first plasmid and the rev gene is provided on a second plasmid (*e.g.*, Figure 2). Accordingly, both 3-vector and 4-vector systems can be used to produce a lentivirus as described herein. In embodiments, the therapeutic vector, at least one envelope plasmid and at least one helper plasmid are transfected into a packaging cell, for example a packaging cell line. A non-limiting example of a packaging cell line is the 293T/17 HEK cell line. When the
25 therapeutic vector, the envelope plasmid, and at least one helper plasmid are transfected into

the packaging cell line, a lentiviral particle is ultimately produced.

[144] In another aspect, a lentiviral vector system for expressing a lentiviral particle is disclosed. The system includes a lentiviral vector as described herein; an envelope plasmid for expressing an envelope protein optimized for infecting a cell; and at least one helper
5 plasmid for expressing gag, pol, and rev genes, wherein when the lentiviral vector, the envelope plasmid, and the at least one helper plasmid are transfected into a packaging cell line, a lentiviral particle is produced by the packaging cell line, wherein the lentiviral particle is capable of inhibiting of producing PAH and/or inhibiting the expression of endogenous PAH.

10 [145] In another aspect, the lentiviral vector, which is also referred to herein as a therapeutic vector, includes the following elements: hybrid 5' long terminal repeat (RSV/5' LTR) (SEQ ID NOs: 15-16), Psi sequence (RNA packaging site) (SEQ ID NO: 17), RRE (Rev-response element) (SEQ ID NO: 18), cPPT (polypurine tract) (SEQ ID NO: 19), Anti alpha trypsin promoter (hAAT) (SEQ ID NO: 6), Phenylalanine hydroxylase (PAH) (SEQ ID
15 NOs: 1-4, Woodchuck Post-Transcriptional Regulatory Element (WPRE) (SEQ ID NOs: 20), and Δ U3 3' LTR (SEQ ID NO: 21). In embodiments, the lentiviral vector, which is also referred to herein as a therapeutic vector, includes the following elements: hybrid 5' long terminal repeat (RSV/5' LTR) (SEQ ID NOs: 15-16), Psi sequence (RNA packaging site) (SEQ ID NO: 17), RRE (Rev-response element) (SEQ ID NO: 18), cPPT (polypurine tract)
20 (SEQ ID NO: 19), H1 promoter (SEQ ID NO: 22), PAH shRNA (SEQ ID NOs: 1-4), Anti alpha trypsin promoter (hAAT) (SEQ ID NO: 6), PAH shRNA (SEQ ID NOs: 1-4), Woodchuck Post-Transcriptional Regulatory Element (WPRE) (SEQ ID NO: 20), and Δ U3 3' LTR (SEQ ID NO: 21). In embodiments, sequence variation, by way of substitution, deletion, addition, or mutation can be used to modify the sequences references herein.

[146] In another aspect, a helper plasmid includes the following elements: CMV enhancer/chicken beta actin enhancer (SEQ ID NO: 23); HIV component gag (SEQ ID NO: 24); HIV component pol (SEQ ID NO: 25); HIV Int (SEQ ID NO: 26); HIV RRE (SEQ ID NO: 27); and HIV Rev (SEQ ID NO: 28). In another aspect, the helper plasmid may be modified to include a first helper plasmid for expressing the gag and pol genes, and a second and separate plasmid for expressing the rev gene. In embodiments, sequence variation, by way of substitution, deletion, addition, or mutation can be used to modify the sequences references herein.

[147] In another aspect, an envelope plasmid includes the following elements: RNA polymerase II promoter (CMV) (SEQ ID NO: 29) and vesicular stomatitis virus G glycoprotein (VSV-G) (SEQ ID NO: 30). In embodiments, sequence variation, by way of substitution, deletion, addition, or mutation can be used to modify the sequences references herein.

[148] In various aspects, the plasmids used for lentiviral packaging are modified by substitution, addition, subtraction or mutation of various elements without loss of vector function. For example, and without limitation, the following elements can replace similar elements in the plasmids that comprise the packaging system: Elongation Factor-1 (EF-1), phosphoglycerate kinase (PGK), and ubiquitin C (UbC) promoters can replace the CMV or CAG promoter. SV40 poly A and bGH poly A can replace the rabbit beta globin poly A. The HIV sequences in the helper plasmid can be constructed from different HIV strains or clades. The VSV-G glycoprotein can be substituted with membrane glycoproteins from human endogenous retroviruses including HERV-W, baboon endogenous retrovirus BaEV, feline endogenous virus (RD114), gibbon ape leukemia virus (GALV), Rabies (FUG), lymphocytic choriomeningitis virus (LCMV), influenza A fowl plague virus (FPV), Ross River alphavirus (RRV), murine leukemia virus 10A1 (MLV), or Ebola virus (EboV).

[149] Various lentiviral packaging systems can be acquired commercially (*e.g.*, Lenti-vpak packaging kit from OriGene Technologies, Inc., Rockville, MD), and can also be designed as described herein. Moreover, it is within the skill of a person ordinarily skilled in the relevant art to substitute or modify aspects of a lentiviral packaging system to improve any number of relevant factors, including the production efficiency of a lentiviral particle.

[150] In another aspect, adeno-associated viral (AAV) vectors can also be used. In embodiments, the AAV vector is an AAV-DJ serotype. In embodiments, the AAV vector is any of serotypes 1-11. In embodiments, the AAV serotype is AAV-2. In embodiments, the AAV vector is a non-natural type engineered for optimal transduction of human hepatocytes.

10 [151] *AAV Vector Construction.* In aspects of the disclosure, the PAH coding sequence (SEQ ID NOs: 1-4) and the prothrombin enhancer (SEQ ID NO: 5) with hAAT promoter (SEQ ID NO: 6) are inserted into the pAAV plasmid (Cell Biolabs, San Diego, CA). The PAH coding sequence with flanking EcoRI and SalI restriction sites is synthesized by Eurofins Genomics (Louisville, KY). The pAAV plasmid and PAH sequence are digested with EcoRI and SalI enzyme and ligated together. Insertion of the PAH sequence is verified by sequencing. Next, the prothrombin enhancer and hAAT promoter are synthesized by Eurofins Genomics (Louisville, KY) with flanking MluI and EcoRI restriction sites. The pAAV plasmid containing the PAH coding sequence and the prothrombin enhancer/hAAT promoter sequence are digested with MluI and EcoRI enzymes and ligated together.

15

20 Insertion of the prothrombin enhancer/hAAT promoter are verified by sequencing.

[152] Further, a representative AAV plasmid system for expressing PAH may comprise an AAV Helper plasmid, an AAV plasmid, and an AAV Rev/Cap plasmid. The AAV Helper plasmid may contain a Left ITR (SEQ ID NO: 31), a Prothrombin enhancer (SEQ ID NO: 5), a human Anti alpha trypsin promoter (SEQ ID NO: 6), a PAH element (SEQ ID NOs: 1-4), a PolyA element (SEQ ID NO: 32), and a Right ITR (SEQ ID NO: 33).

25

The AAV plasmid may contain a suitable promoter element (SEQ ID NO: 23 or SEQ ID NO: 29), an E2A element (SEQ ID NO: 34), an E4 element (SEQ ID NO: 35), a VA RNA element (SEQ ID NO: 36), and a PolyA element (SEQ ID NO: 32). The AAV Rep/Cap plasmid may contain a suitable promoter element, a Rep element (SEQ ID NO: 37), a Cap element (SEQ ID NO: 38), and a PolyA element (SEQ ID NO: 32).

[153] In embodiments, an AAV/DJ plasmid is provided comprising a prothrombin enhancer and a PAH sequence (AAV/DJ-Pro-PAH). In embodiments, an AAV/DJ plasmid is provided comprising a prothrombin enhancer, an intron, and a PAH sequence (AAV/DJ-Pro-Intron-PAH). In embodiments, the intron is a human beta globin intron. In embodiments, the intron is a rabbit beta globin intron. In embodiments, an AAV/DJ plasmid is provided comprising GFP (AAV/DJ-GFP).

[154] In embodiments, an AAV2 plasmid is provided comprising a prothrombin enhancer and a PAH sequence (AAV2-Pro-PAH). In embodiments, an AAV2 plasmid is provided comprising a prothrombin enhancer, an intron, and a PAH sequence (AAV2-Pro-Intron-PAH). In embodiments, the intron is a human beta globin intron. In embodiments, the intron is a rabbit beta globin intron. In embodiments, an AAV2 is provided comprising GFP (AAV2-GFP).

[155] In embodiments, any of the AAV vectors disclosed herein may contain a sequence that expresses a regulatory RNA. In embodiments, the regulatory RNA is a lncRNA. In embodiments, the regulatory RNA is a microRNA. In embodiments, the regulatory RNA is a piRNA. In embodiments, the regulatory RNA is a shRNA. In embodiments, the regulatory RNA is a small RNA sequence comprising a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95% or more percent identity with SEQ ID NOs: 13 or 14.

[156] *Production of AAV particles.* The AAV-PAH plasmid is combined with the plasmids pAAV-RC2 (Cell Biolabs) and pHelper (Cell Biolabs). The pAAV-RC2 plasmid contains the Rep and AAV-2 capsid genes and pHelper contains the adenovirus E2A, E4, and VA genes. The AAV capsid may also consist of the AAV-8 (SEQ ID NO: 39) or AAV-DJ (SEQ ID NO: 40) sequences. To produce AAV particles, these plasmids are transfected in the ratio 1:1:1 (pAAV-PAH: pAAV-RC2: pHelper) into 293T cells. For transfection of cells in 150 mm dishes (BD Falcon), 10 micrograms of each plasmid are added together in 1 ml of DMEM. In another tube, 60 microliters of the transfection reagent PEI (1 microgram/ml) (Polysciences) is added to 1 ml of DMEM. The two tubes are mixed together and allowed to incubate for 15 minutes. Then the transfection mixture is added to cells and the cells are collected after 3 days. The cells are lysed by freeze/thaw lysis in dry ice/isopropanol. Benzonase nuclease (Sigma) is added to the cell lysate for 30 minutes at 37 degrees Celsius. Cell debris are then pelleted by centrifugation at 4 degrees Celsius for 15 minutes at 12,000 rpm. The supernatant is collected and then added to target cells.

15 **Dosage and Dosage Forms**

[157] The disclosed vector compositions allow for short, medium, or long-term expression of genes or sequences of interest and episomal maintenance of the disclosed vectors. Accordingly, dosing regimens may vary based upon the condition being treated and the method of administration.

20 [158] In embodiments, vector compositions may be administered to a subject in need in varying doses. Specifically, a subject may be administered about $\geq 10^6$ infectious doses (where 1 dose is needed on average to transduce 1 target cell). More specifically, a subject may be administered about $\geq 10^7$, about $\geq 10^8$, about $\geq 10^9$, about $\geq 10^{10}$, about $\geq 10^{11}$, or about $\geq 10^{12}$ infectious doses per kilogram of body weight, or any number of doses in-
25 between these values. Upper limits of dosing will be determined for each disease indication,

and will depend on toxicity/safety profiles for each individual product or product lot.

[159] Additionally, vector compositions of the present disclosure may be administered periodically, such as once or twice a day, or any other suitable time period. For example, vector compositions may be administered to a subject in need once a week, once
5 every other week, once every three weeks, once a month, every other month, every three months, every six months, every nine months, once a year, every eighteen months, every two years, every thirty months, or every three years.

[160] In embodiments, the disclosed vector compositions are administered as a pharmaceutical composition. In embodiments, the pharmaceutical composition can be
10 formulated in a wide variety of dosage forms, including but not limited to nasal, pulmonary, oral, topical, or parenteral dosage forms for clinical application. Each of the dosage forms can comprise various solubilizing agents, disintegrating agents, surfactants, fillers, thickeners, binders, diluents such as wetting agents or other pharmaceutically acceptable excipients. The pharmaceutical composition can also be formulated for injection, insufflation, infusion, or
15 intradermal exposure. For instance, an injectable formulation may comprise the disclosed vectors in an aqueous or non-aqueous solution at a suitable pH and tonicity.

[161] The disclosed vector compositions may be administered to a subject via direct injection into the liver with guided injection. In some embodiments, the vectors can be administered systemically via arterial or venous circulation. In some embodiments, the vector
20 compositions can be administered via guided cannulation to tissues immediately surrounding liver including spleen or pancreas. In some embodiments, the vector composition may be delivered by injection into the portal vein or portal sinus, and may be delivered by injection into the umbilical vein.

[162] The disclosed vector compositions can be administered using any
25 pharmaceutically acceptable method, such as intranasal, buccal, sublingual, oral, rectal,

ocular, parenteral (intravenously, intradermally, intramuscularly, subcutaneously, intraperitoneally), pulmonary, intravaginal, locally administered, topically administered, topically administered after scarification, mucosally administered, via an aerosol, in semi-solid media such as agarose or gelatin, or via a buccal or nasal spray formulation.

5 [163] Further, the disclosed vector compositions can be formulated into any pharmaceutically acceptable dosage form, such as a solid dosage form, tablet, pill, lozenge, capsule, liquid dispersion, gel, aerosol, pulmonary aerosol, nasal aerosol, ointment, cream, semi-solid dosage form, a solution, an emulsion, and a suspension. Further, the pharmaceutical composition may be a controlled release formulation, sustained release
10 formulation, immediate release formulation, or any combination thereof. Further, the pharmaceutical composition may be a transdermal delivery system.

[164] In embodiments, the pharmaceutical composition can be formulated in a solid dosage form for oral administration, and the solid dosage form can be powders, granules, capsules, tablets or pills. In embodiments, the solid dosage form can include one or more
15 excipients such as calcium carbonate, starch, sucrose, lactose, microcrystalline cellulose or gelatin. In addition, the solid dosage form can include, in addition to the excipients, a lubricant such as talc or magnesium stearate. In some embodiments, the oral dosage form can be immediate release, or a modified release form. Modified release dosage forms include controlled or extended release, enteric release, and the like. The excipients used in the
20 modified release dosage forms are commonly known to a person of ordinary skill in the art.

[165] In embodiments, the pharmaceutical composition can be formulated as a sublingual or buccal dosage form. Such dosage forms comprise sublingual tablets or solution compositions that are administered under the tongue and buccal tablets that are placed between the cheek and gum.

[166] In embodiments, the pharmaceutical composition can be formulated as a nasal dosage form. Such dosage forms of this disclosure comprise solution, suspension, and gel compositions for nasal delivery.

[167] In embodiments, the pharmaceutical composition can be formulated in a liquid dosage form for oral administration, such as suspensions, emulsions or syrups. In 5 embodiments, the liquid dosage form can include, in addition to commonly used simple diluents such as water and liquid paraffin, various excipients such as humectants, sweeteners, aromatics or preservatives. In embodiments, the composition can be formulated to be suitable for administration to a pediatric patient.

10 [168] In embodiments, the pharmaceutical composition can be formulated in a dosage form for parenteral administration, such as sterile aqueous solutions, suspensions, emulsions, non-aqueous solutions or suppositories. In embodiments, the solutions or suspensions can include propyleneglycol, polyethyleneglycol, vegetable oils such as olive oil or injectable esters such as ethyl oleate.

15 [169] The dosage of the pharmaceutical composition can vary depending on the patient's weight, age, gender, administration time and mode, excretion rate, and the severity of disease.

[170] In embodiments, the treatment of PKU is accomplished by guided direct injection of the disclosed vector constructs into liver, using needle, or intravascular 20 cannulation. In embodiments, the vectors compositions are administered into the cerebrospinal fluid, blood or lymphatic circulation by venous or arterial cannulation or injection, intradermal delivery, intramuscular delivery or injection into a draining organ near the liver.

[171] The following examples are given to illustrate aspects of the present invention.
25 It should be understood, however, that the inventions are not to be limited to the specific

conditions or details described in these examples. All printed publications referenced herein are specifically incorporated by reference.

EXAMPLES

[172] Example 1. Development of a Lentiviral Vector System

5 [173] A lentiviral vector system was developed as summarized in Figure 1 (circularized form). Lentiviral particles were produced in 293T/17 HEK cells (purchased from American Type Culture Collection, Manassas, VA) following transfection with the therapeutic vector, the envelope plasmid, and the helper plasmid. The transfection of 293T/17 HEK cells, which produced functional viral particles, employed the reagent

10 Poly(ethylenimine) (PEI) to increase the efficiency of plasmid DNA uptake. The plasmids and DNA were initially added separately in culture medium without serum in a ratio of 3:1 (mass ratio of PEI to DNA). After 2-3 days, cell medium was collected and lentiviral particles were purified by high-speed centrifugation and/or filtration followed by anion-exchange chromatography. The concentration of lentiviral particles can be expressed in

15 terms of transducing units/ml (TU/ml). The determination of TU was accomplished by measuring HIV p24 levels in culture fluids (p24 protein is incorporated into lentiviral particles), measuring the number of viral DNA copies per transduced cell by quantitative PCR, or by infecting cells and using light (if the vectors encode luciferase or fluorescent protein markers).

20 [174] As mentioned above, a 3-vector system (*i.e.*, which includes a 2-vector lentiviral packaging system) was designed for the production of lentiviral particles. A schematic of the 3-vector system is shown in Figure 1. Briefly, and with reference to Figure 1, the top-most vector is a helper plasmid, which, in this case, includes Rev. The vector appearing in the middle of Figure 1 is the envelope plasmid. The bottom-most vector is the

25 therapeutic vector, as described herein.

[175] Referring to Figure 1, the Helper plus Rev plasmid includes a CMV enhancer with chicken beta actin promoter (SEQ ID NO: 23); a chicken beta actin intron (SEQ ID NO: 41); a HIV Gag (SEQ ID NO: 24); a HIV Pol (SEQ ID NO: 25); a HIV Integrase (SEQ ID NO: 26); a HIV RRE (SEQ ID NO: 27); a HIV Rev (SEQ ID NO: 28); and a rabbit beta globin poly A (SEQ ID NO: 42).

[176] The Envelope plasmid includes a CMV promoter (SEQ ID NO: 29); a beta globin intron (SEQ ID NO: 7 or 8); a VSV-G envelope glycoprotein (SEQ ID NO: 30); and a rabbit beta globin poly A (SEQ ID NO: 42).

[177] Synthesis of a 3-vector system, which includes a 2-vector lentiviral packaging system consisting of Helper (plus Rev) and Envelope plasmids, is disclosed.

[178] **Materials and Methods:**

[179] *Construction of the helper plasmid:* The helper plasmid was constructed by initial PCR amplification of a DNA fragment from the pNL4-3 HIV plasmid (NIH Aids Reagent Program) containing Gag, Pol, and Integrase genes. Primers were designed to amplify the fragment with EcoRI and NotI restriction sites which could be used to insert at the same sites in the pCDNA3 plasmid (Invitrogen). The forward primer was (5'-TAAGCAGAATTCATGAATTTGCCAGGAAGAT-3') (SEQ ID NO: 43) and reverse primer was (5'-CCATACAATGAATGGACACTAGGCGGCCGCACGAAT-3') (SEQ ID NO: 44).

[180] The sequence for the Gag, Pol, Integrase fragment was as follows:

[181] GAATTCATGAATTTGCCAGGAAGATGGAAACCAAAAATGATAGGG
GGAATTGGAGGTTTTATCAAAGTAAGACAGTATGATCAGATACTCATAGAAATCT
GCGGACATAAAGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCAACATAA
TTGGAAGAAATCTGTTGACTCAGATTGGCTGCACTTTAAATTTCCCATAGTCCT
ATTGAGACTGTACCAGTAAAATTAAAGCCAGGAATGGATGGCCCCAAAAGTTAAA

CAATGGCCATTGACAGAAGAAAAAATAAAAAGCATTAGTAGAAATTTGTACAGAA
ATGGAAAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATACAATACT
CCAGTATTTGCCATAAAGAAAAAAGACAGTACTAAATGGAGAAAATTAGTAGAT
TTCAGAGAACTTAATAAGAGAACTCAAGATTTCTGGGAAGTTCAATTAGGAATA
5 CCACATCCTGCAGGGTTAAAACAGAAAAAATCAGTAACAGTACTGGATGTGGGC
GATGCATATTTTTCAGTTCCCTTAGATAAAGACTTCAGGAAGTATACTGCATTTA
CCATACCTAGTATAAAACAATGAGACACCAGGGATTAGATATCAGTACAATGTGC
TTCCACAGGGATGGAAAGGATCACCAGCAATATTCCAGTGTAGCATGACAAAAA
TCTTAGAGCCTTTTAGAAAACAAAATCCAGACATAGTCATCTATCAATACATGGA
10 TGATTTGTATGTAGGATCTGACTTAGAAATAGGGCAGCATAGAACAAAAATAGA
GGAAGTGAAGACAACATCTGTTGAGGTGGGGATTTACCACACCAGACAAAAACA
TCAGAAAGAACCTCCATTCCCTTTGGATGGGTTATGAACTCCATCCTGATAAATGG
ACAGTACAGCCTATAGTGCTGCCAGAAAAGGACAGCTGGACTGTCAATGACATA
CAGAAATTAGTGGGAAAATTGAATTGGGCAAGTCAGATTTATGCAGGGATTAAA
15 GTAAGGCAATTATGTAACTTCTTAGGGGAACCAAAGCACTAACAGAAGTAGTA
CCACTAACAGAAGAAGCAGAGCTAGAACTGGCAGAAAACAGGGAGATTCTAAA
AGAACCGGTACATGGAGTGTATTATGACCCATCAAAAGACTTAATAGCAGAAAT
ACAGAAGCAGGGGCAAGGCCAATGGACATATCAAATTTATCAAGAGCCATTAA
AAATCTGAAAACAGGAAAGTATGCAAGAATGAAGGGTGCCCACTAATGATGT
20 GAAACAATTAACAGAGGCAGTACAAAAAATAGCCACAGAAAGCATAGTAATAT
GGGGAAAGACTCCTAAATTTAAATTACCCATACAAAAGGAAACATGGGAAGCAT
GGTGGACAGAGTATTGGCAAGCCACCTGGATTCTGAGTGGGAGTTTGTCAATAC
CCCTCCCTTAGTGAAGTTATGGTACCAGTTAGAGAAAGAACCATAATAGGAGC
AGAACTTTCTATGTAGATGGGGCAGCCAATAGGGAACTAAATTAGGAAAAGC
25 AGGATATGTAACTGACAGAGGAAGACAAAAAGTTGTCCCCCTAACGGACACAAC

AAATCAGAAGACTGAGTTACAAGCAATTCATCTAGCTTTGCAGGATTCGGGATTA
 GAAGTAAACATAGTGACAGACTCACAAATATGCATTGGGAATCATTCAAGCACAA
 CCAGATAAGAGTGAATCAGAGTTAGTCAGTCAAATAATAGAGCAGTTAATAAAA
 AAGGAAAAAGTCTACCTGGCATGGGTACCAGCACACAAAGGAATTGGAGGAAAT
 5 GAACAAGTAGATAAATTGGTCAGTGCTGGAATCAGGAAAGTACTATTTTTAGAT
 GGAATAGATAAGGCCCAAGAAGAACATGAGAAATATCACAGTAATTGGAGAGC
 AATGGCTAGTGATTTTAACCTACCACCTGTAGTAGCAAAAGAAATAGTAGCCAG
 CTGTGATAAATGTCAGCTAAAAGGGGAAGCCATGCATGGACAAGTAGACTGTAG
 CCCAGGAATATGGCAGCTAGATTGTACACATTTAGAAGGAAAAGTTATCTTGGTA
 10 GCAGTTCATGTAGCCAGTGGATATATAGAAGCAGAAGTAATTCCAGCAGAGACA
 GGGCAAGAAACAGCATACTTCCTCTTAAAATTAGCAGGAAGATGGCCAGTAAAA
 ACAGTACATACAGACAATGGCAGCAATTTACCAGTACTACAGTTAAGGCCGCC
 TGTTGGTGGGCGGGGATCAAGCAGGAATTTGGCATTCCCTACAATCCCCAAAGTC
 AAGGAGTAATAGAATCTATGAATAAAGAATTAAAGAAAATTATAGGACAGGTAA
 15 GAGATCAGGCTGAACATCTTAAGACAGCAGTACAAATGGCAGTATTCATCCACA
 ATTTTAAAAGAAAAGGGGGGATTGGGGGGTACAGTGCAGGGGAAAGAATAGTA
 GACATAATAGCAACAGACATACAACTAAAGAATTACAAAAACAAATTACAAAA
 ATTCAAAATTTTCGGGTTTATTACAGGGACAGCAGAGATCCAGTTTGGAAAGGAC
 CAGCAAAGCTCCTCTGGAAAGGTGAAGGGGCAGTAGTAATACAAGATAATAGTG
 20 ACATAAAAGTAGTGCCAAGAAGAAAAGCAAAGATCATCAGGGATTATGGAAAA
 CAGATGGCAGGTGATGATTGTGTGGCAAGTAGACAGGATGAGGATTAA (SEQ ID
 NO: 45).

[182] Next, a DNA fragment containing the RRE, Rev, and rabbit beta globin poly
 A sequence with XbaI and XmaI flanking restriction sites was synthesized by Eurofins

Genomics. The DNA fragment was then inserted into the plasmid at the XbaI and XmaI restriction sites. The DNA sequence was as follows:

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[183]      TCTAGAATGGCAGGAAGAAGCGGAGACAGCGACGAAGAGCTCATC
AGAACAGTCAGACTCATCAAGCTTCTCTATCAAAGCAACCCACCTCCCAATCCCG
5  AGGGGACCCGACAGGCCCGAAGGAATAGAAGAAGAAGGTGGAGAGAGAGACAG
AGACAGATCCATTGATTAGTGAACGGATCCTTGGCACTTATCTGGGACGATCTG
CGGAGCCTGTGCCTCTTCAGCTACCACCGCTTGAGAGACTTACTCTTGATTGTAA
CGAGGATTGTGGAACCTTCTGGGACGCAGGGGGTGGGAAGCCCTCAAATATTGGT
GGAATCTCCTACAATATTGGAGTCAGGAGCTAAAGAATAGAGGAGCTTTGTTCTT
10  TGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTCAATGACGCTGAC
GGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTGCTG
AGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAG
CAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTC
CTAGATCTTTTTCCCTCTGCCAAAAATTATGGGGACATCATGAAGCCCCCTTGAGC
15  ATCTGACTTCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAA
TTTTTTGTGTCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATTTAAAACAT
CAGAATGAGTATTTGGTTTAGAGTTTGGCAACATATGCCATATGCTGGCTGCCAT
GAACAAAGGTGGCTATAAAGAGGTCATCAGTATATGAAACAGCCCCCTGCTGTC
CATTCTTATTCCATAGAAAAGCCTTGACTTGAGGTTAGATTTTTTTTATATTTTG
20  TTTTGTGTTATTTTTTCTTTAACATCCCTAAAATTTTCCTTACATGTTTTACTAGC
CAGATTTTTCTCTCTCTGACTACTCCAGTCATAGCTGTCCCTCTTCTCTTATG
AAGATCCCTCGACCTGCAGCCCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCC
TGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATA
AAGTGTAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGC
25  GCTCACTGCCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCGGATCCGCATCTC

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AATTAGTCAGCAACCATAGTCCCGCCCCCTAACTCCGCCCATCCCGCCCCCTAACTC
 CGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCA
 GAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTT
 TTGGAGGCCTAGGCTTTTGCAAAAAGCTAACTTGTTTATTGCAGCTTATAATGGT
 5 TACAAATAAAGCAATAGCATCACAAATTTACAAATAAAGCATTTTTTTTCACTGC
 ATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCAGCGGCCGCCCCGG
 G (SEQ ID NO: 46)

[184] Finally, the CMV promoter of pCDNA3.1 was replaced with the CAG
 promoter (CMV enhancer, chicken beta actin promoter plus a chicken beta actin intron
 10 sequence). A DNA fragment containing the CAG enhancer/promoter/intron sequence with
 MluI and EcoRI flanking restriction sites was synthesized by Eurofins Genomics. The DNA
 fragment was then inserted into the plasmid at the MluI and EcoRI restriction sites. The
 DNA sequence was as follows:

[185] ACGCGTTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCAT
 15 AGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCT
 GACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGT
 AACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACTATTTACGGTAAACT
 GCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACG
 TCAATGACGGTAAATGGCCCGCCTGGCATTATGCCAGTACATGACCTTATGGGA
 20 CTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGGTTCGAG
 GTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCCACCCCCAAT
 TTTGTATTTATTTATTTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGG
 GGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCG
 GAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTAT
 25 GGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCGG

GAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGCGCCGCCTCGCGCCGCCC
 GCCCCGGCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGGCCC
 TTCTCCTCCGGGCTGTAATTAGCGCTTGGTTTAATGACGGCTCGTTTCTTTTCTGT
 GGCTGCGTGAAAGCCTTAAAGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGGAGC
 5 GGCTCGGGGGGTGCGTGCGTGTGTGTGTGCGTGGGGAGCGCCGCGTGCGGCCCG
 CGCTGCCCCGGCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGTGCGCTCC
 GCGTGTGCGCGAGGGGAGCGCGGCCGGGGGCGGTGCCCCGCGGTGCGGGGGGG
 CTGCGAGGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAG
 GGGGTGTGGGCGCGGCGGTGCGGGCTGTAACCCCCCTGCACCCCCCTCCCCGAG
 10 TTGCTGAGCACGGCCCCGGCTTCGGGTGCGGGGCTCCGTGCGGGGCGTGCGCGG
 GGCTCGCCGTGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCG
 GGGCCGCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCCGGAGC
 GCCGGCGGCTGTGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTATGGTAATCGT
 GCGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGGCGGAGCCGAAATCTGG
 15 GAGGCGCCGCCGCACCCCCTCTAGCGGGCGCGGGCGAAGCGGTGCGGCGCCGGC
 AGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGCGCCGCGCGTCCCCTT
 CTCCATCTCCAGCCTCGGGGCTGCCGCAGGGGGACGGCTGCCTTCGGGGGGGAC
 GGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGGAATTC (SEQ ID
 NO: 47)

20 [186] Construction of the VSV-G Envelope plasmid:

[187] The vesicular stomatitis Indiana virus glycoprotein (VSV-G) sequence was
 synthesized by Eurofins Genomics with flanking EcoRI restriction sites. The DNA fragment
 was then inserted into the pCDNA3.1 plasmid (Invitrogen) at the EcoRI restriction site and
 the correct orientation was determined by sequencing using a CMV specific primer.

25 [188] The DNA sequence was as follows:

[189] ATGAAGTGCCTTTTGTACTTAGCCTTTTATTTCATTGGGGTGAATTG
CAAGTTCACCATAGTTTTTCCACACAACCAAAAAGGAACTGGAAAAATGTTCC
TCTAATTACCATTATTGCCCCGTCAAGCTCAGATTTAAATTGGCATAATGACTTAAT
AGGCACAGCCTTACAAGTCAAAATGCCCAAGAGTCACAAGGCTATTCAAGCAGA
5 CGGTTGGATGTGTCATGCTTCCAAATGGGTCACTACTTGTGATTTCCGCTGGTATG
GACCGAAGTATATAACACATTCCATCCGATCCTTCACTCCATCTGTAGAACAATG
CAAGGAAAGCATTGAACAAACGAAACAAGGAACTTGGCTGAATCCAGGCTTCCC
TCCTCAAAGTTGTGGATATGCAACTGTGACGGATGCCGAAGCAGTGATTGTCCAG
GTGACTCCTCACCATGTGCTGGTTGATGAATACACAGGAGAATGGGTTGATTAC
10 AGTTCATCAACGGAAAATGCAGCAATTACATATGCCCCACTGTCCATAACTCTAC
AACCTGGCATTCTGACTATAAGGTCAAAGGGCTATGTGATTCTAACCTCATTTC
ATGGACATCACCTTCTTCTCAGAGGACGGAGAGCTATCATCCCTGGGAAAGGAG
GGCACAGGGTTCAGAAGTAACTACTTTGCTTATGAACTGGAGGCAAGGCCTGC
AAAATGCAATACTGCAAGCATTGGGGAGTCAGACTCCCATCAGGTGTCTGGTTTCG
15 AGATGGCTGATAAGGATCTCTTTGCTGCAGCCAGATTCCCTGAATGCCCAGAAGG
GTCAAGTATCTCTGCTCCATCTCAGACCTCAGTGGATGTAAGTCTAATTCAGGAC
GTTGAGAGGATCTTGGATTATTCCCTCTGCCAAGAAACCTGGAGCAAAATCAGA
GCGGGTCTTCCAATCTCTCCAGTGGATCTCAGCTATCTTGCTCCTAAAAACCCAG
GAACCGGTCTGCTTTCACCATAATCAATGGTACCCTAAAATACTTTGAGACCAG
20 ATACATCAGAGTCGATATTGCTGCTCCAATCCTCTCAAGAATGGTCGGAATGATC
AGTGGAACCTACCACAGAAAGGGAAGTGTGGGATGACTGGGCACCATATGAAGAC
GTGGAAATTGGACCCAATGGAGTTCTGAGGACCAGTTCAGGATATAAGTTTCCTT
TATACATGATTGGACATGGTATGTTGGACTCCGATCTTCATCTTAGCTCAAAGGC
TCAGGTGTTGGAACATCCTCACATTCAAGACGCTGCTTCGCAACTTCCTGATGAT
25 GAGAGTTTATTTTTTGGTGATACTGGGCTATCCAAAAATCCAATCGAGCTTGTAG

AAGGTTGGTTCAGTAGTTGGAAAAGCTCTATTGCCTCTTTTTCTTTATCATAGGG
 TTAATCATTGGACTATTCTTGGTTCTCCGAGTTGGTATCCATCTTTGCATTAAATT
 AAAGCACACCAAGAAAAGACAGATTTATACAGACATAGAGATGAACCGACTTGG
 AAAGTGA (SEQ ID NO: 30)

5 [190] A 4-vector system, which includes a 3-vector lentiviral packaging system, has also been designed and produced using the methods and materials described herein. A schematic of the 4-vector system is shown in Figure 2. Briefly, and with reference to Figure 2, the top-most vector is a helper plasmid, which, in this case, does not include Rev. The second vector is a separate Rev plasmid. The third vector is the envelope plasmid. The
 10 bottom-most vector is the therapeutic vector as described herein.

[191] Referring to Figure 2, the Helper plasmid includes a CMV enhancer and chicken beta actin promoter (SEQ ID NO: 23); a chicken beta actin intron (SEQ ID NO: 41); a HIV Gag (SEQ ID NO: 24); a HIV Pol (SEQ ID NO: 25); a HIV Integrase (SEQ ID NO: 26); a HIV RRE (SEQ ID NO: 27); and a rabbit beta globin poly A (SEQ ID NO: 42).

15 [192] The Rev plasmid includes a RSV promoter and HIV Rev (SEQ ID NO: 48); and a rabbit beta globin poly A (SEQ ID NO: 42).

[193] The Envelope plasmid includes a CMV promoter (SEQ ID NO: 29); a beta globin intron (SEQ ID NO: 7 or 8); a VSV-G envelope glycoprotein (SEQ ID NO: 30); and a rabbit beta globin poly A (SEQ ID NO: 42).

20 [194] In one aspect, the therapeutic lentiviral vector expressing PAH includes all of the elements shown in Vector A of Figure 3. In another aspect, the therapeutic lentiviral vector expressing PAH includes all of the elements shown in Vector B of Figure 3. In another aspect, the therapeutic lentiviral vector expressing PAH includes all of the elements shown in Vector C of Figure 3. In another aspect, the therapeutic lentiviral vector expressing PAH
 25 includes all of the elements shown in Vector D of Figure 3. In another aspect, the therapeutic

lentiviral vector expressing PAH includes all of the elements shown in Vector E of Figure 3.

In another aspect, the therapeutic lentiviral vector expressing PAH includes all of the elements shown in Vector F of Figure 3. In another aspect, the therapeutic lentiviral vector expressing PAH includes all of the elements shown in Vector G of Figure 3. In another aspect, the therapeutic lentiviral vector expressing PAH includes all of the elements shown in Vector H of Figure 3.

[195] Synthesis of a 4-vector system, which includes a 3-vector lentiviral packaging system consisting of Helper, Rev, and Envelope plasmids, is disclosed.

[196] **Materials and Methods:**

10 [197] Construction of the Helper plasmid without Rev:

[198] The Helper plasmid without Rev was constructed by inserting a DNA fragment containing the RRE and rabbit beta globin poly A sequence. This sequence was synthesized by Eurofins Genomics with flanking XbaI and XmaI restriction sites. The RRE/rabbit poly A beta globin sequence was then inserted into the Helper plasmid at the XbaI and XmaI restriction sites.

[199] The DNA sequence is as follows:

[200] TCTAGAATGGCAGGAAGAAGCGGAGACAGCGACGAAGAGCTCATCAGAA
CAGTCAGACTCATCAAGCTTCTCTATCAAAGCAACCCACCTCCCAATCCCGAGGGGACCC
GACAGGCCCCGAAGGAATAGAAGAAGAAGGTGGAGAGAGAGACAGAGACAGATCCATTC
20 GATTAGTGAACGGATCCTTGGCACTTATCTGGGACGATCTGCGGAGCCTGTGCCTCTTCA
GCTACCACCGCTTGAGAGACTTACTCTTGATTGTAACGAGGATTGTGGAAGTTCTGGGAC
GCAGGGGGTGGGAAGCCCTCAAATATTGGTGGAAATCTCCTACAATATTGGAGTCAGGAG
CTAAAGAATAGAGGAGCTTTGTTCCCTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGG
CGCAGCGTCAATGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAGC
25 AGCAGAACAATTTGCTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCT
GGGGCATCAAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATACCTAAAGGATCAA

CAGCTCCTAGATCTTTTTCCCTCTGCCAAAATTATGGGGACATCATGAAGCCCCTTGAG
 CATCTGACTTCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAATTTTT
 TGTGTCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATTTAAAACATCAGAATGAG
 TATTTGGTTTAGAGTTTGGCAACATATGCCATATGCTGGCTGCCATGAACAAAGGTGGCT
 5 ATAAAGAGGTCATCAGTATATGAAACAGCCCCCTGCTGTCCATTCCTTATTCCATAGAAA
 AGCCTTGACTTGAGGTTAGATTTTTTTTTATATTTTGTGTTATTTTTTTCTTTAACAT
 CCTAAAATTTTCCTTACATGTTTTACTAGCCAGATTTTTCCTCCTCTCCTGACTACTCCCA
 GTCATAGCTGTCCCTCTTCTCTTATGAAGATCCCTCGACCTGCAGCCCAAGCTTGGCGTA
 ATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATA
 10 CGAGCCGGAAGCATAAAGTGTAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATT
 AATTGCGTTGCGCTCACTGCCCCGCTTTCAGTCGGGAAACCTGTCTGCGCCAGCGGATCCG
 CATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCCTAACT
 CCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTTATTTATGCAGAGG
 CCGAGGCCGCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCC
 15 TAGGCTTTTGCAAAAAGCTAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAA
 TAGCATCACAAATTTACAAATAAAGCATTTTTTTTCACTGCATTCTAGTTGTGGTTTGTCC
 AAATCATCAATGTATCTTATCAGCGGCCGCCCCGGG (SEQ ID NO: 46)

[201] Construction of the Rev plasmid:

[202] The RSV promoter and HIV Rev sequences were synthesized as a single DNA
 20 fragment by Eurofins Genomics with flanking MfeI and XbaI restriction sites. The DNA
 fragment was then inserted into the pCDNA3.1 plasmid (Invitrogen) at the MfeI and XbaI
 restriction sites in which the CMV promoter is replaced with the RSV promoter. The DNA
 sequence was as follows:

[203] CAATTGCGATGTACGGGCCAGATATACGCGTATCTGAGGGGACTAG
 25 GGTGTGTTTAGGCGAAAAGCGGGGCTTCGGTTGTACGCGGTTAGGAGTCCCCTCA
 GGATATAGTAGTTTCGCTTTTGCATAGGGAGGGGGAAATGTAGTCTTATGCAATA
 CACTTGTAGTCTTGCAACATGGTAACGATGAGTTAGCAACATGCCTTACAAGGAG

AGAAAAAGCACCGTGCATGCCGATTGGTGGAAAGTAAGGTGGTACGATCGTGCCT
 TATTAGGAAGGCAACAGACAGGTCTGACATGGATTGGACGAACCACTGAATTCC
 GCATTGCAGAGATAATTGTATTTAAGTGCCTAGCTCGATAACAATAAACGCCATTT
 GACCATTACCCACATTGGTGTGCACCTCCAAGCTCGAGCTCGTTTAGTGAACCGT
 5 CAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGG
 ACCGATCCAGCCTCCCCTCGAAGCTAGCGATTAGGCATCTCCTATGGCAGGAAGA
 AGCGGAGACAGCGACGAAGAACTCCTCAAGGCAGTCAGACTCATCAAGTTTCTC
 TATCAAAGCAACCCACCTCCCAATCCCGAGGGGACCCGACAGGCCCGAAGGAAT
 AGAAGAAGAAGGTGGAGAGAGAGACAGAGACAGATCCATTGATTAGTGAACG
 10 GATCCTTAGCACTTATCTGGGACGATCTGCGGAGCCTGTGCCTCTTCAGCTACCA
 CCGCTTGAGAGACTTACTCTTGATTGTAACGAGGATTGTGGAAGTTCTGGGACGC
 AGGGGGTGGGAAGCCCTCAAATATTGGTGGAAATCTCCTACAATATTGGAGTCAG
 GAGCTAAAGAATAGTCTAGA (SEQ ID NO: 48)

[204] The plasmids used in the packaging systems can be modified with similar
 15 elements, and the intron sequences can potentially be removed without loss of vector
 function. For example, the following elements can replace similar elements in the packaging
 system:

[205] Promoters: Elongation Factor-1 (EF-1) (SEQ ID NO: 49), phosphoglycerate
 kinase (PGK) (SEQ ID NO: 50), and ubiquitin C (UbC) (SEQ ID NO: 51) can replace the
 20 CMV (SEQ ID NO: 29) or CMV enhancer/chicken beta actin promoter (SEQ ID NO: 23).
 These sequences can also be further varied by addition, substitution, deletion or mutation.

[206] Poly A sequences: SV40 poly A (SEQ ID NO: 52) and bGH poly A (SEQ ID
 NO: 53) can replace the rabbit beta globin poly A (SEQ ID NO: 42). These sequences can
 also be further varied by addition, substitution, deletion or mutation.

[207] HIV Gag, Pol, and Integrase sequences: The HIV sequences in the Helper plasmid can be constructed from different HIV strains or clades. For example, HIV Gag (SEQ ID NO: 24); HIV Pol (SEQ ID NO: 25); and HIV Int (SEQ ID NO: 26) from the Bal strain can be interchanged with the gag, pol, and int sequences contained in the helper/helper plus Rev plasmids as outlined herein. These sequences can also be further varied by addition, substitution, deletion or mutation.

[208] Envelope: The VSV-G glycoprotein can be substituted with membrane glycoproteins from feline endogenous virus (RD114) (SEQ ID NO: 54), gibbon ape leukemia virus (GALV) (SEQ ID NO: 55), Rabies (FUG) (SEQ ID NO: 56), lymphocytic choriomeningitis virus (LCMV) (SEQ ID NO: 57), influenza A fowl plague virus (FPV) (SEQ ID NO: 58), Ross River alphavirus (RRV) (SEQ ID NO: 59), murine leukemia virus 10A1 (MLV) (SEQ ID NO: 60), or Ebola virus (EboV) (SEQ ID NO: 61). Sequences for these envelopes are identified in the sequence portion herein. Further, these sequences can also be further varied by addition, substitution, deletion or mutation.

[209] In summary, the 3-vector versus 4-vector systems can be compared and contrasted as follows. The 3-vector lentiviral vector system contains: 1. Helper plasmid: HIV Gag, Pol, Integrase, RRE, and Rev; 2. Envelope plasmid: VSV-G envelope; and 3. Therapeutic vector: RSV, 5'LTR, Psi Packaging Signal, RRE, cPPT, prothrombin enhancer, alpha 1 anti-trypsin promoter, phenylalanine hydroxylase, WPRE, and 3'delta LTR. The 4-vector lentiviral vector system contains: 1. Helper plasmid: HIV Gag, Pol, Integrase, and RRE; 2. Rev plasmid: Rev; 3. Envelope plasmid: VSV-G envelope; and 4. Therapeutic vector: RSV, 5'LTR, Psi Packaging Signal, RRE, cPPT, prothrombin enhancer, alpha 1 anti-trypsin promoter, phenylalanine hydroxylase, WPRE, and 3'delta LTR. Sequences corresponding with the above elements are identified in the sequence listings portion herein.

[210] **Example 2. Therapeutic Vectors**

[211] Exemplary therapeutic vectors have been designed and developed as shown, for example, in Figure 3.

[212] Referring first to Vector A of Figure 3, from left to right, the key genetic
5 elements are as follows: hybrid 5' long terminal repeat (RSV/LTR), Psi sequence (RNA packaging site), RRE (Rev-response element), cPPT (polypurine tract), a prothrombin enhancer, a hAAT promoter, a PAH sequence including the PAH sequences and variants thereof, as detailed herein, Woodchuck Post-Transcriptional Regulatory Element (WPRE), and LTR with a deletion in the U3 region.

10 [213] Referring next to Vector B of Figure 3, from left to right, the key genetic elements are as follows: hybrid 5' long terminal repeat (RSV/LTR), Psi sequence (RNA packaging site), RRE (Rev-response element), cPPT (polypurine tract), a HNF1 (hepatocyte nuclear factor) binding site upstream of a prothrombin enhancer, a hAAT promoter, a PAH sequence including the PAH sequences and variants thereof, as detailed herein, a Woodchuck
15 Post-Transcriptional Regulatory Element (WPRE), and LTR with a deletion in the U3 region.

[214] Referring next to Vector C of Figure 3, from left to right, the key genetic elements are as follows: hybrid 5' long terminal repeat (RSV/LTR), Psi sequence (RNA packaging site), RRE (Rev-response element), cPPT (polypurine tract), a HNF1/4 (hepatocyte nuclear factor) binding site upstream of a prothrombin enhancer, a hAAT
20 promoter, a PAH sequence including the PAH sequences and variants thereof, as detailed herein, a Woodchuck Post-Transcriptional Regulatory Element (WPRE), and LTR with a deletion in the U3 region.

[215] Referring next to Vector D of Figure 3, from left to right, the key genetic elements are as follows: hybrid 5' long terminal repeat (RSV/LTR), Psi sequence (RNA
25 packaging site), RRE (Rev-response element), cPPT (polypurine tract), a prothrombin

enhancer, a HNF1 (hepatocyte nuclear factor), a hAAT promoter, a PAH sequence including the PAH sequences and variants thereof, as detailed herein, a Woodchuck Post-Transcriptional Regulatory Element (WPRE), and LTR with a deletion in the U3 region.

[216] Referring next to Vector E of Figure 3, from left to right, the key genetic elements are as follows: hybrid 5' long terminal repeat (RSV/LTR), Psi sequence (RNA packaging site), RRE (Rev-response element), cPPT (polypurine tract), a prothrombin enhancer, a HNF1/4 (hepatocyte nuclear factor), a hAAT promoter, a PAH sequence including the PAH sequences and variants thereof, as detailed herein, a Woodchuck Post-Transcriptional Regulatory Element (WPRE), and LTR with a deletion in the U3 region.

[217] Referring next to Vector F of Figure 3, from left to right, the key genetic elements are as follows: hybrid 5' long terminal repeat (RSV/LTR), Psi sequence (RNA packaging site), RRE (Rev-response element), cPPT (polypurine tract), five HNF1 (hepatocyte nuclear factor) binding sites upstream of a prothrombin enhancer, a hAAT promoter, a PAH sequence including the PAH sequences and variants thereof, as detailed herein, a Woodchuck Post-Transcriptional Regulatory Element (WPRE), and LTR with a deletion in the U3 region.

[218] Referring next to Vector G of Figure 3, from left to right, the key genetic elements are as follows: hybrid 5' long terminal repeat (RSV/LTR), Psi sequence (RNA packaging site), RRE (Rev-response element), cPPT (polypurine tract), three HNF1/HNF4 (hepatocyte nuclear factor) binding sites upstream of a prothrombin enhancer, a hAAT promoter, a PAH sequence including the PAH sequences and variants thereof, as detailed herein, a Woodchuck Post-Transcriptional Regulatory Element (WPRE), and LTR with a deletion in the U3 region.

[219] Referring first to Vector H of Figure 3, from left to right, the key genetic elements are as follows: hybrid 5' long terminal repeat (RSV/LTR), Psi sequence (RNA

packaging site), RRE (Rev-response element), cPPT (polypurine tract), a prothrombin enhancer, a hAAT promoter, a rabbit beta globin intron, a PAH sequence including the PAH sequences and variants thereof, as detailed herein, Woodchuck Post-Transcriptional Regulatory Element (WPRE), and LTR with a deletion in the U3 region.

5 [220] To produce the vectors outlined generally in Figure 3, the methods and materials described herein and as otherwise as understood by those skilled in the art were employed.

[221] *Inhibitory RNA Design:* The sequence of *Homo sapiens* phenylalanine hydroxylase (PAH) (NM_000277.1) mRNA was used to search for potential shRNA
10 candidates to knockdown PAH levels in human cells. Potential RNA shRNA sequences were chosen from candidates selected by siRNA or shRNA design programs such as from the GPP Web Portal hosted by the Broad Institute (portals.broadinstitute.org/gpp/public/) or the BLOCK-iT RNAi Designer from Thermo Scientific (<https://rnaidesigner.thermofisher.com/rnaiexpress/>). Individual selected shRNA sequences
15 were inserted into a lentiviral vector immediately 3 prime to a RNA polymerase III promoter H1 (SEQ ID NO: 22) to regulate shRNA expression. These lentivirus shRNA constructs were used to transduce cells and measure the change in specific mRNA levels.

[222] *Vector Construction:* For PAH shRNA, oligonucleotide sequences containing BamHI and EcoRI restriction sites were synthesized by Eurofins MWG Operon.
20 Overlapping sense and antisense oligonucleotide sequences were mixed and annealed during cooling from 70 degrees Celsius to room temperature. The lentiviral vector was digested with the restriction enzymes BamHI and EcoRI for one hour at 37 degrees Celsius. The digested lentiviral vector was purified by agarose gel electrophoresis and extracted from the gel using a DNA gel extraction kit from Thermo Scientific. The DNA concentrations were determined
25 and vector to oligo (3:1 ratio) were mixed, allowed to anneal, and ligated. The ligation

reaction was performed with T4 DNA ligase for 30 minutes at room temperature. 2.5 microliters of the ligation mix were added to 25 microliters of STBL3 competent bacterial cells. Transformation was achieved after heat-shock at 42 degrees Celsius. Bacterial cells were spread on agar plates containing ampicillin and drug-resistant colonies (indicating the presence of ampicillin-resistance plasmids) were recovered and expanded in LB broth. To check for insertion of the oligo sequences, plasmid DNA was extracted from harvested bacteria cultures with the Thermo Scientific DNA mini prep kit. Insertion of shRNA sequences in the lentiviral vector was verified by DNA sequencing using a specific primer for the promoter used to regulate shRNA expression. Using the following target sequences, exemplary shRNA sequences were determined to knock-down PAH.

[223] PAH shRNA sequence #1:

TCGCATTTCATCAAGATTAATCTCGAGATTAATCTTGATGAAATGC
GATTTTT (SEQ ID NO: 13)

[224] PAH shRNA sequence #2:

15 ACTCATAAAGGAGCATATAAGCTCGAGCTTATATGCTCCTTTATGA
GTTTTTT (SEQ ID NO: 14)

[225] **Example 3. Liver specific prothrombin enhancer/hAAT promoter**

[226] Hepal-6 mouse hepatoma cells were transduced with lentiviral vectors containing a liver-specific prothrombin enhancer (SEQ ID NO: 5), and a human alpha-1 anti-
20 trypsin promoter (SEQ ID NO: 6). The resulting DNA sequence is as follows:
GCGAGAACTTGTGCCTCCCCGTGTTCTGCTCTTTGTCCCTCTGTCCTACTTAGAC
TAATATTTGCCTTGGTACTGCAAACAGGAAATGGGGGAGGGACAGGAGTAGGG
CGGAGGGTAGCCCGGGGATCTTGCTACCACTGGAACAGCCACTAAGGATTCTGC
AGTGAGAGCAGAGGGCCAGCTAAGTGGTACTCTCCAGAGACTGTCTGACTCAC
25 GCCACCCCTCCACCTTGGACACAGGACGCTGTGGTTTCTGAGCCAGGTACAATG

ACTCCTTTTCGGTAAGTGCAGTGGAAGCTGTACACTGCCCAGGCAAAGCGTCCGG
 GCAGCGTAGGCGGGCGACTCAGATCCCAGCCAGTGGACTTAGCCCCCTGTTTGCTC
 CTCCGATAACTGGGGTGACCTTGGTTAATATTCACCAGCAGCCTCCCCCGTTGCC
 CCTCTGGATCCACTGCTTAAATACGGACGAGGACAGGGCCCTGTCTCCTCAGCTT

5 CAGGCACCACCACTGACCTGGGACAGTGAAT (SEQ ID NO: 63). Results for these
 infections are detailed in further Examples herein.

[227] **Example 4. hAAT promoter with prothrombin enhancer and hepatocyte
 nuclear factor (HNF) binding sites**

[228] Hepal-6 mouse hepatoma cells were transduced with lentiviral vectors
 10 containing a liver-specific prothrombin enhancer (SEQ ID NO: 5), a human alpha-1 anti-
 trypsin promoter (SEQ ID NO: 6), and one or more hepatocyte nuclear factor (HNF) binding
 sites. The resulting DNA sequence that includes five HNF1 binding sites (designated in
 underlined font) was as follows:

GTTAATCATTAACGTTAATCATTAACGTTAATCATTAACGTTAATCATTAACGTTA
 15 ATCATTAACATCGATGCGAGAACTTGTGCCTCCCCGTGTTCTCTGCTCTTTGTCCCT
 CTGTCCTACTTAGACTAATATTTGCCTTGGGTACTGCAAACAGGAAATGGGGGAG
 GGACAGGAGTAGGGCGGAGGGTAGGATTCTGCAGTGAGAGCAGAGGGCCAGCT
 AAGTGGTACTCTCCCAGAGACTGTCTGACTCACGCCACCCCTCCACCTTGGACA
 CAGGACGCTGTGGTTTCTGAGCCAGGTACAATGACTCCTTTTCGGTAAGTGCAGTG
 20 GAAGCTGTACACTGCCCAGGCAAAGCGTCCGGGCAGCGTAGGCGGGCGACTCAG
 ATCCCAGCCAGTGGACTTAGCCCCCTGTTTGCTCCTCCGATAACTGGGGTGACCTT
 GGTAAATATTCACCAGCAGCCTCCCCCGTTGCCCTCTGGATCCACTGCTTAAAT
 ACGGACGAGGACAGGGCCCTGTCTCCTCAGCTTCAGGCACCACCACTGACCTGG
 GACAGTGAAT (SEQ ID NO: 64). The resulting DNA sequence that includes three
 25 HNF1/HNF4 binding sites (HNF1 designated in underlined font; HNF4 designated in bold

font) is as follows:

GTTAATCATTAACGCTTGTACTTTGGTACAGTTAATCATTAACGCTTGTACTTT
GGTACAGTTAATCATTAACGCTTGTACTTTGGTACAATCGATGCGAGAACTTGT
 GCCTCCCCGTGTTCCCTGCTCTTTGTCCCTCTGTCCTACTTAGACTAATATTTGCCTT
 5 GGGTACTGCAAACAGGAAATGGGGGAGGGACAGGAGTAGGGCGGAGGGTAGCC
 CGGGGATTCTGCAGTGAGAGCAGAGGGCCAGCTAAGTGGTACTCTCCCAGAGAC
 TGTCTGACTCACGCCACCCCCTCCACCTTGGACACAGGACGCTGTGGTTTCTGAG
 CCAGGTACAATGACTCCTTTTCGGTAAGTGCAGTGGAAGCTGTACACTGCCCAGGC
 AAAGCGTCCGGGCAGCGTAGGCGGGCGACTCAGATCCCAGCCAGTGGACTTAGC
 10 CCCTGTTTGCTCCTCCGATAACTGGGGTGACCTTGGTTAATATTCACCAGCAGCCT
 CCCCCGTTGCCCTCTGGATCCACTGCTTAAATACGGACGAGGACAGGGCCCTGT
 CTCCTCAGCTTCAGGCACCACCACTGACCTGGGACAGTGAAT (SEQ ID NO: 65).

The expression of PAH from these vectors is detailed in further Examples herein.

[229] **Example 5. Materials and Methods for PAH**

15 [230] The sequence of *Homo sapiens* phenylalanine hydroxylase (hPAH) mRNA
 (Gen Bank: NM_000277.1) was chemically synthesized with EcoRI and SalI restriction
 enzyme sites located at distal and proximal ends of the gene by Eurofins Genomics
 (Louisville, KY). hPAH treated with EcoRI and SalI restriction enzymes was ligated into the
 pCDH lentiviral plasmids (System Biosciences, Palo Alto, CA) under control of a hybrid
 20 promoter comprising parts of ApoE (NM_000001.11, U35114.1) or prothrombin
 (AF478696.1) and hAAT (HG98385.1) locus control regions. Additionally, human PAH was
 synthesized to include 289 nucleotides of the 3' untranslated region (UTR).

[231] The lentiviral vector and hPAH sequences were digested with the restriction
 enzymes BamHI and EcoRI (NEB, Ipswich, MA) for two hours at 37 degrees Celsius. The
 25 digested lentiviral vector was purified by agarose gel electrophoresis and extracted from the

gel using a DNA gel extraction kit from ThermoFisher (Waltham, MA). The DNA concentration was determined and then mixed with the PAH sequence (hPAH) using an insert to vector ratio of 3:1. The mixture was ligated with T4 DNA ligase (NEB) for 30 minutes at room temperature. 2.5 microliters of the ligation mix were added to 25 microliters of STBL3
5 competent bacterial cells (ThermoFisher). Transformation was carried out by heat-shock at 42 degrees Celsius. Bacterial cells were streaked onto agar plates containing ampicillin and then colonies were expanded in LB broth. To check for insertion of the PAH sequences, Plasmid DNA was extracted from harvested bacteria cultures with the ThermoFisher DNA mini prep kit. Insertion of the PAH sequence in the lentiviral vector (LV) was verified by
10 DNA sequencing. Next, the ApoE enhancer/hAAT promoter or prothrombin enhancer/hAAT promoter sequences with ClaI and EcoRI restriction sites were synthesized by Eurofins Genomics. The lentiviral vector containing a PAH coding sequence and the hybrid promoters were digested with ClaI and EcoRI enzymes and ligated together. The plasmids containing the hybrid promoters were verified by DNA sequencing. The lentiviral vector containing
15 hPAH and a hybrid promoter sequence were then used to package lentiviral particles to test for their ability to express PAH in transduced cells. Mammalian cells were transduced with lentiviral particles. Cells were collected after 3 days and protein was analyzed by immunoblot for PAH expression.

[232] *Modifications of the hPAH Sequence:*

20 [233] Several modifications of the hPAH sequence were incorporated to improve cellular expression levels as regulated by the ApoE enhancer/hAAT promoter. First, 289 nucleotides of the hPAH 3' untranslated region (UTR) was inserted after the PAH coding region and before the mRNA terminus. This created LV-ApoE/hAAT-hPAH-UTR.

[234] Next, liver-specific ApoE enhancer was exchanged with the liver-specific
25 prothrombin enhancer. The expression of PAH was analyzed with either the ApoE or

prothrombin enhancer/hAAT promoter combination with the hPAH coding sequence and 289 nucleotides of the UTR. Next, the expression of PAH was assessed with the prothrombin enhancer/hAAT promoter and hPAH coding sequence without the UTR region. The combination of the prothrombin enhancer/hAAT promoter obviated the requirement of the UTR region as was required with the ApoE enhancer/hAAT promoter combination. Therefore, the prothrombin enhancer/hAAT promoter combination can regulate high levels of PAH expression in a liver-specific manner without the requirement of the UTR region. This important advance in understanding liver-specific regulatory elements to regulate the hPAH gene allows for the generation of constructs for specific expression in liver tissue while still achieving high-level production of hPAH. Restricting transgene expression to liver cells is an important consideration for vector safety and target specificity in a genetic medicine for phenylketonuria.

[235] **Example 6. Lentivirus-delivered expression of hPAH with variations of the prothrombin enhancer and with or without the 3' UTR region in Hepa1-6 cells and 293T cells**

[236] This Example illustrates that expression of human PAH is increased in Hepa1-6 carcinoma cells and 293T human embryonic kidney cells with a lentiviral vector containing the hAAT promoter in combination with the prothrombin enhancer as compared to the ApoE enhancer as shown in Figures 4A and 4B, respectively. The 3' UTR is not required for hPAH expression when the prothrombin enhancer is combined with the hAAT promoter. The 3' UTR actually decreases PAH expression in the prothrombin containing vector as shown in Figures 4A and 4B, respectively. This Example also illustrates that a lentivirus vector expressing Hepatocyte Nuclear Factor 1 and 4 (HNF1/4) binding sites in combination with the prothrombin enhancer increases the levels of PAH protein in Hepa1-6 cells and 293T cells as shown in Figures 4A and 4B, respectively.

[237] Human PAH, the prothrombin and ApoE enhancer, and hAAT promoter were synthesized by Eurofins Genomics (Louisville, KY) and inserted into a lentiviral vector. Insertion of the sequences was verified by DNA sequencing. The lentiviral vectors containing a verified hPAH sequence were then used to transduce Hepa1-6 mouse liver cancer cells or
5 293T human embryonic kidney cells (American Type Culture Collection, Manassas, VA). The lentiviral vectors incorporated a human PAH gene with or without its 3' UTR. In addition, hPAH expression in these constructs was driven by the hAAT promoter containing either the liver specific prothrombin or ApoE enhancer. Cells were transduced with lentiviral particles and after 3 days protein was analyzed by immunoblot for hPAH expression. The
10 relative expression of human PAH was detected by immunoblot using an anti-PAH antibody (Abcam, Cambridge, MA) and an anti-beta actin antibody (SigmaMillipore, Billerica, MA) was used for a loading control.

[238] As shown in Figures 4A and 4B, six groups are compared: a control comprising Hepa 1-6 cells or 293T cells alone (lane 1), a lentiviral vector expressing the
15 coding region of hPAH by the prothrombin enhancer/hAAT promoter (lane 2), a lentiviral vector expressing hPAH by the prothrombin enhancer/hAAT promoter containing 5X HNF1 binding sites upstream of the prothrombin enhancer (lane 3), a lentiviral vector expressing hPAH by the prothrombin enhancer/hAAT promoter containing 3X HNF1 and 3X HNF4 binding sites upstream of the prothrombin enhancer (lane 4), a lentiviral vector expressing
20 hPAH with the 3' UTR by the prothrombin enhancer/hAAT promoter (lane 5), and a lentiviral vector expressing hPAH with the 3' UTR by the ApoE enhancer/hAAT promoter (lane 6). Figures 4A and 4B demonstrate that expression of PAH is increased in both Hepa1-6 carcinoma cells and 293T cells when the prothrombin enhancer is combined with the hAAT promoter as compared with the ApoE enhancer. Additionally, the PAH 3' UTR is not
25 required for hPAH expression when the prothrombin enhancer is included in the vector.

[239] **Example 7. Lentivirus-delivered expression of hPAH with an intron sequence, a codon optimized PAH sequence, and the prothrombin enhancer containing HNF-1 or HNF1/4 binding sites in Hepa1-6 cells**

[240] This Example illustrates that expression of human PAH is increased in Hepa1-6 carcinoma cells with a lentiviral vector containing the hAAT promoter in combination with the prothrombin enhancer and a rabbit beta globin intron sequence as shown in Figure 5A. hPAH is not expressed when the intron sequence is inserted in the reverse direction. In Figure 5B, this shows that a codon-optimized version of the hPAH coding sequence expresses less than the non-optimized hPAH coding region sequence. This Example also illustrates that a lentiviral vector expressing Hepatocyte Nuclear Factor-1 and -4 (HNF1 and HNF1/4) binding sites in combination with the prothrombin enhancer increases the levels of hPAH protein in Hepa1-6 cells as shown in Figure 5C.

[241] Human PAH (optimized and non-optimized), the prothrombin enhancer, hAAT promoter, and a rabbit beta globin sequence were synthesized by Eurofins Genomics (Louisville, KY) and inserted into a lentiviral vector. Insertion of the sequences was verified by DNA sequencing. The lentiviral vectors containing a verified hPAH sequence were then used to transduce Hepa1-6 mouse liver cancer cells (American Type Culture Collection, Manassas, VA). The lentiviral vectors incorporated a human PAH gene with or without a rabbit beta globin intron. In addition, hPAH expression in these constructs was driven by the hAAT promoter containing the liver-specific prothrombin enhancer with HNF1 or HNF1/4 binding sites, either upstream or downstream of the prothrombin enhancer. Cells were transduced with lentiviral particles and after 3 days protein was analyzed by immunoblot for PAH expression. The relative expression of human PAH was detected by immunoblot using an anti-PAH antibody (Abcam, Cambridge, MA) and an anti-beta actin antibody (SigmaMillipore, Billerica, MA) was used for a loading control.

[242] As shown in Figure 5A, four groups are compared: a control comprising Hepa 1-6 cells alone (lane 1), a lentiviral vector expressing the coding region of hPAH by the prothrombin enhancer/hAAT promoter (lane 2), a lentiviral vector expressing hPAH by the prothrombin enhancer/hAAT promoter and containing a rabbit beta globin intron in the forward direction (lane 3), and a lentiviral vector expressing hPAH by the prothrombin enhancer/hAAT promoter and containing a rabbit beta globin intron in the reverse direction (lane 4). As shown in Figure 5B, three groups are compared: a control comprising Hepa 1-6 cells alone (lane 1), a lentiviral vector expressing only the coding region of hPAH by the prothrombin enhancer/hAAT promoter (lane 2 and 3), and a lentiviral vector expressing a codon-optimized sequence of hPAH by the prothrombin enhancer/hAAT promoter (lane 4). As shown in Figure 5C, six groups are compared: a control comprising Hepa 1-6 cells alone (lane 1), a lentiviral vector only expressing the coding region of hPAH by the prothrombin enhancer/hAAT promoter (lane 2 and 3), a lentiviral vector expressing hPAH by the prothrombin enhancer/hAAT promoter and containing a rabbit beta globin intron (lane 4), a lentiviral vector expressing hPAH by the prothrombin enhancer/hAAT promoter and a HNF1 binding site upstream of the prothrombin enhancer (lane 5), a lentiviral vector expressing hPAH by the prothrombin enhancer/hAAT promoter and a HNF1 binding site downstream of the prothrombin enhancer (lane 6), a lentiviral vector expressing hPAH by the prothrombin enhancer/hAAT promoter and a HNF1/4 binding site upstream of the prothrombin enhancer (lane 7), and a lentiviral vector expressing hPAH by the prothrombin enhancer/hAAT promoter and a HNF1/4 binding site downstream of the prothrombin enhancer (lane 8). Figures 5A-5C demonstrate that expression of hPAH is increased in Hepa1-6 carcinoma cells when a rabbit beta globin intron sequence is added upstream of the hPAH coding sequence. Additionally, a codon-optimized hPAH coding sequence expresses less than a non-optimized sequence. Finally, the addition of HNF1 or HNF1/4 binding sites upstream, but not

downstream, of the prothrombin enhancer increases the expression of hPAH as compared with the lentiviral vector containing only the prothrombin enhancer/hAAT promoter.

[243] **Example 8. Lentivirus-delivered expression of hPAH RNA with the hAAT promoter and prothrombin enhancer containing HNF-1 and HNF-1/4 binding sites in**

5 **Hepa1-6 cells**

[244] As shown in Figure 6, this Example illustrates that expression of human PAH RNA is increased in Hepa1-6 carcinoma cells transduced at a multiplicity of infection (MOI) of 1 and 5 with a lentiviral vector containing the hAAT promoter in combination with the prothrombin enhancer and binding sites for HNF1 and HNF1/4.

10 [245] Human PAH, the prothrombin enhancer, and hAAT promoter were synthesized by Eurofins Genomics (Louisville, KY) and inserted into a lentiviral vector. Insertion of the sequences was verified by DNA sequencing. The lentiviral vectors containing a verified hPAH sequence were then used to transduce Hepa1-6 mouse liver cancer cells (American Type Culture Collection, Manassas, VA). The lentiviral vectors incorporated a

15 human PAH gene. In addition, hPAH expression in these constructs was driven by the hAAT promoter containing the liver-specific prothrombin enhancer with upstream HNF1 or HNF1/4 binding sites. Cells were transduced with lentiviral particles and after 3 days RNA was extracted with the RNeasy kit (Qiagen, Germantown, MD) and analyzed by qPCR analysis with a TaqMan probe (5'-TCGTGAAAGCTCATGGACAGTGGC-3') (SEQ ID NO: 66) and

20 primer set Fwd: 5'-AGATCTTGAGGCATGACATTGG-3' (SEQ ID NO: 67) and Rev: 5'-GTCCAGCTCTTGAATGGTTCTT-3' (SEQ ID NO: 68) for hPAH. Total RNA (100 ng) was normalized with an actin probe (5'-AGCGGGAAATCGTGCGTGAC-3') (SEQ ID NO: 69) and primer set (Fwd: 5'-GGACCTGACTGACTACCTCAT-3' (SEQ ID NO: 70) and Rev: 5'-CGTAGCACAGCTTCTCCTTAAT-3') (SEQ ID NO: 71).

[246] As shown in Figure 6, four groups are compared: a control comprising Hepa1-6 cells alone (bar 1), a lentiviral vector expressing the coding region of hPAH by the prothrombin enhancer/hAAT promoter at 1 MOI (bar 2) and 5 MOI (bar 5), a lentiviral vector expressing hPAH by the prothrombin enhancer/hAAT promoter and a HNF1 binding site upstream of the prothrombin enhancer at 1 MOI (bar 3) and 5 MOI (bar 6), and a lentiviral vector expressing hPAH by the prothrombin enhancer/hAAT promoter and a HNF1/4 binding site upstream of the prothrombin enhancer at 1 MOI (bar 4) and 5 MOI (bar 7). Figure 6 demonstrates that expression of PAH RNA is increased from 1 to 4.7 pg (1-5 MOI) with a vector expressing hPAH by a prothrombin enhancer/hAAT promoter. When a HNF1 binding site is included upstream of the prothrombin enhancer there is an increase from 2.3 to 10.7 pg (1-5 MOI) and an increase from 3 to 17.8 pg (1-5 MOI) when a HNF1/4 binding site is inserted upstream of the prothrombin enhancer.

[247] **Example 9. Lentivirus-delivered expression of hPAH with the prothrombin enhancer and either the hAAT or thyroxine binding globulin (TBG) promoter in Hepa1-6 cells**

[248] This Example illustrates that expression of human PAH is increased in Hepa1-6 carcinoma cells with a lentiviral vector containing the prothrombin enhancer in combination with the hAAT promoter as compared to the TBG promoter (SEQ ID NO: 62) as shown in Figure 7.

[249] Human PAH, the prothrombin enhancer, and hAAT and TBG promoter were synthesized by Eurofins Genomics (Louisville, KY) and inserted into a lentiviral vector. Insertion of the sequences was verified by DNA sequencing. The lentiviral vectors containing a verified hPAH sequence were then used to transduce Hepa1-6 mouse liver cancer cells (American Type Culture Collection, Manassas, VA). The lentiviral vectors incorporated a human PAH gene. In addition, hPAH expression in these constructs was driven by either the

liver-specific hAAT or TBG promoter. Cells were transduced with lentiviral particles and after 3 days protein was analyzed by immunoblot for PAH expression. The relative expression of human PAH was detected by immunoblot using an anti-PAH antibody (Abcam, Cambridge, MA) and an anti-beta actin antibody (SigmaMillipore, Billerica, MA) was used for a loading control.

[250] As shown in Figure 7, four groups are compared: a lentiviral vector expressing the coding region of hPAH by the prothrombin enhancer/hAAT promoter (lane 1), a lentiviral vector expressing hPAH by the prothrombin enhancer/hAAT promoter and containing a rabbit beta globin intron (lane 2), a control with a lentiviral vector containing only the prothrombin enhancer/hAAT promoter (lane 3), and a lentiviral vector expressing hPAH by the prothrombin enhancer/TBG promoter (lane 4). Figure 7 demonstrates that expression of PAH is substantially increased in Hepa1-6 carcinoma cells when the prothrombin enhancer is combined with the hAAT promoter as compared with the TBG promoter.

[251] **Example 10. Lentivirus-delivered expression of hPAH with either a rabbit or human beta globin intron sequence upstream of the PAH gene in Hepa1-6 cells or Hep3B cells.**

[252] This Example illustrates that expression of human PAH in Hepa1-6 and Hep3B carcinoma cells with a lentiviral vector containing the prothrombin enhancer in combination with the hAAT promoter and either a rabbit or human beta globin intron is not increased with the human beta globin intron as shown in Figures 8A and 8B.

[253] Human PAH, the prothrombin enhancer, hAAT promoter, and rabbit or human beta globin intron were synthesized by Eurofins Genomics (Louisville, KY) and inserted into a lentiviral vector. Insertion of the sequences was verified by DNA sequencing. The lentiviral vectors containing a verified hPAH sequence were then used to transduce Hepa1-6

mouse liver cancer cells or Hep3B human hepatocellular carcinoma cells (American Type Culture Collection, Manassas, VA). The lentiviral vectors incorporated a human PAH gene. In addition, hPAH expression in these constructs was driven by the liver-specific hAAT promoter and either a rabbit or human beta globin intron. Cells were transduced with
5 lentiviral particles and after 3 days protein was analyzed by immunoblot for PAH expression. The relative expression of human PAH was detected by immunoblot using an anti-PAH antibody (Abcam, Cambridge, MA) and an anti-beta actin antibody (SigmaMillipore, Billerica, MA) was used for a loading control.

[254] As shown in Figures 8A and 8B, four groups are compared: no lentivirus
10 (lane 1), a lentiviral vector expressing hPAH by the prothrombin enhancer/hAAT promoter (lane 2), a lentiviral vector expressing hPAH by the prothrombin enhancer/hAAT promoter and a rabbit beta globin intron sequence (lane 3), and a lentiviral vector expressing hPAH by the prothrombin enhancer/hAAT promoter and a human beta globin intron (lane 4). Figures 8A and 8B demonstrate that expression of PAH is increased in Hepa1-6 and Hep3B
15 carcinoma cells with the prothrombin enhancer and hAAT promoter. Addition of the rabbit beta globin intron improves expression in Hepa1-6 cells but not Hep3B cells. The human beta globin intron does not improve expression in either Hepa1-6 or Hep3B cells.

[255] **Example 11. Lentivirus-delivered expression of hPAH with the prothrombin enhancer/hAAT promoter in primary human hepatocytes**

20 [256] This Example illustrates that expression of human PAH is substantially increased in primary human hepatocytes with lentiviral vectors containing the prothrombin enhancer in combination with the hAAT promoter as shown in Figure 9.

[257] Human PAH, the prothrombin and ApoE enhancer, and hAAT promoter were synthesized by Eurofins Genomics (Louisville, KY) and inserted into a lentiviral vector.
25 Insertion of the sequences was verified by DNA sequencing. The lentiviral vectors containing

a verified hPAH sequence were then used to transduce primary human hepatocytes (Triangle Research Labs, North Carolina). The lentiviral vectors incorporated the coding sequence of human PAH or the coding sequence and 3' UTR. In addition, hPAH expression in these constructs was driven by the liver-specific prothrombin or ApoE enhancer and hAAT promoter. Cells were transduced with lentiviral particles and after 4 days protein was analyzed by immunoblot for PAH expression. The relative expression of human PAH was detected by immunoblot using an anti-PAH antibody (Abcam, Cambridge, MA) and an anti-beta actin antibody (SigmaMillipore, Billerica, MA) was used for a loading control.

[258] As shown in Figure 9, five groups are compared: a control with a lentiviral vector containing only the prothrombin enhancer/hAAT promoter (lane 1), a lentiviral vector expressing the coding region of hPAH by the prothrombin enhancer/hAAT promoter (lane 2), a lentiviral vector expressing hPAH by the prothrombin enhancer/hAAT promoter and 5X HNF1 binding sites upstream of the prothrombin enhancer (lane 3), a lentiviral vector expressing hPAH by the prothrombin enhancer/hAAT promoter and containing a rabbit beta globin intron (lane 4), and a lentiviral vector expressing hPAH with the 3' UTR by the ApoE/hAAT promoter (lane 5). Figure 9 demonstrates that expression of PAH is increased in primary human hepatocytes when the prothrombin enhancer is combined with the hAAT promoter as compared with the ApoE enhancer. Also, inclusion of a rabbit beta globin intron sequence upstream of the hPAH coding sequence enhances hPAH expression.

[259] **Example 12. Enzymatic activity of lentivirus-delivered hPAH in Hepa1-6 cells**

[260] This Example illustrates that lentiviral-delivered human PAH is enzymatically active as indicated by a decrease in phenylalanine (Phe) levels in the cell media and cell lysate when hPAH is expressed in Hepa1-6 cells as shown in Figures 10A and 10C, and 10B, respectively. A precursor of the cofactor BH₄, sepiapterin, was required for PAH activity in Hepa1-6 cells.

[261] Human PAH, the prothrombin and ApoE enhancer, hAAT promoter, and rabbit beta globin intron were synthesized and inserted into a lentiviral vector. Insertion of the sequences was verified by DNA sequencing. The lentiviral vectors containing a verified hPAH sequence were then used to transduce Hepal-6 mouse liver cancer cells (American
5 Type Culture Collection, Manassas, VA). The lentiviral vectors incorporated the coding sequence of human PAH. In addition, hPAH expression in these constructs was driven by the liver-specific prothrombin enhancer/hAAT promoter and included the rabbit beta globin intron sequence. Cells were transduced with lentiviral particles and after 4 days phenylalanine levels were measured from either cell media or cell lysate using a
10 Phenylalanine Assay kit (SigmaMillipore, Billerica, MA).

[262] As shown in Figures 10A and 10B, four groups are compared from either cell media in Figure 10A or from cell lysate in Figure 10B: a control with a lentiviral vector containing only the prothrombin enhancer/hAAT promoter without sepiapterin (bar 1) or with sepiapterin (bar 3), a lentiviral vector expressing the coding region of hPAH by the
15 prothrombin enhancer/hAAT promoter and containing a rabbit beta globin intron sequence without sepiapterin (bar 2) or with sepiapterin (bar 4). As shown in Figure 10C, ten groups are compared from cell media: a control with a lentiviral vector containing only the prothrombin enhancer/hAAT promoter without sepiapterin (bar 1) or with sepiapterin (bar 6), a lentiviral vector expressing the coding region of hPAH by the prothrombin enhancer/hAAT
20 promoter without sepiapterin (bar 2) or with sepiapterin (bar 7), a lentiviral vector expressing the coding region of hPAH by the prothrombin enhancer/hAAT promoter and 5X HNF1 binding sites upstream of the prothrombin enhancer without sepiapterin (bar 3) or with sepiapterin (bar 8), a lentiviral vector expressing the coding region of hPAH by the prothrombin enhancer/hAAT promoter and containing a rabbit beta globin intron sequence
25 without sepiapterin (bar 4) or with sepiapterin (bar 9), a lentiviral vector expressing the

coding region of hPAH including the 3' UTR by the ApoE enhancer/hAAT promoter without sepiapterin (bar 5) or with sepiapterin (bar 10). Figures 10A and 10B show that hPAH is enzymatically active in Hepal-6 cells in the presence of sepiapterin. There was a 38% decrease in cell media phenylalanine levels in Hepal-6 cells transduced with a lentiviral vector expressing hPAH as shown in Figure 10A. In cell lysate, there was an 88% decrease in phenylalanine levels in Hepal-6 cells transduced with a lentiviral vector expressing hPAH as shown in Figure 10B. Figure 10C shows that hPAH is enzymatically active in vectors containing different liver-specific promoter elements. There was a 41% decrease in Phe levels with the vector containing the prothrombin enhancer/hAAT promoter and hPAH, a 43% decrease in Phe levels with the vector containing 5X HNF1 binding sites upstream of the prothrombin enhancer/hAAT promoter and hPAH, a 48% decrease in Phe levels with the vector containing the prothrombin enhancer/hAAT promoter including the rabbit beta globin intron and hPAH, and a 36% decrease in Phe levels with the vector containing the ApoE enhancer/hAAT promoter and hPAH including the 3' UTR.

Example 13. Lentiviral delivered PAH decreases blood phenylalanine levels in blood of PAH mutant mice

This Example illustrates that lentiviral-delivered PAH decreases phenylalanine levels in the blood of Pah^{enu2} mutant mice. The Pah^{enu2} mutant mouse is described and characterized in Shedlovsky *et al.* (Mouse Models of Human Phenylketonuria, Genetics 134: 1205-1210 (August, 1993)), the entirety of which is incorporated by reference herein. Pah^{enu2} mutant mice (n=4) were injected with 150 µl volume of the LV-Pro-hAAT-PAH (AGT323) vector via the tail vein. Control treated animals were injected via the tail vein with saline that did not contain a lentivirus vector. The titer of LV-Pro-hAAT-PAH (AGT323) was 1x10¹⁰ as determined by qPCR detection of integrated vector copies in transduced 293T cells. At the time of injection, the mice were 6-8 weeks old. Blood was collected before vector injection

(T=0) and at 1 and 2 weeks post-injection.

[265] For measurement of phenylalanine levels, blood was collected via the facial vein. A lancet was used to puncture the cheek and 400 µl of whole blood was collected in serum tubes. The blood was rested for one hour and then the tubes were centrifuged. The plasma/serum was separated on the top layer and collected. The plasma was then frozen until it was analyzed on a clinical amino acid analyzer instrument.

[266] Introduction of the LV-Pro-hAAT-PAH (AGT323) vector caused a reduction in blood phenylalanine levels at both one week and two weeks post-injection. As shown in Table 1 and Figure 11, the mean micro-molar levels of blood phenylalanine at the 1-week and 2-week time points was 1674.7 and 1890, respectively. This is compared to the mean micro-molar blood phenylalanine levels of control treated mice of between ~2400 and ~2500 (Table 1 and Figure 11).

[267] In addition, introduction of the LV-Pro-hAAT-PAH (AGT 323) vector resulted in genetic modification of the liver cells. Vector copy number studies on livers collected during necropsy showed the lentivirus vector copy number was approximately 0.2 per cell in these studies. As shown in Figure 12, there is roughly a linear relationship between increasing vector copy number in liver and decreasing levels of phenylalanine (Pearson product correlation coefficient = -0.326).

[268] A decrease in blood Phenylalanine levels of $\geq 20\%$ would provide therapeutic benefit to human patients with Phenylketonuria disease, potentially shifting their diagnosis from classic PKU to mild Phenylalanemia.

Table 1

Blood Phenylalanine Suppression by Tail Vein Injection of AGT323 in Juvenile enu2 mice

Control Mice (n=6)	Control Week 1	Control Week 2	Treated Mice (n=12)	Treated/ Week 1	Treated/ Week 2
Mouse 1	2943*	2165	Mouse 7	1863	1448
Mouse 2	2305	2155	Mouse 8	1489	1412

Mouse 3	2275	2480	Mouse 9	1473	1741
Mouse 4	2756	2432	Mouse 10	1910	1999
Mouse 5	2263	2701	Mouse 11	1606	1753
Mouse 6	2483	2477	Mouse 12	1851	2337
			Mouse 13	1187	1065
			Mouse 14	2042	2266
			Mouse 15	1841	2174
			Mouse 16	1532	1498
			Mouse 17	1768	1758
			Mouse 18	1534	1534
Mean	2504.2	2401.7		1674.7	1890
Std Dev. of Mean	230.2	161.1		204.5	299.1

*Blood [Phe] in micromole/L

[269] **Example 14. AAV-delivered expression of hPAH with either a DJ or AAV2 serotype in HEK293T cells**

[270] This Example illustrates that human PAH is expressed in HEK293T cells with
5 an AAV vector containing the prothrombin enhancer in combination with the hAAT promoter, and with or without a rabbit beta globin intron.

[271] Human PAH, prothrombin enhancer, hAAT promoter, and rabbit beta globin intron were synthesized by Eurofins Genomics (Louisville, KY) and inserted into an AAV vector. Insertion of the sequences was verified by DNA sequencing. The AAV vectors
10 containing a verified hPAH sequence were then used to transduce HEK293T cells (American Type Culture Collection, Manassas, VA). The AAV vectors incorporated a human PAH gene as disclosed herein. In addition, hPAH expression in these constructs was driven by the liver-specific hAAT promoter and a rabbit beta globin intron. Cells were transduced with AAV particles and after three days, protein or RNA was analyzed by immunoblot or qPCR for
15 PAH expression. The expression of human PAH protein was detected by immunoblot using an anti-PAH antibody (Abcam, Cambridge, MA) and an anti-beta actin antibody (MilliporeSigma, Billerica, MA) was used for a loading control. PAH RNA expression was detected by qPCR using a TaqMan Fam-labeled probe (SEQ ID NO: 66) and PAH primers (Fwd: SEQ ID NO: 67; Rev: SEQ ID NO: 68).

[272] Figure 13A shows a comparison of six groups: an AAV/DJ-GFP vector (lane 1), an AAV/DJ vector expressing hPAH by the prothrombin enhancer/hAAT promoter (lane 2), an AAV/DJ vector expressing hPAH by the prothrombin enhancer/hAAT promoter and a rabbit beta globin intron sequence (lane 3), an AAV2-GFP vector (lane 4), an AAV2 vector
5 expressing hPAH by the prothrombin enhancer/hAAT promoter (lane 5), an AAV2 vector expressing hPAH by the prothrombin enhancer/hAAT promoter and a rabbit beta globin intron sequence (lane 6).

[273] Figures 13B and 13C show blots after increased exposure the PAH bands in Figure 13A. Exposure was increased so that the band densities were brought into a range to
10 be analyzed by quantitative imaging. The original band intensities were much lower on the blot showing treatment with the AAV/2 serotype vector relative to the blot showing treatment with AAV/DJ serotype vector. As a result, a longer exposure time was needed on the AAV/2 serotype blot relative to the AAV/DJ serotype blot, in order to make quantitative measurements of PAH protein.

[274] Results of quantitative imaging showed that PAH protein expression was
15 increased by 2-fold using either an AAV/DJ vector that includes a rabbit beta globin intron or an AAV/DJ vector that lacks a rabbit beta globin intron (Figure 13B; the numbers below the bands show relative fold increase). Using the AAV/2 vector with a rabbit beta globin intron suppressed expression of PAH, whereas using the AAV2 vector without a rabbit beta globin
20 intron resulted in a 50-fold increase of PAH (Figure 13C; the numbers below the bands show relative fold increase). Comparing PAH expression between AAV serotypes revealed that use of the AAV/DJ PAH vector results in higher expression of PAH protein relative to using the AAV2 PAH vector.

[275] Figure 13D demonstrates that PAH RNA expression is higher with the
25 AAV/DJ PAH vector as compared with the AAV2 PAH vector, and there is similar

expression with and without the rabbit beta globin intron.

[276] **Example 15. Lentivirus Vector Therapy for PKU in Neonatal enu2/enu2 mice**

[277] Neonatal enu2/enu2 mice (day 3 after birth) were treated with 10 μ l of vector stock LV-Pro-hAAT-PAH via direct injection into the liver. Untreated animals received
5 saline without vector (sham control). The vector stock was approximately 5×10^8 transducing units per mL in sterile saline (measured in HEK293 cells) for a final dose of $\sim 5 \times 10^6$ transducing units per mouse ($\sim 10^9$ transducing units per kg).

[278] As shown in Table 2 and Figure 14, micro-molar concentrations of blood phenylalanine levels was significantly reduced in LV-Pro-hAAT-PAH treated enu2/enu2
10 mice (1390 $\pm$ 127) relative to untreated enu2/enu2 mice (2063 $\pm$ 185).

Table 2

	Wild-type (n=7)	enu2/enu2 treated (n=5)	enu2/enu2 untreated (n=10)
Blood Phe ($\pm$ standard error of them mean)	60.7 $\pm$ 3.4	1390 $\pm$ 127	2063 $\pm$ 185
Treated vs. untreated		Unpaired t test P = 0.032	
		Difference between means blood [Phe] 674 $\pm$ 280	

[279] **Example 16. Expression of shPAH targeting the 3'UTR of the PAH gene does not suppress PAH expression by LV-H1-shPAH-Prothrombin-hAAT-hPAH in**
15 **Hep3B cells**

[280] This Example illustrates that shPAH does not suppress PAH expression from the lentiviral vector, LV-Pro-hAAT-PAH-shPAH sequence # 1 (SEQ ID NO: 13), or from the

lentiviral vector, LV-Pro-hAAT-PAH-shPAH sequence # 2 (SEQ ID NO: 14), in Hep3B cells.

[281] Human PAH as disclosed herein was synthesized and inserted into lentiviral vectors containing either PAH shRNA sequence #1 (SEQ ID NO: 13) or PAH shRNA sequence #2 (SEQ ID NO: 14). Insertion of the sequences was verified by DNA sequencing. Lentiviral vectors containing either PAH alone or in combination with either PAH shRNA sequence #1 (SEQ ID NO: 13) or PAH shRNA sequence #2 (SEQ ID NO: 14) were then used to transduce human Hep3B cells (purchased from American Type Culture Collection, Manassas, VA). Cells were transduced with lentiviral particles and after 3 days protein was analyzed by western blot for PAH expression. The relative expression of human PAH was detected by immunoblot using an anti-PAH antibody (Abcam) and the loading control beta-actin. hPAH expression was driven by a prothrombin enhancer and hAAT promoter. The lentiviral vectors incorporated, in various instances, a human PAH gene and PAH shRNA sequence #1 (SEQ ID NO: 13) or PAH shRNA sequence #2 (SEQ ID NO: 14). Insertion of a shRNA sequence in the lentiviral vector (LV) was verified by DNA sequencing using a primer complementary to the promoter used to regulate shPAH-2 expression. In this case, an H1 promoter was used to regulate PAH shRNA expression. The target sequences for shPAH—(PAH shRNA sequence #1 (SEQ ID NO: 13) or PAH shRNA sequence # 2 (SEQ ID NO: 14))—are in the PAH 3'UTR that are not present in the LV-PAH vector.

[282] As shown in Figure 15, expression of PAH derived from a lentivirus is not suppressed with either PAH shRNA sequence #1 (SEQ ID NO: 13) or PAH shRNA sequence # 2 (SEQ ID NO: 14).

[283] Sequence Listings

SEQ ID NO:	Description	Sequence
1	PAH	ATGTCCACTGCGGTCCTGGAAAACCCAGGCTTGGGCAGGAACTCT CTGACTTTGGACAGGAAACAAGCTATATTGAAGACAACTGCAATCA AAATGGTGCCATATCACTGATCTTCTCACTCAAAGAAGAAGTTGGTG CATTGGCCAAAGTATTGCGCTTATTTGAGGAGAATGATGTAAACCTG ACCCACATTGAATCTAGACCTTCTCGTTTAAAGAAAGATGAGTATGA ATTTTTCACCCATTTGGATAAACGTAGCCTGCCTGCTCTGACAAACA TCATCAAGATCTTGAGGCATGACATTGGTGCCACTGTCCATGAGCTT TCACGAGATAAGAAGAAAGACACAGTGGCCTGGTTCCCAAGAACCA TTCAAGAGCTGGACAGATTTGCCAATCAGATTCTCAGCTATGGAGCG GAACTGGATGCTGACCACCCTGGTTTTAAAGATCCTGTGTACCGTGC AAGACGGAAGCAGTTTGCTGACATTGCCTACAACCTACCGCCATGGG CAGCCCATCCCTCGAGTGGAATACATGGAGGAAGAAAAGAAAACAT GGGGCACAGTGTTCAAGACTCTGAAGTCCTTGTATAAAACCCATGCT TGCTATGAGTACAATCACATTTTCCACTTCTTGAAAAGTACTGTGG CTTCCATGAAGATAACATTCCCCAGCTGGAAGACGTTTCTCAATTCC TGCAGACTTGCACTGGTTTCCGCCTCCGACCTGTGGCTGGCCTGCTTT CCTCTCGGGATTCTTGGGTGGCCTGGCCTTCCGAGTCTTCCACTGCA CACAGTACATCAGACATGGATCCAAGCCCATGTATACCCCCGAACCT GACATCTGCCATGAGCTGTTGGGACATGTGCCCTTGTTTTAGATCG CAGCTTTGCCAGTTTTCCAGGAAATTGGCCTTGCTCTCTGGGTG CACCTGATGAATACATTGAAAAGCTCGCCACAATTTACTGGTTTACT GTGGAGTTTGGGCTCTGCAACAAGGAGACTCCATAAAGGCATATG GTGCTGGGCTCCTGTCATCCTTTGGTGAATTACAGTACTGCTTATCA GAGAAGCCAAAGCTTCTCCCCCTGGAGCTGGAGAAGACAGCCATCC AAAATTACACTGTCACGGAGTTCCAGCCCCTGTATTACGTGGCAGAG AGTTTTAATGATGCCAAGGAGAAAAGTAAGGAACCTTGCTGCCACAA TACCTCGGCCCTTCTCAGTTGCTACGACCCATACACCCAAAGGATT GAGGTCTTGGACAATAACCCAGCAGCTTAAGATTTTGGCTGATTCCAT TAACAGTGAAATTGGAATCCTTTGCAGTGCCCTCCAGAAAATAAAGT AA
2	Codon optimized PAH (Opt2)	ATGAGTACGGCTGTGCTCGAGAATCCAGGTTTGGGCCGAAAGCTGT CTGATTTTGGACAGGAGACATCTTATATTGAAGACAACTGCAACCAG AATGGTGCGATATCCCTTATTTTTTCTCTGAAAGAAGAAGTAGGTGC GCTGGCAAAGGTCTTGCGGCTGTTTGAAGAGAACGATGTTAATCTTA CTCATATTGAGTCCAGACCATCACGGCTGAAAAAAGACGAGTACGA ATTTTTTACTCACTTGGACAAACGAAGCTTGCCGGCTCTTACTAATA TCATTAAGATCCTCCGGCATGACATAGGGGCGACAGTGCATGAGCTT TCAAGGGATAAAAAGAAAGATACCGTCCCCTGGTTTCCAAGGACCA TACAAGAACTCGACCGATTTCGGAACCAAGATCCTTTCATATGGTGT GAGTTGGATGCTGACCACCCCGGCTTCAAAGACCCGGTCTACCGAG CGCGGCGGAAACAATTTGCTGACATCGCATACAATTACAGGCATGG CCAGCCAATTCCTAGAGTAGAATACATGGAAGAAGAGAAAAAAACC TGGGGTACCGTCTTCAAGACGCTGAAATCATTGTATAAAACTCATGC ATGTTACGAATATAACCATATTTTTCCGTTGCTCGAGAAATATTGCG GGTTCCACGAAGATAACATCCCACAACCTCGAGGATGTATCTCAGTTC CTCCAGACCTGTACGGGGTTTCGACTTAGGCCTGTGCGGGGTTTGCT CAGTTCTCGAGACTTCCTGGGTGGATTGGCGTTTCGGGTATTCCATT

		GCACGCAGTATATCCGACACGGAAGTAAGCCAATGTACACGCCAGA ACCCGATATCTGTACGAATTGCTTGGACACGTTCTCTGTTTTCTGA TCGATCATTGCTCAGTTTTACAGGAAATCGGCCTGGCATCTTTGG GAGCGCCGGATGAATATATTGAGAAGCTCGCTACAATTTACTGGTTC ACGGTAGAATTTGGGTTGTGCAAGCAGGGTGATAGTATTAAAGCAT ACGGTGCGGGATTGCTGTCCTCATTTCGGGGAGCTTCAGTATTGCCTG TCCGAGAAACCCAAGCTGTTGCCGTTGGAATTGGAAAAAACCGCTA TCCAAAATTACACAGTAACGGAGTTCCAACCTTTGTACTACGTAGCC GAGTCATTTAACGATGCAAAGGAGAAGGTCAGAAATTTTGCTGCGA CGATACCCAGACCGTTCTCAGTAAGGTACGATCCTTACACTCAGAGG ATTGAAGTCCTGGATAATACGCAACAGCTCAAGATCCTGGCAGACT CCATAAATTCTGAAATCGGCATCTTGTGTTCAGCACTGCAAAAGATA AAATAA
3	PAH 3'UTR sequence (897 nucleotides)	AGCCATGGACAGAATGTGGTCTGTCAGCTGTGAATCTGTTGATGGAG ATCCAACATTTCTTTTCATCAGAAAAAGTCCGAAAAGCAAACCTTAA TTTGAAATAACAGCCTTAAATCCTTTACAAGATGGAGAAACAACAA ATAAGTCAAAATAATCTGAAATGACAGGATATGAGTACATACTCAA GAGCATAATGGTAAATCTTTTGGGGTCATCTTTGATTTAGAGATGAT AATCCCATACTCTCAATTGAGTTAAATCAGTAATCTGTCGCATTTCA TCAAGATTAATTAATAATTTGGGACCTGCTTCATTCAAGCTTCATATA TGCTTTGCAGAGAACTCATAAAGGAGCATATAAGGCTAAATGTAAA ACCCAAGACTGTCATTAGAATTGAATTATTGGGCTTAATATAAATCG TAACCTATGAAGTTTATTTTTTATTTTAGTTAACTATGATTCCAATTA CTACTTTGTTATTGTACCTAAGTAAATTTTCTTTAAGTCAGAAGCCCA TTAAAATAGTTACAAGCATTGAACCTCTTTAGTATTATATTAATATA AAAACATTTTTGTATGTTTTATTGTAATCATAAATACTGCTGTATAAG GTAATAAAACTCTGCACCTAATCCCCATAACTTCCAGTATCATTTTC CAATTAATTATCAAGTCTGTTTTGGGAAACACTTTGAGGACATTTAT GATGCAGCAGATGTTGACTAAAGGCTTGGTTGGTAGATATTCAGGA AATGTTCACTGAATAAATAAGTAAATACATTATTGAAAAGCAAATCT GTATAAATGTGAAATTTTTATTTGTATTAGTAATAAAACATTAGTAG TTTAAACAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAACTCGACT CTAGATT
4	PAH 3'UTR sequence (289 nucleotides)	AGCCATGGACAGAATGTGGTCTGTCAGCTGTGAATCTGTTGATGGAG ATCCAACATTTCTTTTCATCAGAAAAAGTCCGAAAAGCAAACCTTAA TTTGAAATAACAGCCTTAAATCCTTTACAAGATGGAGAAACAACAA ATAAGTCAAAATAATCTGAAATGACAGGATATGAGTACATACTCAA GAGCATAATGGTAAATCTTTTGGGGTCATCTTTGATTTAGAGATGAT AATCCCATACTCTCAATTGAGTTAAATCAGTAATCTGTCGCATTTCA TCAAGATTA
5	Prothrombin enhancer (Pro)	GCGAGAACTTGTGCCTCCCCGTGTTCTCTGCTCTTTGTCCCTCTGTCCT ACTTAGACTAATATTTGCCTTGGGTACTGCAAACAGGAAATGGGGG AGGGACAGGAGTAGGGCGGAGGGTAG
6	Human alpha- 1 antitrypsin promoter (hAAT)	GATCTTGCTACCAGTGGAAACAGCCACTAAGGATTCTGCAGTGAGAG CAGAGGGCCAGCTAAGTGGTACTCTCCAGAGACTGTCTGACTCAC GCCACCCCTCCACCTTGGACACAGGACGCTGTGGTTTCTGAGCCAG GTACAATGACTCCTTTCGGTAAGTGCAGTGGAAGCTGTACACTGCCC AGGCAAAGCGTCCGGGCAGCGTAGGCGGGCGACTCAGATCCCAGCC AGTGGACTTAGCCCCTGTTTGCTCCTCCGATAACTGGGGTGACCTTG GTTAATATTACACAGCAGCCTCCCCCGTTGCCCTCTGGATCCACTG CTTAAATACGGACGAGGACAGGGCCCTGTCTCCTCAGCTTCAGGCAC CACCCTGACCTGGGACAGTGAAT
7	Rabbit beta globin intron	GTGAGTTTGGGGACCCTTGATTGTTCTTTCTTTTCGCTATTGTAAAA TTCATGTTATATGGAGGGGGCAAAGTTTTTCAGGGTGTTGTTTAGAAT

		GGGAAGATGTCCCTTGTATCACCATGGACCCTCATGATAAATTTGTT TCTTTCACCTTTCTACTCTGTTGACAACCATTGTCTCCTCTTATTTTCTT TTCATTTTCTGTAACCTTTTTCGTTAACTTTAGCTTGCATTTGTAACG AATTTTAAATTCACCTTTTGTATTTGTCAGATTGTAAGTACTTTCT CTAATCACTTTTTTTCAAGGCAATCAGGGTATATTATATTGTACTTC AGCACAGTTTTAGAGAACAATTGTTATAATTAATGATAAGGTAGA ATATTTCTGCATATAAATCTGGCTGGCGTGGAAATATTCTTATTGGT AGAAACAACCTACACCCTGGTCATCATCCTGCCTTTCTCTTTATGGTTA CAATGATATACACTGTTTGAGATGAGGATAAAATACTCTGAGTCCAA ACCGGGCCCCCTCTGCTAACCATGTTTCATGCCTTCTTCTTTCTCTACA G
8	Human beta globin intron	GGATCCTGAGAACTTCAGGGTGAGTCTATGGGACGCTTGATGTTTTT TTTCCCCTTCTTTTCTATGGTTAAGTTTCATGTCATAGGAAGGGGATAA GTAACAGGGTACACATATTGACCAAAATCAGGGTAATTTTGCATTTGT AATTTTAAAAAATGCTTTCCTTTTAAATATACTTTTTGTTTATCTTA TTTCTAATACTTTCCCTAATCTCTTTCTTTCAGGGCAATAATGATACA ATGTATCATGCCTCTTTGCACCATTCTAAAGAATAACAGTGATAATT TCTGGGTAAAGGCAATAGCAATATTTCTGCATATAAATATTTCTGCA TATAAATTGTAAGTATGTAAGAGGTTTCATATTGCTAATAGCAGCT ACAATCCAGCTACCATTCTGCTTTTATTTTATGGTTGGGATAAGGCT GGATTATTCTGAGTCCAAGCTAGGCCCTTTTGCTAATCATGTTTCATA CCTCTTATCTTCTCCACAGCTCCTGGGCAACGTGCTGGTCTGTGTG CTGGCCCATCACTTTGGCAAAG
9	1X Hepatocyte Nuclear Factor 1 (1XHNF1)	GTAAATCATTAAC
10	5X Hepatocyte Nuclear Factor 1 (5XHNF1)	GTAAATCATTAACGTTAATCATTAACGTTAATCATTAACGTTAATCA TTAACGTTAATCATTAAC
11	1X Hepatocyte Nuclear Factor 1/4 (1XHNF1/4)	GTAAATCATTAACGCTTGTACTTTGGTACA
12	3X Hepatocyte Nuclear Factor 1/4 (3XHNF1/4)	GTAAATCATTAACGCTTGTACTTTGGTACAGTTAATCATTAACGCTTG TACTTTGGTACAGTTAATCATTAACGCTTGTACTTTGGTACA
13	PAH shRNA sequence #1	TCGCATTTTCATCAAGATTAATCTCGAGATTAATCTTGATGAAATGCG ATTTTT
14	PAH shRNA sequence #2	ACTCATAAAGGAGCATATAAGCTCGAGCTTATATGCTCCTTTATGAG TTTTTT
15	Rous Sarcoma virus (RSV) promoter	GTAGTCTTATGCAATACTCTTGTAGTCTTGCAACATGGTAACGATGA GTTAGCAACATGCCTTACAAGGAGAGAAAAAGCACCGTGCATGCCG ATTGGTGGAAGTAAGGTGGTACGATCGTGCCTTATTAGGAAGGCAA CAGACGGGTCTGACATGGATTGGACGAACCACTGAATTGCCGCATT GCAGAGATATTGTATTAAAGTGCCTAGCTCGATACAATAAACG

16	5' Long terminal repeat (LTR)	GGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCCTCAGACCCTTTTAGTCAGTGTGGAAAATCTCTAGCA
17	Psi Packaging signal	TACGCCAAAAATTTTACTAGCGGAGGCTAGAAGGAGAGAG
18	Rev response element (RRE)	AGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCCTCAATGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTGCTGAGGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTC
19	Central polypurine tract (cPPT)	TTTTAAAAGAAAAGGGGGGATTGGGGGGTACAGTGCAGGGGAAAGAAATAGTAGACATAATAGCAACAGACATACAAATAAAGAATTACAAAAACAATTACAAAATTCAAAATTTTA
20	Long WPRE sequence	AATCAACCTCTGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAATATGTTGCTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTCTTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGGCGTGGTGTGCACTGTGTTTGCTGACGCAACCCCCAC TGGTTGGGGCATTGCCACCACCTGTCAGCTCCTTTCCGGGACTTTTCGCTTTCCCCCTCCCTATTGCCACGGCGGAACATCGCCGCCTGCCTTGCCGCTGCTGGACAGGGGCTCGGCTGTTGGGCACTGACAATTCCGTGTGTTGTGCGGGGAAATCATCGTCCTTTTCTTGGCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGGACGTCCTTCTGCTACGTCCTTTCGGCCCTCAATCCAGCGGACCTTCCCTTCCCGCGGCCTGCTGCCGGCTCTGCGCCTCTTCCGCGTCTTCGCTTTCGCCCTCAGACGAGTCGGATCTCCCTTTGGGCCGCTCCCCGCCT
21	delta U3 3'LTR	TGGAAGGGCTAATTCCTCCCAACGAAGATAAGATCTGCTTTTGTCTTGACTGGGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCCGTCTGTTGTGTGACTCTGGTAAC TAGAGATCCCTCAGACCCTTTTAGTCAGTGTGGAAAATCTCTAGCAGTAGTAGTTCATGTCA
22	H1 Promoter	GAACGCTGACGTCAATCAACCCGCTCCAAGGAATCGCGGGCCAGTG TCACTAGGCGGGAACACCCAGCGCGCTGCGCCCTGGCAGGAAGATGGCTGTGAGGGACAGGGGAGTGGCGCCCTGCAATATTTGCATGTCTATGTGTTCTGGGAAATCACCATAAACGTGAAATGTCTTTGGATTGGGAATCTTATAAGTTCTGTATGAGACCACTT
23	CMV enhancer/chicken beta actin promoter	TAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCGCCATTGACGTCAATAATGACGTATGT TCCCATAGTAACGCCAATAGGGACTTTCATTGACGTCAATGGGTGGACTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGGTGCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTATTTTAAATATTTTGTGACGCGATGGGGGCGGGGGGGGGGGGGCGCGGCCAGGCGGGGCGGGGCGGGGCGAGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGCGGCCCTATAAAAAGCGAAGCGCGCGGGCGG

24	HIV Gag	ATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAGAATTAGATCGAT GGGAAAAAATTCGGTTAAGGCCAGGGGGAAAGAAAAAATATAAAT TAAAACATATAGTATGGGCAAGCAGGGAGCTAGAACGATTTCGAGT TAATCCTGGCCTGTTAGAAACATCAGAAGGCTGTAGACAAATACTG GGACAGCTACAACCATCCCTTCAGACAGGATCAGAAGAACTTAGAT CATTATATAATACAGTAGCAACCCTCTATTGTGTGCATCAAAGGATA GAGATAAAAGACACCAAGGAAGCTTTAGACAAGATAGAGGAAGAG CAAAACAAAAGTAAGAAAAAAGCACAGCAAGCAGCAGCTGACACA GGACACAGCAATCAGGTCAGCCAAAATTACCCTATAGTGCAGAACA TCCAGGGGCAAATGGTACATCAGGCCATATCACCTAGAACTTTAAAT GCATGGGTAAAAGTAGTAGAAGAGAAGGCTTTCAGCCCAGAAGTGA TACCCATGTTTTTCAGCATTATCAGAAGGAGCCACCCACAAAGATTTA AACACCATGCTAAACACAGTGGGGGGACATCAAGCAGCCATGCAAA TGTTAAAAGAGACCATCAATGAGGAAGCTGCAGATGGGATAGAGT GCATCCAGTGCATGCAGGGCCTATTGCACCAGGCCAGATGAGAGAA CCAAGGGGAAGTGACATAGCAGGAACCTACTAGTACCCTTCAGGAAC AAATAGGATGGATGACACATAATCCACCTATCCCAGTAGGAGAAAT CTATAAAAGATGGATAATCCTGGGATTAAATAAAATAGTAAGAATG TATAGCCCTACCAGCATTCTGGACATAAGACAAGGACCAAAGGAAC CCTTTAGAGACTATGTAGACCGATTCTATAAACTCTAAGAGCCGAG CAAGCTTCACAAGAGGTAAAAAATTGGATGACAGAAACCTTGTTGG TCCAAAATGCGAACCCAGATTGTAAGACTATTTTAAAAGCATTGGG ACCAGGAGCGACACTAGAAGAAATGATGACAGCATGTCAGGGAGTG GGGGGACCCGGCCATAAAGCAAGAGTTTTGGCTGAAGCAATGAGCC AAGTAACAAATCCAGCTACCATAATGATACAGAAAGGCAATTTTAG GAACCAAGAAAGACTGTTAAGTGTTTCAATTGTGGCAAAAGAGGG CACATAGCCAAAAATTGCAGGGCCCCTAGGAAAAAGGGCTGTTGGA AATGTGGAAAGGAAGGACACCAATGAAAGATTGTACTGAGAGAC AGGCTAATTTTTTAGGGAAGATCTGGCCTTCCCACAAGGGAAGGCC AGGGAATTTTCTCAGAGCAGACCAGAGCCAACAGCCCCACCAGAA GAGAGCTTCAGGTTTTGGGGAAGAGACAACAACCTCTCAGAAGC AGGAGCCGATAGACAAGGAACTGTATCCTTTAGCTTCCCTCAGATCA CTCTTTGGCAGCGACCCCTCGTCACAATAA
25	HIV Pol	ATGAATTTGCCAGGAAGATGGAAACCAAAAATGATAGGGGGAATTG GAGGTTTTATCAAAGTAGGACAGTATGATCAGATACTCATAGAAAT CTGCGGACATAAAGCTATAGGTACAGTATTAGTAGGACCTACACCT GTCAACATAAATTGGAAGAAATCTGTTGACTCAGATTGGCTGCACCTT AAATTTTCCCATTAGTCCTATTGAGACTGTACCAGTAAAATTAAGC CAGGAATGGATGGCCCAAAAGTTAAACAATGGCCATTGACAGAAGA AAAAATAAAAGCATTAGTAGAAATTTGTACAGAAATGGAAAAGGAA GGAAAAATTTCAAAAATTGGGCCTGAAAATCCATACAATACTCCAG TATTTGCCATAAAGAAAAAAGACAGTACTAAATGGAGAAAATTAGT AGATTTTCAGAGAACTTAATAAGAGAACTCAAGATTTCTGGGAAGTT CAATTAGGAATACCACATCCTGCAGGGTTAAAAACAGAAAAAATCAG TAACAGTACTGGATGTGGGCGATGCATATTTTTTCAGTTCCCTTAGAT AAAGACTTCAGGAAGTATACTGCATTTACCATACCTAGTATAAACAA TGAGACACCAGGGATTAGATATCAGTACAATGTGCTTCCACAGGGA TGGAAGGATCACACGCAATATTCCAGTGTAGCATGACAAAAAATCT TAGAGCCTTTTAGAAAAACAAAATCCAGACATAGTCATCTATCAATAC ATGGATGATTTGTATGTAGGATCTGACTTAGAAATAGGGCAGCATA GAACAAAAATAGAGGAACTGAGACAACATCTGTTGAGGTGGGGATT TACCACACCAGACAAAAAACATCAGAAAGAACCTCCATTCCCTTTGG ATGGGTTATGAACTCCATCCTGATAAATGGACAGTACAGCCTATAGT GCTGCCAGAAAAGGACAGCTGGACTGTCAATGACATACAGAAATTA GTGGGAAAATTGAATTGGGCAAGTCAGATTTATGCAGGGATTAAAG

		TAAGGCAATTATGTAACTTCTTAGGGGAACCAAAGCACTAACAGA AGTAGTACCACTAACAGAAGAAGCAGAGCTAGAACTGGCAGAAAA CAGGGAGATTCTAAAAGAACCGGTACATGGAGTGTATTATGACCCA TCAAAAGACTTAATAGCAGAAATACAGAAGCAGGGGCAAGGCCAAT GGACATATCAAATTTATCAAGAGCCATTTAAAAATCTGAAAACAGG AAAATATGCAAGAATGAAGGGTGCCACACTAATGATGTGAAACAA TTAACAGAGGCAGTACAAAAAATAGCCACAGAAAGCATAGTAATAT GGGGAAGACTCCTAAATTTAAATTACCCATACAAAAGGAAACATG GGAAGCATGGTGGACAGAGTATTGGCAAGCCACCTGGATTCTGAG TGGGAGTTTGTCAATACCCCTCCCTTAGTGAAGTTATGGTACCAGTT AGAGAAAGAACCCATAATAGGAGCAGAACTTTCTATGTAGATGGG GCAGCCAATAGGGAACTAAATTAGGAAAAGCAGGATATGTAAGT ACAGAGGAAGACAAAAAGTTGTCCCCCTAACGGACACAACAAATCA GAAGACTGAGTTACAAGCAATTCATCTAGCTTTGCAGGATTCGGGAT TAGAAGTAAACATAGTGACAGACTACAATATGCATTGGGAATCAT TCAAGCACAAACCAGATAAGAGTGAATCAGAGTTAGTCAGTCAAATA ATAGAGCAGTTAATAAAAAAGGAAAAAGTCTACCTGGCATGGGTAC CAGCACACAAAGGAATTGGAGGAAATGAACAAGTAGATGGGTTGGT CAGTGCTGGAATCAGGAAAGTACTA
26	HIV Int	TTTTTAGATGGAATAGATAAGGCCCAAGAAGAACATGAGAAATATC ACAGTAATTGGAGAGCAATGGCTAGTGATTTAACCTACCACCTGTA GTAGCAAAAGAAATAGTAGCCAGCTGTGATAAATGTCAGCTAAAAG GGGAAGCCATGCATGGACAAGTAGACTGTAGCCCAGGAATATGGCA GCTAGATTGTACACATTTAGAAGGAAAAGTTATCTTGGTAGCAGTTC ATGTAGCCAGTGGATATATAGAAGCAGAAGTAATTCCAGCAGAGAC AGGGCAAGAAACAGCATACTTCCTCTTAAATTAGCAGGAAGATGG CCAGTAAAAACAGTACATACAGACAATGGCAGCAATTTACCAGTA CTACAGTTAAGGCCGCCTGTTGGTGGGCGGGGATCAAGCAGGAATT TGGCATTCCCTACAATCCCCAAAGTCAAGGAGTAATAGAATCTATGA ATAAAGAATTAAAGAAAATTATAGGACAGGTAAGAGATCAGGCTGA ACATCTTAAGACAGCAGTACAAATGGCAGTATTCATCCACAATTTTA AAAGAAAAGGGGGGATTGGGGGTACAGTGCAGGGGAAAGAATAG TAGACATAATAGCAACAGACATACAACTAAAGAATTACAAAAACA AATTACAAAAATTCAAAATTTTCGGGTTTATTACAGGGACAGCAGA GATCCAGTTTGGAAAGGACCAGCAAAGCTCCTCTGGAAAGGTGAAG GGGCAGTAGTAATACAAGATAATAGTGACATAAAAGTAGTGCCAAG AAGAAAAGCAAAGATCATCAGGGATTATGGAAAACAGATGGCAGG TGATGATTGTGTGGCAAGTAGACAGGATGAGGATTAA
27	HIV RRE	AGGAGCTTTGTTCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATGG GCGCAGCGTCAATGACGCTGACGGTACAGGCCAGACAATTATTGTC TGGTATAGTGCAGCAGCAGAACAAATTTGCTGAGGGCTATTGAGGCG CAACAGCATCTGTTGCAATCACAGTCTGGGGCATCAAGCAGCTCCA GGCAAGAATCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTC CT
28	HIV Rev	ATGGCAGGAAGAAGCGGAGACAGCGACGAAGAAGTCTCTCAAGGCA GTCAGACTCATCAAGTTTCTCTATCAAAGCAACCCACCTCCCAATCC CGAGGGGACCCGACAGGCCCGAAGGAATAGAAGAAGAAGGTGGAG AGAGAGACAGAGACAGATCCATTTCGATTAGTGAACGGATCCTTAGC ACTTATCTGGGACGATCTGCGGAGCCTGTGCCTCTTCAGTACTACCAC GCTTGAGAGACTTACTCTTGATTGTAACGAGGATTGTGGAACCTCTG GGACGCAGGGGGTGGGAAGCCCTCAAATATTGGTGGAATCTCCTAC AATATTGGAGTCAGGAGCTAAAGAATAG
29	CMV Promoter	ACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCAT TAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTA

		AATGGCCCCGCTGGCTGACCGCCCCAACGACCCCCGCCCATTTGACGTC AATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATT GACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTA CATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGA CGGTAAATGGCCCCGCTGGCATTATGCCCAGTACATGACCTTATGGG ACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCA TGGTGATGCGGTTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTT GACTCACGGGGATTTC AAGTCTCCACCCCATTTGACGTCAATGGGAG TTTTTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACA ACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGA GGTCTATATAAGCAGAGCTCTCTGGCTAACTAGAGAACCCACTGCTT ACTG
30	VSV-G Envelope Glycoprotein	ATGAAGTGCCTTTTGTACTTAGCCTTTTTTATTCATTGGGGTGAATTGC AAGTTCACCATAGTTTTTCCACACAACCAAAAAGGAAACTGGAAAA ATGTTCCCTTCTAATTACCATTATTGCCCGTCAAGCTCAGATTTAAATT GGCATAATGACTTAATAGGCACAGCCTTACAAGTCAAAATGCCCAA GAGTCACAAGGCTATTCAAGCAGACGGTTGGATGTGTTCATGCTTCCA AATGGGTCACTACTTGTGATTTCGCTGGTATGGACCGAAGTATATA ACACATTCCATCCGATCCTTCACTCCATCTGTAGAACAATGCAAGGA AAGCATTGAACAAACGAAACAAGGAACCTGGCTGAATCCAGGCTTC CCTCCTCAAAGTTGTGGATATGCAACTGTGACGGATGCCGAAGCAGT GATTGTCCAGGTGACTCCTCACCATGTGCTGGTTGATGAATACACAG GAGAATGGGTTGATTACAGTTCATCAACGGAAAATGCAGCAATTA CATATGCCCCACTGTCCATAACTCTACAACCTGGCATTCTGACTATA AGGTCAAAGGGCTATGTGATTCTAACCTCATTTCATGGACATCACC TTCTTCTCAGAGGACGGAGAGCTATCATCCCTGGGAAAGGAGGGCA CAGGGTTCAGAAGTAACTACTTTGCTTATGAACTGGAGGCAAGGC CTGCAAAATGCAATACTGCAAGCATTGGGGAGTCAGACTCCCATCA GGTGTCTGGTTCGAGATGGCTGATAAGGATCTCTTTGCTGCAGCCAG ATTCCCTGAATGCCCAGAAGGGTCAAGTATCTCTGCTCCATCTCAGA CCTCAGTGGATGTAAGTCTAATTCAGGACGTTGAGAGGATCTTGGAT TATTCCCTCTGCCAAGAAACCTGGAGCAAAATCAGAGCGGGTCTTCC AATCTCTCCAGTGGATCTCAGCTATCTTGCTCCTAAAAACCCAGGAA CCGGTCTGCTTTTACCATAATCAATGGTACCCTAAAATACTTTGAG ACCAGATACATCAGAGTCGATATTGCTGCTCCAATCCTCTCAAGAAT GGTCGGAATGATCAGTGGAACCTACCACAGAAAGGGAACCTGTGGGAT GACTGGGCACCATATGAAGACGTGGAAATTGGACCCAATGGAGTTC TGAGGACCAGTTCAGGATATAAGTTTCCTTTATACATGATTGGACAT GGTATGTTGGACTCCGATCTTCATCTTAGCTCAAAGGCTCAGGTGTT CGAACATCCTCACATTCAAGACGCTGCTTCGCAACTCCTGATGATG AGAGTTTATTTTTTGGTGATACTGGGCTATCCAAAATCCAATCGAG CTTGTAGAAGGTTGGTTCAGTAGTTGGAAAAGCTCTATTGCCTCTTT TTTCTTTATCATAGGGTTAATCATTGGACTATTCTTGGTTCTCCGAGT TGGTATCCATCTTTGCATTAAATTAAGCACACCAAGAAAAGACAG ATTTATACAGACATAGAGATGAACCGACTTGGAAGTGA
31	Left ITR	CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGC AAAGCCCGGGCGTCGGGCGACCTTTGGTCGCCCCGGCCTCAGTGAGC GAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTT CCT
32	Poly A	GACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGGCCCTCCCCCGT GCCTTCCTTGACCCTGGAAGGTGCCACTCCCCTGTCCTTTCCTAATA AAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTTC TGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAG ACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGC

33	Right ITR	AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGC TCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCCGACGCCCGGGCT TTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGCTGCCTGCA GG
34	E2A	TTAAAAGTCGAAGGGGTTCTCGCGCTCGTCGTTGTGCGCCGCGCTGG GGAGGGCCACGTTGCGGAACCTGGTACTTGGGCTGCCACTTGAACCTC GGGGATCACCAGTTTGGGCACTGGGGTCTCGGGGAAGGTCCTCGCTC CACATGCGCCGGCTCATCTGCAGGGCGCCCAGCATGTCAGGCGCGG AGATCTTGAAATCGCAGTTGGGGCCGGTGCTCTGCGCGCGCGAGTTG CGGTACACTGGGTTGCAGCACTGGAACACCATCAGACTGGGGTACT TCACACTAGCCAGCACGCTCTTGTCGCTGATCTGATCCTTGTCAGG TCCTCGGC GTTGCTCAGGCCGAACGGGGTCATCTTGACACAGCTGGCG GCCCAGGAAGGGCACGCTCTGAGGCTTGTTGGTTACACTCGCAGTGC ACGGGCATCAGCATCATCCCCGCGCCGCGCTGCATATTCGGGTAGA GGGCCTTGACGAAGGCCGCGATCTGCTTGAAAGCTTGCTGGGCCTTG GCCCCCTCGCTGAAAAACAGGCCGCGAGCTCTTCCCGCTGAACTGATT ATTCCCGCACCCGGCATCATGGACGCAGCAGCGCGCTCATGGCTG GTCAGTTGCACCACGCTCCGTCCCCAGCGGTTCTGGGTACCTTGGC CTTGCTGGGTTGCTCCTTCAGCGCACGCTGCCCGTTCTCACTGGTCAC ATCCATCTCCACCACGTGGTCCTTGTTGGATCATCACCGTCCCATGCA GACACTTGAGCTGGCCTTCCACCTCGGTGCAGCCGTGGTCCCACAGG GCACTGCCGGTGCACTCCCAGTTCTTGTCGCGGATCCCGCTGTGGCT GAAGATGTAACCTTGCAACAGGCGACCCATGATGGTGCTAAAGCTC TTCTGGGTGGTGAAGGTCAGTTGCAGACCGCGGGCCTCCTCGTTTCAT CCAGGTCTGGCACATCTTTTGGAAGATCTCGGTCTGCTCGGGCATGA GCTTGTAAGCATCGCGCAGGCCGCTGTCGACGCGGTAGCGTTCATC AGCACATTCATGGTATCCATGCCCTTCTCCAGGACGAGACCAGAGG CAGACTCAGGGGGTTGCGCACGTTCAAGGACACCGGGGGTCCGCGGC TCGACGATGCGTTTTCCGTCTTGCCTTCCTTCAACAGAACCGGCGG CTGGCTGAATCCCACTCCACGATCACGGCTTCTTCTGGGGCATCT CTTCGTCTGGGTCTACCTTGGTCACATGCTTGGTCTTTCTGGCTTGCT TCTTTTTTGGAGGGCTGTCCACGGGGACCACGTCTCTCCTCGGAAGAC CCGGATCCCACCCGCTGATACTTTCGGCGCTTGGTTGGCAGAGGAGG TGGCGGCGAGGGGCTCCTCTCCTGCTCCGGCGGATAGCGCGCTGAA CCGTGGCCCCGGGGCGGAGTGGCTCTCGGTCCATGAACCGGCGCA CGTCCTGACTGCCGCCGGCCAT
35	E4	TCATGTATCTTTATTGATTTTTACACCAGCACGGGTAGTCAGTCTCCC ACCACCAGCCCATTTCACAGTGTAACAATTCTCTCAGCACGGGTGG CCTTAAATAGGGCAATATTCTGATTAGTGCGGGAACCTGGACTTGGGG TCTATAATCCACACAGTTTCTGGCGAGCCAAACGGGGGTCCGTGAT TGAGATGAAGCCGTCTCTGAAAAGTCATCCAAGCGAGCCTCACAG TCCAAGGTCACAGTATTATGATAATCTGCATGATCACAATCGGGCAA CAGGGGATGTTGTTCAAGTCAGTGAAGCCCTGGTTTCCTCATCAGATC GTGGTAAACGGGGCCTGCGATATGGATGATGGCGGAGCGAGCTGGA TTGAATCTCGGTTTGAT
36	VA RNA	AGCGGGCACTCTCCGTGGTCTGGTGGATAAATTCGCAAGGGTATCA TGCGCGACGACCGGGGTTTCGAGCCCCGTATCCGGCCGTCCGCCGTG ATCCATGCGGTTACCGCCCGCGTGTGCAACCCAGGTGTGCGACGTCA GACAACGGGGGAGTGCTCCTT
37	AAV2 Rep	ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACACTCTCTC TGAAGGAATAAGACAGTGGTGGAAGCTCAAACCTGGCCCACCACCA CCAAAGCCCGCAGAGCGGCATAAGGACGACAGCAGGGGTCTTGTC TTCTGGGTACAAGTACCTCGGACCCTTCAACGGACTCGACAAGGG AGAGCCGGTCAACGAGGCAGACGCCGCGGCCCTCGAGCACGACAAA

		GCCTACGACCGGCAGCTCGACAGCGGAGACAACCCGTACCTCAAGT ACAACCACGCCGACGCGGAGTTTCAGGAGCGCCTTAAAGAAGATAC GTCTTTTGGGGGCAACCTCGGACGAGCAGTCTTCCAGGCGAAAAAG AGGGTTCTTGAACCTCTGGGCCTGGTTGAGGAACCTGTTAAGACGGC TCCGGGAAAAAAGAGGCCGGTAGAGCACTCTCCTGTGGAGCCAGAC TCCTCCTCGGGAACCGGAAAGGCGGGCCAGCAGCCTGCAAGAAAAA GATTGAATTTTGGTCAGACTGGAGACGCAGACTCAGTACCTGACCCC CAGCCTCTCGGACAGCCACCAGCAGCCCCCTCTGGTCTGGGAATAA TACGATGGCTACAGGCAGTGGCGCACCAATGGCAGACAATAACGAG GGCGCCGACGGAGTGGGTAATTCTCGGGAATTGGCATTGCGATT CCACATGGATGGGCGACAGAGTCATCACCACCAGCACCCGAACCTG GGCCCTGCCACCTACAACAACCACCTCTACAAACAAATTTCCAGCC AATCAGGAGCCTCGAACGACAATCACTACTTTGGCTACAGCACCCCT TGGGGGTATTTGACTTCAACAGATTCCACTGCCACTTTTCACCACG TGACTGGCAAAGACTCATCAACAACAACCTGGGGATTCCGACCCAAG AGACTCAACTTCAAGCTCTTTAACATTCAAGTCAAAGAGGTACGCA GAATGACGGTACGACGACGATTGCCAATAACCTTACCAGCACGGTT CAGGTGTTTACTGACTCGGAGTACCAGTCCCCGTACGTCCTCGGCTC GGCGCATCAAGGATGCCTCCCGCCGTTCCCGAGCAGACGTCTTCATGG TGCCACAGTATGGATACCTCACCTGAACAACGGGAGTCAGGCAGT AGGACGCTCTTCATTTTACTGCCTGGAGTACTTTCCTTCTCAGATGCT GCGTACCGGAAACAACCTTTACCTTCAGCTACACTTTTGAGGACGTTT CTTTCCACAGCAGCTACGCTCACAGCCAGAGTCTGGACCGTCTCATG AATCCTCTCATCGACCAGTACCTGTATTACTTGAGCAGAACAAACAC TCCAAGTGGAACCACCACGCAGTCAAGGCTTCAGTTTCTCAGGCCG GAGCGAGTGACATTCCGGGACCAGTCTAGGAACTGGCTTCTTGACC CTGTTACCGCCAGCAGCGAGTATCAAAGACATCTGCGGATAACAAC AACAGTGAATACTCGTGGACTGGAGCTACCAAGTACCACCTCAATG GCAGAGACTCTCTGGTGAATCCGGGGCCCGCCATGGCAAGCCACAA GGACGATGAAGAAAAGTTTTTCTCAGAGCGGGGTTCTCATCTTTG GGAAGCAAGGCTCAGAGAAAAACAATGTGGACATTGAAAAGGTCAT GATTACAGACGAAGAGGAAATCAGGACAACCAATCCCGTGGCTACG GAGCAGTATGGTTCTGTATCTACCAACCTCCAGAGAGGCAACAGAC AAGCAGCTACCGCAGATGTCAACACACAAGGCGTTCTTCCAGGCAT GGTCTGGCAGGACAGAGATGTGTACCTTCAGGGGCCCATCTGGGCA AAGATTCCACACACGGACGGACATTTTACCCCTCTCCCCTCATGGG TGGATTCCGACTTAAACACCCTCCTCCACAGATTCTCATCAAGAACA CCCCGGTACCTGCGAATCCTTCGACCACCTTCAGTGCGGCAAAAGTTT GCTTCCTTCATCACACAGTACTCCACGGGACAGGTCAGCGTGGAGAT CGAGTGGGAGCTGCAGAAGGAAAACAGCAAACGCTGGAATCCCGA AATTCAGTACACTTCCAACATAACAAGTCTGTAAATGTGGACTTTA CTGTGGACACTAATGGCGTGTATTCAGAGCCTCGCCCCATTGGCACC AGATACCTGACTCGTAATCTGTAA
38	AAV2 Cap	ATGCCGGGGTTTTACGAGATTGTGATTAAGGTCCCCAGCGACCTTGA CGAGCATCTGCCCGGCATTTCTGACAGCTTTGTGAAGTGGGTGGCCG AGAAGGAATGGGAGTTGCCGCCAGATTCTGACATGGATCTGAATCT GATTGAGCAGGCACCCCTGACCGTGGCCGAGAAGCTGCAGCGCGAC TTTCTGACGGAATGGCGCCGTGTGAGTAAGGCCCCGGAGGCCCTTTT CTTTGTGCAATTTGAGAAGGGAGAGAGCTACTTCCACATGCACGTGC TCGTGGAAACCACCGGGGTGAAATCCATGGTTTTGGGACGTTTCTG AGTCAGATTTCGCGAAAACTGATTCAGAGAATTTACCGCGGGATCG AGCCGACTTTGCCAAACTGGTTCGCGGTCACAAAGACCAGAAATGG CGCCGGAGGCGGGAACAAGGTGGTGGATGAGTGCTACATCCCCAAT TACTTGCTCCCCAAAACCCAGCCTGAGCTCCAGTGGGCGTGGACTAA TATGGAACAGTATTTAAGCGCCTGTTTGAATCTCACGGAGCGTAAAC

		GGTTGGTGGCGCAGCATCTGACGCACGTGTGCGCAGACGCAGGAGCA GAACAAAGAGAATCAGAATCCCAATTCTGATGCGCCGGTGATCAGA TCAAAAACCTTCAGCCAGGTACATGGAGCTGGTCGGGTGGCTCGTGG ACAAGGGGATTACCTCGGAGAAGCAGTGGATCCAGGAGGACCAGGC CTCATACATCTCCTTCAATGCGGCCTCCAACCTCGCGGTCCCAAATCA AGGCTGCCTTGGACAATGCGGGAAAGATTATGAGCCTGACTAAAC CGCCCCGACTACCTGGTGGGCCAGCAGCCCGTGGAGGACATTTCC AGCAATCGGATTTATAAAATTTTGAACTAAACGGGTACGATCCCCA ATATGCGGCTTCCGTCTTTCTGGGATGGGCCACGAAAAAGTTCGGCA AGAGGAACACCATCTGGCTGTTTGGGCCTGCAACTACCGGGAAGAC CAACATCGCGGAGGCCATAGCCCACTGTGCCCTTCTACGGGTGCG TAACTGGACCAATGAGAACTTTCCCTTCAACGACTGTGTGACAAG ATGGTGATCTGGTGGGAGGAGGGGAAGATGACCGCCAAGGTGCGTGG AGTCGGCCAAAGCCATTCTCGGAGGAAGCAAGGTGCGCGTGGACCA GAAATGCAAGTCCTCGGCCAGATAGACCCGACTCCCGTGATCGTC ACCTCCAACACCAACATGTGCGCCGTGATTGACGGGAACCTCAACGA CCTTCGAACACCAGCAGCCGTTGCAAGACCGGATGTTCAAATTTGAA CTCACCCGCCGTCTGGATCATGACTTTGGGAAGGTCACCAAGCAGG AAGTCAAAGACTTTTTCCGGTGGGCAAAGGATCACGTGGTTGAGGT GGAGCATGAATTCTACGTCAAAAAGGGTGGAGCCAAGAAAAGACCC GCCCCAGTGACGCAGATATAAGTGAGCCCAAACGGGTGCGCGAGT CAGTTGCGCAGCCATCGACGTCAGACGCGGAAGCTTCGATCAACTA CGCAGACAGGTACCAAAACAAATGTTCTCGTCACGTGGGCATGAAT CTGATGCTGTTTCCCTGCAGACAATGCGAGAGAATGAATCAGAATTC AAATATCTGCTTCACTCACGGACAGAAAGACTGTTTAGAGTGCTTTC CCGTGTCAGAATCTCAACCCGTTTCTGTCTGTCAAAAAGGCGTATCAG AACTGTGCTACATTCATCATATCATGGGAAAGGTGCCAGACGCTTG CACTGCCTGCGATCTGGTCAATGTGGATTTGGATGACTGCATCTTTG ACAATAA
39	AAV8 Cap	ATGGCTGCAGGCGGTGGCGCACCAATGGCAGACAATAACGAAGGCG CCGACGGAGTGGGTAGTTCCTCGGGAATTGGCATTGCGATTCCACA TGGCTGGGGCAGCAGAGTCATCACCACCAGCACCCGAACCTGGGCC TGCCACCTACAACAACCACCTCTACAAGCAAATCTCCAACGGGAC ATCGGGAGGAGCCACCAACGACAACACCTACTTCGGCTACAGCACC CCCTGGGGGTATTTTGACTTTAACAGATTCCACTGCCACTTTTACCA CGTGACTGGCAGCGACTCATCAACAACAACCTGGGGATTCCGGCCCA AGAGACTCAGCTTCAAGCTCTTCAACATCCAGGTCAAGGAGGTCAC GCAGAAATGAAGGCACCAAGACCATCGCCAATAACCTCACCAGCACC ATCCAGGTGTTTACGGACTCGGAGTACCAGCTGCCGTACGTTCTCGG CTCTGCCCACCAGGGCTGCCTGCCTCCGTTCCCGGCGGACGTGTTCA TGATTCCCCAGTACGGCTACCTAACACTCAACAACGGTAGTCAGGCC GTGGGACGCTCCTCCTTCTACTGCCTGGAATACTTTCTTTCGAGAT GCTGAGAACCAGGCAACAACCTCCAGTTTACTTACACCTTCGAGGACG TGCTTTTCCACAGCAGCTACGCCACAGCCAGAGCTTGGACCGGCTG ATGAATCCTCTGATTGACCAGTACCTGTACTACTTGTCTCGGACTCA AACAACAGGAGGCACGGCAAATACGCAGACTCTGGGCTTCAGCCAA GGTGGGCCTAATAAATGGCCAATCAGGCAAAGAACTGGCTGCCAG GACCCTGTTACCGCCAACAACGCGTCTCAACGACAACCGGGCAAAA CAACAATAGCAACTTTGCCTGGACTGCTGGGACCAAAATACCATCTGA ATGGAAGAAATTCAATTGGCTAATCCTGGCATCGCTATGGCAACACAC AAAGACGACGAGGAGCGTTTTTTTTCCAGTAACGGGATCCTGATTTT TGGCAAACAAAATGCTGCCAGAGACAATGCGGATTACAGCGATGTC ATGCTCACCAGCGAGGAAGAAATCAAAACCACTAACCTGTGGCTA CAGAGGAATACGGTATCGTGGCAGATAACTTGCAGCAGCAAAACAC GGCTCCTCAAATTGGAAGTGTCAACAGCCAGGGGGCCTTACCCGGT

		ATGGTCTGGCAGAACCGGGACGTGTACCTGCAGGGTCCCATCTGGG CCAAGATTCTTCACACGGACGGCAACTTCCACCCGTCTCCGCTGATG GGCGGCTTTGGCCTGAAACATCCTCCGCCTCAGATCCTGATCAAGAA CACGCCTGTACCTGCGGATCCTCCGACCACCTTCAACCAGTCAAAGC TGAACCTTTTCATCACGCAATACAGCACCGGACAGGTGACGCTGGA AATTGAATGGGAGCTGCAGAAGGAAAACAGCAAGCGCTGGAACCCC GAGATCCAGTACACCTCCAACCTACTACAAATCTACAAGTGTGGACTT TGCTGTTAATACAGAAGGCGTGTACTCTGAACCCCGCCCCATTGGCA CCCGTTACCTCACCCGTAATCTGTAA
40	AAV DJ Cap	ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACACTCTCTC TGAAGGAATAAGACAGTGGTGGAAAGCTCAAACCTGGCCCACCACCA CCAAAGCCCGCAGAGCGGCATAAGGACGACAGCAGGGGTCTTGTGC TTCTTGGGTACAAGTACCTCGGACCCTTCAACGGACTCGACAAGGG AGAGCCGGTCAACGAGGCAGACGCCGCGGCCCTCGAGCACGACAAA GCCTACGACCGGCAGCTCGACAGCGGAGACAACCCGTACCTCAAGT ACAACCACGCCGACGCCGAGTTCCAGGAGCGGCTCAAAGAAGATAC GTCTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAAAAG AGGCTTCTTGAACCTCTTGGTCTGGTTGAGGAAGCGGCTAAGACGGC TCCTGGAAAGAAGAGGCCTGTAGAGCACTCTCCTGTGGAGCCAGAC TCCTCCTCGGGAACCGGAAAGGCGGGCCAGCAGCCTGCAAGAAAAA GATTGAATTTTGGTCAGACTGGAGACGCAGACTCAGTCCCAGACCCT CAACCAATCGGAGAACCCTCCCGCAGCCCCCTCAGGTGTGGGATCTCT TACAATGGCTGCAGGCGGTGGCGCACCAATGGCAGACAATAACGAG GGCGCCGACGGAGTGGGTAAATCCTCGGGAAATTGGCATTGCGATT CCACATGGATGGGCGACAGAGTCATCACCACCAGCACCCGAACCTG GGCCCTGCCCACCTACAACAACCACCTCTACAAGCAAATCTCCAACA GCACATCTGGAGGATCTTCAAATGACAACGCCTACTTCGGCTACAGC ACCCCCTGGGGGTATTTTGAAGTTTAAACAGATTCCACTGCCACTTTTCA CCACGTGACTGGCAGCGACTCATCAACAACAACCTGGGGATTCCGGC CCAAGAGACTCAGCTTCAAGCTCTTCAACATCCAGGTCAAGGAGGT CACGCAGAATGAAGGCACCAAGACCATCGCCAATAACCTCACCAGC ACCATCCAGTGTTTACGGACTCGGAGTACCAGCTGCCGTACGTTCT CGGCTCTGCCCACCAGGGCTGCCTGCCTCCGTTCCCGGCGGACGTGT TCATGATTCCCCAGTACGGCTACCTAACACTCAACAACGGTAGTCAG GCCGTGGGACGCTCCTCCTTCTACTGCCTGGAATACTTTCTTCGCA GATGCTGAGAACCGGCAACAACCTCCAGTTTACTTACACCTTCGAGG ACGTGCCTTTCCACAGCAGCTACGCCCACAGCCAGAGCTTGGACCG GCTGATGAATCCTCTGATTGACCAGTACCTGTACTACTTGTCTCGGA CTCAAACAACAGGAGGCACGACAAATACGCAGACTCTGGGCTTCAG CCAAGGTGGGCCTAATACAATGGCCAATCAGGCAAAGAAGTGGCTG CCAGGACCCTGTTACCGCCAGCAGCGAGTATCAAAGACATCTGCGG ATAACAACAACAGTGAATACTCGTGGACTGGAGCTACCAAGTACCA CCTCAATGGCAGAGACTCTCTGGTGAATCCGGGGCCCGGCCATGGCA AGCCACAAGGACGATGAAGAAAAGTTTTTCTCAGAGCGGGGTTTC TCATCTTTGGGAAGCAAGGCTCAGAGAAAACAAATGTGGACATTGA AAAGGTCATGATTACAGACGAAGAGGAAATCAGGACAACCAATCCC GTGGCTACGGAGCAGTATGGTTCTGTATCTACCAACCTCCAGAGAGG CAACAGACAAGCAGCTACCGCAGATGTCAACACACAAGGCGTTCTT CCAGGCATGGTCTGGCAGGACAGAGATGTGTACCTTCAGGGGCCCA TCTGGGCAAGATTCCACACACGGACGGACATTTTACCCCTCTCCC CTCATGGGTGGATTTCGGAATTAAACACCCCTCCGCTCAGATCCTGAT CAAGAACACGCCTGTACCTGCGGATCCTCCGACCACCTTCAACCAGT CAAAGCTGAACTCTTTCATCACCCAGTATTCTACTGGCCAAGTCAGC GTGGAGATCGAGTGGGAGCTGCAGAAGGAAAACAGCAAGCGCTGG AACCCCGAGATCCAGTACACCTCCAACCTACTACAAATCTACAAGTGT

		GGACTTTGCTGTTAATACAGAAGGCGTGTACTCTGAACCCCGCCCCA TTGGCACCCGTTACCTCACCCGTAATCTGTAA
41	Chicken beta actin intron	GGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGCGCCGCCTC GCGCCGCCCCGCCCCGGCTCTGACTGACCGCGTTACTCCCACAGGTGA GCGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGTT TAATGACGGCTCGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTAAAGG GCTCCGGGAGGGCCCTTTGTGCGGGGGGGAGCGGCTCGGGGGGTGC GTGCGTGTGTGTGTGCGTGGGGAGCGCCGCGTGCGGCCCGCGCTGC CCGGCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGTGCGCT CCGCGTGTGCGCGAGGGGAGCGCGGCCGGGGCGGTGCCCCGCGGT GCGGGGGGGCTGCGAGGGGAACAAGGCTGCGTGCGGGGTGTGTGC GTGGGGGGGTGAGCAGGGGTGTGGGCGCGCGGTGCGGGCTGTAAC CCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGCACGGCCCCGCTTC GGGTGCGGGGCTCCGTGCGGGGCGTGGCGCGGGGCTCGCCGTGCCG GGCGGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCCG CCTCGGGCCGGGAGGGCTCGGGGAGGGGCGCGGCGGCCCGGA GCGCCGGCGGCTGTGAGGCGCGGCGAGCCGAGCCATTGCCTTTT ATGGTAATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCT GGCGGAGCCGAAATCTGGGAGGCGCCGCCGCACCCCTCTAGCGGG CGCGGGCGAAGCGGTGCGGCGCCGCGAGGAAGGAAATGGGCGGGG AGGGCCTTCGTGCGTCCGCGCGCCGCGTCCCTTCTCCATCTCCAG CCTCGGGGCTGCCGAGGGGGACGGCTGCCTTCGGGGGGGACGGGG CAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGG
42	Rabbit beta globin poly A	AGATCTTTTTCCCTCTGCCAAAAATTATGGGGACATCATGAAGCCCC TTGAGCATCTGACTTCTGGCTAATAAAGGAAATTTATTTTCATTGCA ATAGTGTGTTGGAATTTTTGTGTCTCTCACTCGGAAGGACATATGG GAGGGCAAATCATTTAAACATCAGAATGAGTATTTGGTTTAGAGTT TGGCAACATATGCCATATGCTGGCTGCCATGAACAAAGGTGGCTAT AAAGAGGTCATCAGTATATGAAACAGCCCCCTGCTGTCCATTCTTA TTCCATAGAAAAGCCTTGACTTGAGGTAGATTTTTTTTTATATTTTGT TTTGTGTTATTTTTTTCTTTAACATCCCTAAAATTTTCTTACATGTTT TACTAGCCAGATTTTTCTCTCTCTGACTACTCCAGTCATAGCTG TCCCTCTTCTTATGAAGATC
43	Primer	TAAGCAGAATTCATGAATTTGCCAGGAAGAT
44	Primer	CCATACAATGAATGGACACTAGGCGGCCGCACGAAT
45	Gag, Pol, Integrase fragment	GAATTCATGAATTTGCCAGGAAGATGGAAACCAAAAATGATAGGGG GAATTGGAGGTTTTATCAAAGTAAGACAGTATGATCAGATACTCATA GAAATCTGCGGACATAAAGCTATAGGTACAGTATTAGTAGGACCTA CACCTGTCAACATAATTGGAAGAAATCTGTTGACTCAGATTGGCTGC ACTTTAAATTTTCCCATTAGTCTATTGAGACTGTACCAGTAAAATT AAAGCCAGGAATGGATGGCCCAAAAGTTAAACAATGGCCATTGACA GAAGAAAAAATAAAAGCATTAGTAGAAATTTGTACAGAAATGGAAA AGGAAGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATACAATAC TCCAGTATTTGCCATAAAGAAAAAAGACAGTACTAAATGGAGAAAA TTAGTAGATTTAGAGAACTTAATAAGAGAACTCAAGATTTCTGGGA AGTTCAATTAGGAATACCACATCCTGCAGGGTTAAACAGAAAAAA TCAGTAACAGTACTGGATGTGGGCGATGCATATTTTCAGTTCCCTT AGATAAAGACTTCAGGAAGTATACTGCATTTACCATACCTAGTATAA ACAATGAGACACCAGGGATTAGATATCAGTACAATGTGCTTCCACA GGGATGGAAAGGATCACCAGCAATATTCCAGTGTAGCATGACAAAA ATCTTAGAGCCTTTTAGAAAAACAAAATCCAGACATAGTCATCTATCA ATACATGGATGATTTGTATGTAGGATCTGACTTAGAAATAGGGCAGC

		ATAGAACAAAAATAGAGGAACTGAGACAACATCTGTTGAGGTGGGG ATTTACCACACCAGACAAAAAACATCAGAAAGAACCTCCATTCTTT GGATGGGTTATGAACTCCATCCTGATAAATGGACAGTACAGCCTATA GTGCTGCCAGAAAAGGACAGCTGGACTGTCAATGACATACAGAAAT TAGTGGGAAAATTGAATTGGGCAAGTCAGATTTATGCAGGGATTAA AGTAAGGCAATTATGTAACTTCTTAGGGGAACCAAAGCACTAACA GAAGTAGTACCCTAACAGAAGAAGCAGAGCTAGAACTGGCAGAA AACAGGGAGATTCTAAAAGAACCGGTACATGGAGTGTATTATGACC CATCAAAAGACTTAATAGCAGAAATACAGAAGCAGGGGCAAGGCC AATGGACATATCAAATTTATCAAGAGCCATTTAAAAATCTGAAAAC AGGAAAGTATGCAAGAATGAAGGGTGCCCACTAATGATGTGAAA CAATTAACAGAGGCAGTACAAAAAATAGCCACAGAAAGCATAGTAA TATGGGGAAAGACTCCTAAATTTAAATTACCCATACAAAAGGAAAC ATGGGAAGCATGGTGGACAGAGTATTGGCAAGCCACCTGGATTCCCT GAGTGGGAGTTTGTCAATACCCCTCCCTTAGTGAAGTTATGGTACCA GTTAGAGAAAAGAACCCTAATAGGAGCAGAACTTTCTATGTAGAT GGGGCAGCCAATAGGGAACTAAATTAGGAAAAGCAGGATATGTA ACTGACAGAGGAAGACAAAAAGTTGTCCCCCTAACGGACACAACAA ATCAGAAGACTGAGTTACAAGCAATTCATCTAGCTTTGCAGGATTCTG GGATTAGAAGTAAACATAGTGACAGACTCACAAATATGCATTGGGAA TCATTCAAGCACAAACCAGATAAGAGTGAATCAGAGTTAGTCAGTCA AATAATAGAGCAGTTAATAAAAAAGGAAAAAGTCTACCTGGCATGG GTACCAGCACACAAAGGAATTGGAGGAAATGAACAAGTAGATAAAT TGGTCAGTGCTGGAATCAGGAAAGTACTATTTTTAGATGGAATAGAT AAGGCCCAAGAAGAACATGAGAAATATCACAGTAATTGGAGAGCA ATGGCTAGTGATTTTAACCTACCACCTGTAGTAGCAAAAGAAATAGT AGCCAGCTGTGATAAATGTCAGCTAAAAGGGGAAGCCATGCATGGA CAAGTAGACTGTAGCCCAGGAATATGGCAGCTAGATTGTACACATTT AGAAGGAAAAGTTATCTTGGTAGCAGTTCATGTAGCCAGTGGATAT ATAGAAGCAGAAGTAATTCCAGCAGAGACAGGGCAAGAAACAGCA TACTCTCTTAAAAATTAGCAGGAAGATGGCCAGTAAAAACAGTAC ATACAGACAATGGCAGCAATTTACCAGTACTACAGTTAAGGCCGC CTGTTGGTGGGCGGGGATCAAGCAGGAATTTGGCATTCCCTACAATC CCCAAAGTCAAGGAGTAATAGAATCTATGAATAAAGAATTAAAGAA AATTATAGGACAGGTAAGAGATCAGGCTGAACATCTTAAGACAGCA GTACAAATGGCAGTATTCATCCACAATTTTAAAGAAAAGGGGGGA TTGGGGGGTACAGTGCAGGGGAAAGAATAGTAGACATAATAGCAAC AGACATACAACTAAAGAATTACAAAAACAAATTACAAAAATTCAA AATTTTCGGGTTTATTACAGGGACAGCAGAGATCCAGTTTGGAAAG GACCAGCAAAGCTCCTCTGGAAAGGTGAAGGGGCAGTAGTAATACA AGATAATAGTGACATAAAAGTAGTGCCAAGAAGAAAAGCAAAGAT CATCAGGGATTATGGAACAGATGGCAGGTGATGATTGTGTGGCA AGTAGACAGGATGAGGATTAA
46	DNA Fragment	TCTAGAATGGCAGGAAGAAGCGGAGACAGCGACGAAGAGCTCATC AGAACAGTCAGACTCATCAAGCTTCTCTATCAAAGCAACCCACCTCC CAATCCCGAGGGGACCCGACAGGCCCCGAAGGAATAGAAGAAGAAG GTGGAGAGAGAGACAGAGACAGATCCATTTCGATTAGTGAACGGATC CTTGGCACTTATCTGGGACGATCTGCGGAGCCTGTGCCTCTTCAGCT ACCACCGCTTGAGAGACTTACTCTTGATTGTAACGAGGATTGTGGAA CTTCTGGGACGCAGGGGGTGGGAAGCCCTCAAATATTGGTGGGAATC TCCTACAATATTGGAGTCAGGAGCTAAAGAATAGAGGAGCTTTGTTC CTTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTCAA TGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAG CAGCAGAACAATTTGCTGAGGGCTATTGAGGCGCAACAGCATCTGT TGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCT

		GGCTGTGGAAAGATACCTAAAGGATCAACAGCTCCTAGATCTTTTTTC CCTCTGCCAAAAATTATGGGGACATCATGAAGCCCCTTGAGCATCTG ACTTCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTTG GAATTTTTTGTGTCTCTCACTCGGAAGGACATATGGGAGGGCAAATC ATTTAAAACATCAGAATGAGTATTTGGTTTAGAGTTTGGCAACATAT GCCATATGCTGGCTGCCATGAACAAAGGTGGCTATAAAGAGGGTCAT CAGTATATGAAACAGCCCCCTGCTGTCCATTCCCTATTCCATAGAAA AGCCTTGACTTGAGGTTAGATTTTTTTTTTATATTTTGTTTTGTGTTATTT TTTTCTTTAACATCCCTAAAATTTTCTTACATGTTTTACTAGCCAGA TTTTCTCTCTCTCCTGACTACTCCAGTCATAGCTGTCCCTCTTCTCT TATGAAGATCCCTCGACCTGCAGCCCAAGCTTGGCGTAATCATGGTC ATAGCTGTTTCTGTGTGAAATTGTTATCCGCTCACAATTCCACACA ACATACGAGCCGGAAGCATAAAGTGTAAGCCTGGGGTGCCATAATG AGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCC AGTCGGGAAACCTGTCTGCCAGCGGATCCGCATCTCAATTAGTCAG CAACCATAGTCCCGCCCCCTAACTCCGCCCATCCCGCCCCCTAACTCCG CCCAGTTCCGCCCATTTCTCCGCCCATGGCTGACTAATTTTTTTTTTATT TATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGT AGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAGCTAACT TGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACA AATTTACAAAATAAAGCATTTTTTTCTACTGCATTCTAGTTGTGGTTTG TCCAAACTCATCAATGTATCTTATCAGCGGCCGCCCGGG
47	DNA fragment	ACGCGTTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTTCATA GCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCG CCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGAC GTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAAT GGGTGGACTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTG TATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATG GCCCCGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCTTA CTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATTGGGTGCA GGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCC ACCCCCAATTTTGTATTTATTTATTTTAAATTTTGTGACGCGAT GGGGGCGGGGGGGGGGGGGGGCGCGGCCAGGCGGGGGGGGGGGGG GCGAGGGGGCGGGGGGGGGGGGAGGCGGAGAGGTGCGGCGGCAGCCA ATCAGAGCGGCGCGCTCCGAAAGTTTCTTTTATGGCGAGGCGGCG GCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCGGGAGT CGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGCGCCGCTCGCGCC GCCCCCCCCGGCTCTGACTGACCGCGTTACTCCACAGGTGAGCGGG CGGGACGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTTAATG ACGGCTCGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTAAAGGGCTCC GGGAGGGCCCTTTGTGCGGGGGGAGCGGCTCGGGGGGTGCGTGCG TGTGTGTGTGCGTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCGGC GGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGTGCGCTCCGCG TGTGCGCGAGGGGAGCGCGGCCGGGGGCGGTGCCCGCGGTGCGGG GGGGTGCAGGGGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGTGGG GGGTGAGCAGGGGGTGTGGGCGCGGCGGTGCGGCTGTAAACCCCC CCTGCACCCCCCTCCCCGAGTTGCTGAGCACGGCCCCGGCTTCGGGTG CGGGGCTCCGTGCGGGGCGTGCGCGGGGCTCGCCGTGCCGGGCGG GGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCGCCCTCG GGCCGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCCGGAGCGCC GGCGGCTGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTATGGTA ATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGGCGG AGCCGAAATCTGGGAGGCGCCGCCGCACCCCCCTTAGCGGGCGCGG GCGAAGCGGTGCGGCGCCGCGCAGGAAGGAAATGGGCGGGGAGGGC CTTCGTGCGTCGCCGCGCCCGCTCCCTTCTCCATCTCCAGCCTCGG

		GGCTGCCGCAGGGGGACGGCTGCCTTCGGGGGGGACGGGGCAGGGC GGGGTTCGGCTTCTGGCGTGTGACCGGCGGGAATTC
48	RSV promoter and HIV Rev	CAATTGCGATGTACGGGCCAGATATACGCGTATCTGAGGGGACTAG GGTGTGTTTAGGCGAAAAGCGGGGCTTCGGTTGTACGCGTTAGGA GTCCCTCAGGATATAGTAGTTTCGCTTTTGCATAGGGAGGGGAAA TGTAGTCTTATGCAATACACTTGTAGTCTTGCAACATGGTAACGATG AGTTAGCAACATGCCTTACAAGGAGAGAAAAAGCACCGTGCATGCC GATTGGTGGAAGTAAGGTGGTACGATCGTGCCTTATTAGGAAGGCA ACAGACAGGTCTGACATGGATTGGACGAACCACTGAATTCGCGATT GCAGAGATAATTGTATTTAAGTGCCTAGCTCGATACAATAAACGCCA TTTGACCATTACACCATTTGGTGTGCACCTCCAAGCTCGAGCTCGTT TAGTGAACCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGAC CTCCATAGAAGACACCGGGACCGATCCAGCCTCCCCTCGAAGCTAG CGATTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAA GAACTCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAA CCCACCTCCCAATCCCGAGGGGACCCGACAGGCCCGAAGGAATAGA AGAAGAAGGTGGAGAGAGAGACAGAGACAGATCCATTGATTAGT GAACGGATCCTTAGCACTTATCTGGGACGATCTGCGGAGCCTGTGCC TCTTCAGCTACCACCGCTTGAGAGACTTACTCTTGATTGTAACGAGG ATTGTGGAATTCTGGGACGCAGGGGGTGGGAAGCCCTCAAATATT GGTGAATCTCCTACAATATTGGAGTCAGGAGCTAAAGAATAGTCT AGA
49	Elongation Factor-1 alpha (EF1-alpha) promoter	CCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGT CGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATA TAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCC GCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGCCTGGCC TCTTTACGGGTTATGGCCCTTGCGTGCCTTGAATTACTTCCACGCCCC TGGCTGCAGTACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTGG GTGGGAGAGTTTCGAGGCCTTGCGCTTAAGGAGCCCCCTTCGCTCGTG CTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCCGCCGCTGCGAAT CTGGTGGCACCTTCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGC CATTTAAAATTTTTGATGACCTGCTGCGACGCTTTTTTTCTGGCAAGA TAGTCTTGTAATGCGGGGCCAAGATCTGCACACTGGTATTTTCGTTT TTGGGGCCCGGGCGGCGACGGGGCCCGTGCCTCCAGCGCACATG TTCGGCGAGGCGGGGCCCTGCGAGCGCGGCCACCGAGAATCGGACGG GGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCC GCCGTGTATCGCCCCGCCCTGGGCGGCAAGGCTGGCCCGGTGCGCA CCAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAG GGAGCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTG AGTACCCACACAAAGGAAAAGGGCCTTCCGTCCTCAGCCGTCGCT TCATGTGACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTA GTTCTCGAGCTTTTGGAGTACGTCTCTTTAGGTTGGGGGGAGGGGT TTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGAAGTT AGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAATTTGCCCTTTTT GAGTTTGGATCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAG TTTTTTTCTTCCATTTCAAGGTGTCGTGA
50	Promoter; PGK	GGGGTTGGGGTTGCGCCTTTTCCAAGGCAGCCCTGGGTTTGCGCAGG GACGCGGCTGCTCTGGGCGTGGTTCCGGGAAACGCAGCGGCGCCGA CCCTGGGTCTCGCACATTCTTACGTCCGTTTCGACGCTCACCCGGA TCTTCGCCGCTACCTTGTGGGCCCCCGGCGACGCTTCTGCTCCG CCCCTAAGTCGGGAAGGTTCCCTTGCAGTTTCGCGGCTGCCGAGCTG ACAAACGGAAGCCGCACGTCTCACTAGTACCCTCGCAGACGGACAG CGCCAGGGAGCAATGGCAGCGCGCCGACCGCGATGGGCTGTGGCCA ATAGCGGCTGCTCAGCAGGGCGCGCCGAGAGCAGCGGCCGGGAAG

		GGGCGGTGCGGGAGGCGGGGTGTGGGGCGGTAGTGTGGGCCCTGTT CCTGCCCCGCGCGGTGTTCCGCATTCTGCAAGCCTCCGGAGCGCACGT CGGCAGTCGGCTCCCTCGTTGACCGAATCACCGACCTCTCTCCCCAG
51	Promoter; UbC	GCGCCGGGTTTTGGCGCCTCCCGCGGGCGCCCCCTCCTCACGGCGA GCGCTGCCACGTCAGACGAAGGGCGCAGGAGCGTTTCTGATCCTTC CGCCCGGACGCTCAGGACAGCGGCCCGCTGCTCATAAGACTCGGCC TTAGAACCCCAGTATCAGCAGAAGGACATTTTAGGACGGGACTTGG GTGACTCTAGGGCACTGGTTTTCTTTCCAGAGAGCGGAACAGGCGA GGAAAAGTAGTCCCTTCTCGGCGATTCTGCGGAGGGATCTCCGTGGG GCGGTGAACGCCGATGATTATATAAGGACGCGCCGGGTGTGGCACA GCTAGTTCCGTCGCAGCCGGGATTTGGGTCGCGGTTCTTGTGTGG ATCGCTGTGATCGTCACTTGGTGAGTTGCGGGCTGCTGGGCTGGCCG GGGCTTTCGTGGCCGCCGGGCCGCTCGGTGGGACGGAAGCGTGTGG AGAGACCGCCAAGGGCTGTAGTCTGGGTCCGCGAGCAAGGTTGCC TGAAGTGGGGGTGGGGGGAGCGCACAAAATGGCGGCTGTTCCCGA GTCTTGAATGGAAGACGCTTGTAAGGCGGGCTGTGAGGTCGTTGAA ACAAGGTGGGGGGCATGGTGGGCGGCAAGAACCCAAAGGTCTTGAGG CCTTCGCTAATGCGGGAAAGCTCTTATTCGGGTGAGATGGGCTGGGG CACCATCTGGGGACCCTGACGTGAAGTTTGTCACTGACTGGAGA CGGGTTTGTCTGTCTGGTTGCGGGGGCGGCAGTTATGCGGTGCCGTTG GGCAGTGCACCCGTACCTTTGGGAGCGCGCGCCTCGTCGTGTCTGA CGTCACCCGTTCTGTTGGCTTATAATGCAGGGTGGGGCCACCTGCCG GTAGGTGTGCGGTAGGCTTTTCTCCGTGCAGGACGCAGGGTTCGGG CCTAGGGTAGGCTCTCCTGAATCGACAGGCGCCGACCTCTGGTGA GGGGAGGGATAAGTGAGGCGTCAGTTTCTTTGGTCGGTTTTATGTAC CTATCTTCTTAAGTAGCTGAAGCTCCGGTTTTGAACTATGCGCTCGG GGTTGGCGAGTGTGTTTTGTGAAGTTTTTTAGGCACCTTTTGAAATGT AATCATTTGGGTCAATATGTAATTTTCAGTGTTAGACTAGTAAA
52	Poly A; SV40	GTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAA ATTTACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTGT CCAAACTCATCAATGTATCTTATCA
53	Poly A; bGH	GACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCCTCCCCCGT GCCTTCCTTGACCTTGAAGGTGCCACTCCCACTGTCCTTTCTTAATA AAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTC TGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAG ACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGG
54	Envelope; RD114	ATGAAACTCCCAACAGGAATGGTCATTTTATGTAGCCTAATAATAGT TCGGGCAGGGTTTTGACGACCCCCGCAAGGCTATCGCATTAGTACAA AAACAACATGGTAAACCATGCGAATGCAGCGGAGGGCAGGTATCCG AGGCCCCACCGAACTCCATCCAACAGGTAAGTTGCCAGGCAAGAC GGCCTACTTAATGACCAACCAAAAATGGAAATGCAGAGTCACTCCA AAAAATCTCACCCCTAGCGGGGGAGAACTCCAGAACTGCCCTGT ACACTTTCAGGACTCGATGCACAGTTCTTGTATATACTGAATACCGG CAATGCAGGGCGAATAATAAGACATACTACACGGCCACCTTGCTTA AAATACGGTCTGGGAGCCTCAACGAGGTACAGATATTACAAAACCC CAATCAGCTCCTACAGTCCCCCTGTAGGGGGCTCTATAAATCAGCCCCG TTTGCTGGAGTGCCACAGCCCCCATCCATATCTCCGATGGTGGAGGA CCCCTCGATACTAAGAGAGTGTGGACAGTCCAAAAAAGGCTAGAAC AAATTCATAAGGCTATGCATCCTGAAGTTCAATACCACCCCTTAGCC CTGCCCAAAGTCAGAGATGACCTTAGCCTTGATGCACGGAAGTTTGA TATCCTGAATACCACTTTTAGGTTACTCCAGATGTCCAATTTTAGCCT TGCCCAAGATTGTTGGCTCTGTTTAAAACTAGGTACCCCTACCCCTC TTGCGATACCCACTCCCTCTTTAACCTACTCCCTAGCAGACTCCCTAG CGAATGCCTCCTGTCAGATTATACCTCCCCCTCTTGGTTCAACCGATG

		CAGTTCTCCAACCTCGTCCTGTTTATCTTCCCCTTTTCATTAACGATACG GAACAAATAGACTTAGGTGCAGTCACCTTTACTAACTGCACCTCTGT AGCCAATGTCAGTAGTCCTTTATGTGCCCTAAACGGGTGTCAGTCTTCC TCTGTGGAAATAACATGGCATAACACCTATTTACCCCAAACTGGACA GGACTTTGCGTCCAAGCCTCCCTCCTCCCCGACATTGACATCATCCC GGGGGATGAGCCAGTCCCCATTCTGCCATTGATCATTATATACATA GACCTAAACGAGCTGTACAGTTCATCCCTTTACTAGCTGGACTGGGA ATCACCGCAGCATTACCAACCGGAGCTACAGGCCTAGGTGTCTCCGT CACCCAGTATACAAAATTATCCCATCAGTTAATATCTGATGTCCAAG TCTTATCCGGTACCATAACAAGATTTACAAGACCAGGTAGACTCGTTA GCTGAAGTAGTTCTCCAAAATAGGAGGGGACTGGACCTACTAACGG CAGAACAAGGAGGAATTTGTTTAGCCTTACAAGAAAAATGCTGTTTT TATGCTAACAAGTCAGGAATTGTGAGAAACAAAATAAGAACCCCTAC AAGAAGAATTACAAAAACGCAGGGAAAGCCTGGCATCCAACCCCTCT CTGGACCGGGCTGCAGGGCTTTCTTCCGTACCTCCTACCTCTCCTGG GACCCCTACTCACCCCTCCTACTCATACTAACCATTTGGGCCATGCGTT TTCAATCGATTGGTCCAATTTGTTAAAGACAGGATCTCAGTGGTCCA GGCTCTGGTTTTGACTCAGCAATATCACCAGCTAAACCCATAGAGT ACGAGCCATGA
55	Envelope; GALV	ATGCTTCTCACCTCAAGCCCGCACCACTTCGGGCACCAGATGAGTCC TGGGAGCTGGAAAAGACTGATCATCCTCTTAAGCTGCGTATTCTGGAG ACGGCAAAACGAGTCTGCAGAATAAGAACCCCCACCAGCCTGTGAC CCTCACCTGGCAGGTACTGTCCCAAACTGGGGACGTTGTCTGGGACA AAAAGGCAGTCCAGCCCCCTTTGGACTTGGTGGCCCTCTCTTACACCT GATGTATGTGCCCTGGCGGCCCGGTCTTGAGTCCTGGGATATCCCGGG ATCCGATGTATCGTCCTCTAAAAGAGTTAGACCTCCTGATTACAGACT ATACTGCCGCTTATAAGCAAATCACCTGGGGAGCCATAGGGTGCAG CTACCCTCGGGCTAGGACCAGGATGGCAAATCCCCCTTCTACGTGT GTCCCCGAGCTGGCCGAACCCATTGAGAAGCTAGGAGGTGTGGGGG GCTAGAATCCCTATACTGTAAAGAATGGAGTTGTGAGACCACGGGT ACCGTTTATTGGCAACCCAAGTCCTCATGGGACCTCATAACTGTAAA ATGGGACCAAAATGTGAAATGGGAGCAAAAATTTCAAAAGTGTGAA CAAACCGGCTGGTGTAAACCCCTCAAGATAGACTTCACAGAAAAAG GAAAACCTCTCCAGAGATTGGATAACGGAAAAAACCTGGGAATTAAG GTTCTATGTATATGGACACCCAGGCATACAGTTGACTATCCGCTTAG AGGTCATAACATGCCGGTTGTGGCAGTGGGCCAGACCTGTCTT GCGGAACAGGGACCTCCTAGCAAGCCCCCTCACTCTCCCTCTCTCCCC ACGGAAAGCGCCGCCACCCCTCTACCCCCGGCGGCTAGTGAGCAA ACCCCTGCGGTGCATGGAGAACTGTTACCCTAAACTCTCCGCCTCC CACCAGTGGCGACCGACTCTTTGGCCTTGTGCAGGGGGCCTTCCTAA CCTTGAATGCTACCAACCCAGGGGCCACTAAGTCTTGCTGGCTCTGT TTGGGCATGAGCCCCCTTATTATGAAGGGATAGCCTCTTCAGGAGA GGTCGCTTATACCTCCAACCATACCCGATGCCACTGGGGGGCCCAAG GAAAGCTTACCCCTACTGAGGTCTCCGGACTCGGGTCATGCATAGGG AAGGTGCCTCTTACCCATCAACATCTTTGCAACCAGACCTTACCCAT CAATTCCTCTAAAAACCATCAGTATCTGCTCCCTCAAACCATAGCT GGTGGGCCTGCAGCACTGGCCTCACCCCTGCCTCTCCACCTCAGTT TTAATCAGTCTAAAGACTTCTGTGTCCAGGTCCAGCTGATCCCCCG CATCTATTACATTCTGAAGAAACCTTGTTACAAGCCTATGACAAAT CACCCCCCAGGTTTAAAGAGAGCCTGCCTCACTTACCCTAGCTGTC TTCCTGGGGTTAGGGATTGCGGCAGGTATAGGTACTGGCTCAACCGC CCTAATTAAGGGCCCATAGACCTCCAGCAAGGCCTAACCCAGCCTC CAAATCGCCATTGACGCTGACCTCCGGGGCCCTTCAGGACTCAATCAG CAAGCTAGAGGACTCACTGACTTCCCTATCTGAGGTAGTACTCCAAA ATAGGAGAGGCCTTGACTTACTATTCTTAAAGAAGGAGGCCTCTGC

		GCGGCCCTAAAAGAAGAGTGCTGTTTTTATGTAGACCACTCAGGTGC AGTACGAGACTCCATGAAAAAACTTAAAGAAAGACTAGATAAAAAGA CAGTTAGAGCGCCAGAAAAACCAAACTGGTATGAAGGGTGGTTCA ATAACTCCCCTTGGTTTACTACCCTACTATCAACCATCGCTGGGCCC CTATTGCTCCTCCTTTTGTACTCACTCTTGGGCCCTGCATCATCAAT AAATTAATCCAATTCATCAATGATAGGATAAGTGCAGTCAAAATTTT AGTCCTTAGACAGAAATATCAGACCCTAGATAACGAGGAAAACCTT TAA
56	Envelope; FUG	ATGGTTCGCGCAGGTTCTTTTGTGTACTCCTTCTGGGTTTTTCGTTGT GTTTCGGGAAGTTCCCATTTACACGATACCAGACGAACCTTGGTCCC TGGAGCCCTATTGACATACACCATCTCAGCTGTCCAAATAACCTGGT TGTGGAGGATGAAGGATGTACCAACCTGTCCGAGTTCTCCTACATGG AACTCAAAGTGGGATACATCTCAGCCATCAAAGTGAACGGGTTTAC TTGCACAGGTGTTGTGACAGAGGCAGAGACCTACACCAACTTTGTG GTTATGTCACAACCACATTCAAGAGAAAGCATTTCCGCCCCACCCCA GACGCATGTAGAGCCGCGTATAACTGGAAGATGGCCGGTGACCCCA GATATGAAGAGTCCCTACACAATCCATACCCCGACTACCACTGGCTT CGAACTGTAAGAACCACCAAAAGAGTCCCTCATTATCATATCCCCAAG TGTGACAGATTTGGACCCATATGACAAATCCCTTCACTCAAGGGTCT TCCCTGGCGGAAAGTGCTCAGGAATAACGGTGTCTCTACCTACTGC TCAACTAACCATGATTACACCATTTGGATGCCCCGAGAATCCGAGACC AAGGACACCTTGTGACATTTTACCAATAGCAGAGGGAAGAGAGCA TCCAACGGGAACAAGACTTGC GGCTTTGTGGATGAAAGAGGCCTGT ATAAGTCTCTAAAAGGAGCATGCAGGCTCAAGTTATGTGGAGTTCTT GGACTTAGACTTATGGATGGAACATGGGTGCGGATGCAACATCAG ATGAGACCAAATGGTGCCCTCCAGATCAGTTGGTGAATTTGCACGAC TTTCGCTCAGACGAGATCGAGCATCTCGTTGTGGAGGAGTTAGTTAA GAAAAGAGAGGAATGTCTGGATGCATTAGAGTCCATCATGACCACC AAGTCAGTAAGTTTCAGACGTCTCAGTCACCTGAGAAAACCTTGTCCT AGGGTTTGGAAAAGCATATACCATATTCAACAAAACCTTGATGGAG GCTGATGCTCACTACAAGTCAGTCCGGACCTGGAATGAGATCATCCC CTCAAAAGGGTGTTTGAAAGTTGGAGGAAGGTGCCATCCTCATGTG AACGGGGTGTTTTTCAATGGTATAATATTAGGGCCTGACGACCATGT CCTAATCCCAGAGATGCAATCATCCCTCCTCCAGCAACATATGGAGT TGTTGGAATCTTCAGTTATCCCCCTGATGCACCCCTGGCAGACCTT TCTACAGTTTTCAAAGAAGGTGATGAGGCTGAGGATTTTGTGAGT TCACCTCCCCGATGTGTACAAACAGATCTCAGGGGTTGACCTGGGTC TCCCGAACTGGGGAAAGTATGTATTGATGACTGCAGGGGCCATGAT TGGCCTGGTGTGATATTTTCCCTAATGACATGGTGCAGAGTTGGTA TCCATCTTTGCATTAAATTAAAGCACACCAAGAAAAGACAGATTTAT ACAGACATAGAGATGAACCGACTTGGAAAGTAA
57	Envelope; LCMV	ATGGGTCAGATTGTGACAAATGTTTGAGGCTCTGCCTCACATCATCGA TGAGGTGATCAACATTGTCATTATTGTGCTTATCGTGATCACGGGTA TCAAGGCTGTCTACAATTTTGCCACCTGTGGGATATTGCGATTGATC AGTTTCCTACTTCTGGCTGGCAGGTCCTGTGGCATGTACGGTCTTAA GGGACCCGACATTTACAAAGGAGTTTACCAATTTAAGTCAGTGGAG TTTGATATGTCACATCTGAACCTGACCATGCCCAACGCATGTTTACG CAACAACTCCCACCATTACATCAGTATGGGGACTTCTGGACTAGAAT TGACCTTCACCAATGATTCCATCATCAGTCACAACCTTTTGCAATCTG ACCTCTGCCTTCAACAAAAGACCTTTGACCACACACTCATGAGTAT AGTTTCGAGCCTACACCTCAGTATCAGAGGGAACCTCCAACATAAG GCAGTATCCTGCGACTTCAACAATGGCATAACCATCCAATACAACCTT GACATTCTCAGATCGACAAAGTGCTCAGAGCCAGTGTAGAACCCTT AGAGGTAGAGTCCTAGATATGTTTAGAACTGCCTTCGGGGGGAAAT ACATGAGGAGTGGCTGGGGCTGGACAGGCTCAGATGGCAAGACCAC

		CTGGTGTAGCCAGACGAGTTACCAATACCTGATTATACAAAATAGA ACCTGGGAAAACCACTGCACATATGCAGGTCCTTTTGGGATGTCCAG GATTCTCCTTTCCCAAGAGAAGACTAAGTTCTTCACTAGGAGACTAG CGGGCACATTACCTGGACTTTGTCAGACTCTTCAGGGGTGGAGAAT CCAGGTGGTTATTGCCTGACCAAATGGATGATTCTTGCTGCAGAGCT TAAGTGTTCGGGAACACAGCAGTTGCGAAATGCAATGTAAATCAT GATGCCGAATTCTGTGACATGCTGCGACTAATTGACTACAACAAGGC TGCTTTGAGTAAGTTCAAAGAGGACGTAGAATCTGCCTTGCACTTAT TCAAAACAACAGTGAATTCCTTGATTTTCAATCACTACTGATGAGG AACCCTTGAGAGATCTGATGGGGGTGCCATATTGCAATTACTCAAA GTTTTGGTACCTAGAACATGCAAAGACCGGCGAAACTAGTGTCCTCC AAGTGCTGGCTTGTACCAATGGTTCTTACTTAAATGAGACCCACTT CAGTGATCAAATCGAACAGGAAGCCGATAACATGATTACAGAGATG TTGAGGAAGGATTACATAAAGAGGCAGGGGAGTACCCCTTAGCAT TGATGGACCTTCTGATGTTTTCCACATCTGCATATCTAGTCAGCATCT TCCTGCACCTTGTCAAATACCAACACACAGGCACATAAAAGGTGG CTCATGTCCAAAGCCACACCGATTAAACCAACAAAGGAATTTGTAGTT GTGGTGCATTTAAGGTGCCTGGTGTAAAAACCGTCTGGAAAAGACG CTGA
58	Envelope; FPV	ATGAACACTCAAATCCTGGTTTTTCGCCCTTGTGGCAGTCATCCCCAC AAATGCAGACAAAATTTGTCTTGGACATCATGCTGTATCAAATGGCA CCAAAGTAAACACACTCACTGAGAGAGGAGTAGAAGTTGTCAATGC AACGGAAACAGTGGAGCGGACAAACATCCCCAAAATTTGCTCAAAA GGGAAAAGAACCACTGATCTTGGCCAATGCGGACTGTTAGGGACCA TTACCGGACCACCTCAATGCGACCAATTTCTAGAATTTTCAGCTGAT CTAATAATCGAGAGACGAGAAGGAAATGATGTTTTGTTACCCGGGGA AGTTTGTAAATGAAGAGGCATTGCGACAAATCCTCAGAGGATCAGG TGGGATTGACAAAGAAACAATGGGATTCACATATAGTGGAATAAGG ACCAACGGAACAACACTAGTGCATGTAGAAGATCAGGGTCTTCATTCT ATGCAGAAATGGAGTGGCTCCTGTCAAATACAGACAATGCTGCTTTC CCACAAATGACAAAATCATAAAAAACACAAGGAGAGAATCAGCTC TGATAGTCTGGGGAATCCACCATTTCAGGATCAACACCGAACAGAC CAAACCTATATGGGAGTGGAAATAAACTGATAACAGTCGGGAGTTCC AAATATCATCAATCTTTTGTGCCGAGTCCAGGAACACGACCGCAGAT AAATGGCCAGTCCGGACGGATTGATTTTCATTGGTTGATCTTGGATC CCAATGATACAGTTACTTTTAGTTTCAATGGGGCTTTTCATAGCTCCA AATCGTGCCAGCTTCTTGAGGGGAAAGTCCATGGGGATCCAGAGCG ATGTGCAGGTTGATGCCAATTGCGAAGGGGAATGCTACCACAGTGG AGGGACTATAACAAGCAGATTGCCTTTTCAAACATCAATAGCAGA GCAGTTGGCAAATGCCAAGATATGTAAAACAGGAAAGTTTATTAT TGGCAACTGGGATGAAGAACGTTCCCGAACCTTCCAAAAAAGGAA AAAAAGAGGCCTGTTTGGCGCTATAGCAGGGTTTATTGAAAATGGTT GGGAAGGTCTGGTCGACGGGTGGTACGGTTTCAGGCATCAGAATGC ACAAGGAGAAGGAACCTGCAGCAGACTACAAAAGCACCCAATCGGC AATTGATCAGATAACCGGAAAGTTAAATAGACTCATTGAGAAAACC AACCAGCAATTTGAGCTAATAGATAATGAATTCCTGAGGTGAAAA AGCAGATTGGCAATTTAATTAACCTGGACCAAGACTCCATCACAGA AGTATGGTCTTACAATGCTGAACCTTCTTGTTGGCAATGGAAAACGAGC ACACTATTGATTGGCTGATTGAGAGATGAACAAGCTGTATGAGCGA GTGAGGAAACAATTAAGGGAAAATGCTGAAGAGGATGGCACTGGTT GCTTTGAAATTTTTCATAAATGTGACGATGATTGTATGGCTAGTATA AGGAACAATACTTATGATCACAGCAAATACAGAGAAGAAGCGATGC AAAATAGAATACAAATTGACCCAGTCAAATTGAGTAGTGGCTACAA AGATGTGATACTTTGGTTTAGCTTCGGGGCATCATGCTTTTTGCTTCT TGCCATTGCAATGGGCCTTGTTTTCATATGTGTGAAGAACGGAAACA

		TGCGGTGCACTATTTGTATATAA
59	Envelope; RRV	AGTGTAACAGAGCACTTTAATGTGTATAAGGCTACTAGACCATACCT AGCACATTGCGCCGATTGCGGGGACGGGTACTTCTGCTATAGCCCAG TTGCTATCGAGGAGATCCGAGATGAGGCGTCTGATGGCATGCTTAA GATCCAAGTCTCCGCCCAAATAGGTCTGGACAAGGCAGGCACCCAC GCCCACACGAAGCTCCGATATATGGCTGGTCATGATGTTTCAGGAATC TAAGAGAGATTCCCTTGAGGGTGTACACGTCCGCAGCGTGCTCCATAC ATGGGACGATGGGACACTTCATCGTCGCACACTGTCCACCAGGCGA CTACCTCAAGGTTTCGTTTCGAGGACGCAGATTTCGCACGTGAAGGCAT GTAAGGTCCAATACAAGCACAAATCCATTGCCGGTGGGTAGAGAGAA GTTCTGGTTAGACCACACTTTGGCGTAGAGCTGCCATGCACCTCAT ACCAGCTGACAACGGCTCCCACCGACGAGGAGATTGACATGCATAC ACCGCCAGATATACCGGATCGCACCCCTGCTATCACAGACGGCGGGC AACGTCAAAATAACAGCAGGCGGCAGGACTATCAGGTACAACCTGTA CCTGCGGCCGTGACAACGTAGGCACTACCAGTACTGACAAGACCAT CAACACATGCAAGATTGACCAATGCCATGCTGCCGTACCAGCCAT GACAAATGGCAATTTACCTCTCCATTTGTTCCAGGGCTGATCAGAC AGCTAGGAAAGGCAAGGTACACGTTCCGTTCCCTCTGACTAACGTCA CCTGCCGAGTGCCGTTGGCTCGAGCGCCGGATGCCACCTATGGTAAG AAGGAGGTGACCCTGAGATTACACCCAGATCATCCGACGCTCTTCTC CTATAGGAGTTTAGGAGCCGAACCGCACCCGTACGAGGAATGGGTT GACAAGTTCTCTGAGCGCATCATCCAGTGACGGAAGAAGGGATTG AGTACCAGTGGGGCAACAACCCGCCGGTCTGCCTGTGGGCGCAACT GACGACCGAGGGCAAACCCCATGGCTGGCCACATGAAATCATTAG TACTATTATGGAATAACCCCGCCGCCACTATTGCCGCAGTATCCGG GGCGAGTCTGATGGCCCTCCTAACTCTGGCGGCCACATGCTGCATGC TGGCCACCGCGAGGAGAAAGTGCCTAACACCGTACGCCCTGACGCC AGGAGCGGTGGTACCGTTGACACTGGGGCTGCTTTGCTGCGCACCG AGGGCGAATGCA
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62	Thyroxin binding globulin promoter (TBG)	CTTTCTCTTTTGTTTTACATGAAGGGTCTGGCAGCCAAAGCAATCAC TCAAAGTTCAAACCTTATCATTTTTTGTCTTGTTCCTCTTGGCCTTGG TTTTGTACATCAGCTTTGAAAATACCATCCAGGGTTAATGCTGGGG TTAATTTATACTAAGAGTGCTCTAGTTTTGCAATACAGGACATGCT ATAAAAATGGAAAGATGTTGCTTTCTGAG
63	DNA fragment containing prothrombin enhancer and human alpha-1 anti-trypsin promoter	GCGAGAACTTGTGCCTCCCCGTGTTCTGCTCTTTGTCCCTCTGTCCT ACTTAGACTAATATTTGCCTTGGGTACTGCAAACAGGAAATGGGGG AGGGACAGGAGTAGGGCGGAGGGTAGCCCCGGGGATCTTGCTACCAG TGGAACAGCCACTAAGGATTCTGCAGTGAGAGCAGAGGGGCCAGCTA AGTGGTACTCTCCCAGAGACTGTCTGACTCACGCCACCCCTCCACC TTGGACACAGGACGCTGTGGTTTCTGAGCCAGGTACAATGACTCCTT TCGGTAAGTGCAGTGGAAGCTGTACACTGCCAGGCCAAAGCGTCCG GGCAGCGTAGGCGGGCGACTCAGATCCCAGCCAGTGGACTTAGCCC CTGTTTGCTCCTCCGATAACTGGGGTGACCTTGTTAATATTCACCA GCAGCCTCCCCCGTTGCCCTCTGGATCCACTGCTTAAATACGGACG AGGACAGGGCCCTGTCTCCTCAGCTTCAGGCACCACCACTGACCTGG GACAGTGAAT
64	DNA fragment containing prothrombin enhancer, human alpha-1 anti-trypsin promoter, and five HNF1 binding sites	GTTAATCATTAAACGTTAATCATTAAACGTTAATCATTAAACGTTAATCA TTAACGTTAATCATTAAACATCGATGCGAGAACTTGTGCCTCCCCGTG TTCCTGCTCTTTGTCCCTCTGTCCTACTTAGACTAATATTTGCCTTGG GTACTGCAAACAGGAAATGGGGGAGGGACAGGAGTAGGGCGGAGG GTAGGATTCTGCAGTGAGAGCAGAGGGGCCAGCTAAGTGGTACTCTC CCAGAGACTGTCTGACTCACGCCACCCCTCCACCTTGACACAGGA CGCTGTGGTTTCTGAGCCAGGTACAATGACTCCTTTTCGGTAAGTGCA GTGGAAGCTGTACACTGCCAGGCCAAAGCGTCCGGGCAGCGTAGGC GGGCGACTCAGATCCCAGCCAGTGGACTTAGCCCCCTGTTTGCTCCTC CGATAACTGGGGTGACCTTGTTAATATTCACCAGCAGCCTCCCCCG TTGCCCTCTGGATCCACTGCTTAAATACGGACGAGGACAGGGCCCT GTCTCCTCAGCTTCAGGCACCACCACTGACCTGGGACAGTGAAT
65	DNA fragment containing prothrombin enhancer, human alpha- 1, anti-trypsin promoter, and three HNF1/HNF4 binding sites	GTTAATCATTAAACGCTTGTACTTTGGTACAGTTAATCATTAAACGCTTG TACTTTGGTACAGTTAATCATTAAACGCTTGTACTTTGGTACAATCGAT GCGAGAACTTGTGCCTCCCCGTGTTCTGCTCTTTGTCCCTCTGTCCT ACTTAGACTAATATTTGCCTTGGGTACTGCAAACAGGAAATGGGGG AGGGACAGGAGTAGGGCGGAGGGTAGCCCCGGGGATTCTGCAGTGA GAGCAGAGGGCCAGCTAAGTGGTACTCTCCCAGAGACTGTCTGACT CACGCCACCCCTCCACCTTGACACAGGACGCTGTGGTTTCTGAGC CAGGTACAATGACTCCTTTTCGGTAAGTGCAGTGGAAGCTGTACACTG CCCAGGCCAAAGCGTCCGGGCAGCGTAGGCGGGCGACTCAGATCCCA GCCAGTGGACTTAGCCCCCTGTTTGCTCCTCCGATAACTGGGGTGACC TTGGTTAATATTCACCAGCAGCCTCCCCCGTTGCCCTCTGGATCCA CTGCTTAAATACGGACGAGGACAGGGCCCTGTCTCCTCAGCTTCAGG CACCACCACTGACCTGGGACAGTGAAT
66	Taqman Probe	TCGTGAAAGCTCATGGACAGTGGC
67	Taqman Forward Primer	AGATCTTGAGGCATGACATTGG

68	Taqman Reverse Primer	GTCCAGCTCTTGAATGGTTCTT
69	Actin Probe	AGCGGGAAATCGTGCGTGAC
70	Actin Forward Primer	GGACCTGACTGACTACCTCAT
71	Actin Reverse Primer	CGTAGCACAGCTTCTCCTTAAT

CLAIMS

WHAT IS CLAIMED IS:

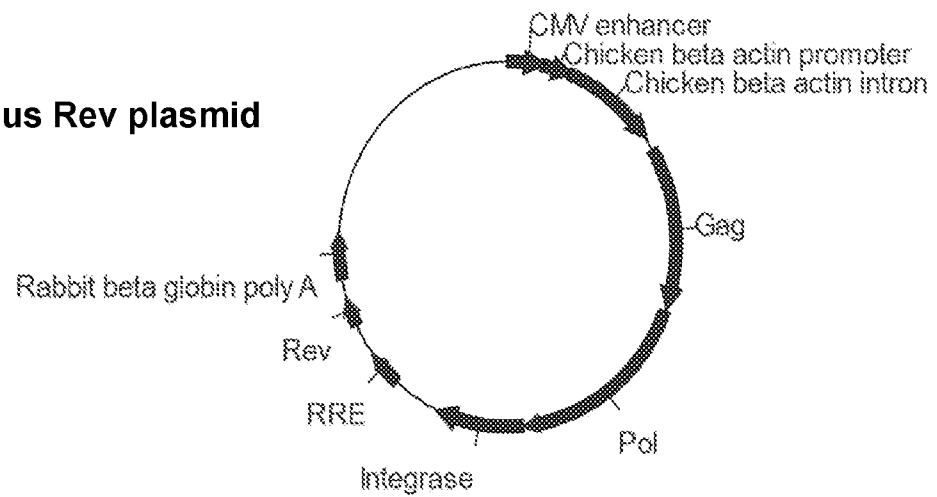
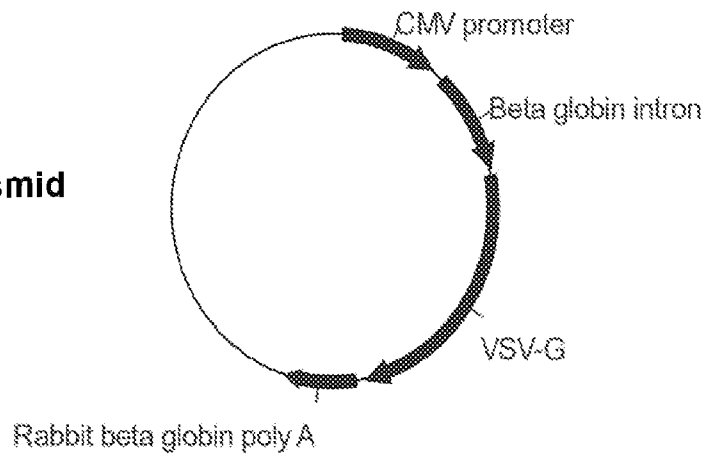
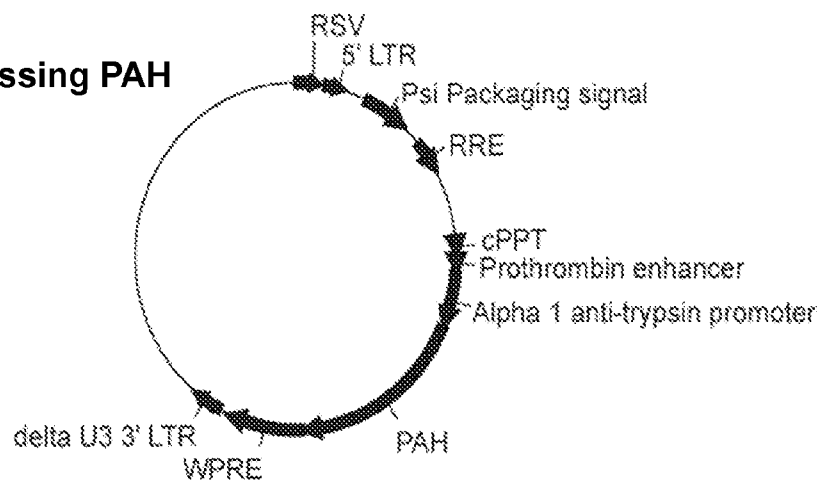
1. A viral vector comprising a therapeutic cargo portion, wherein the therapeutic
5 cargo portion comprises:
a PAH sequence or a variant thereof;
a promoter; and
a liver-specific enhancer,
wherein the PAH sequence or the variant thereof is operatively controlled by both
10 the promoter and the liver-specific enhancer.
2. The viral vector of claim 1, wherein the liver-specific enhancer comprises a
prothrombin enhancer.
3. The viral vector of claim 2, wherein the promoter comprises a liver-specific
promoter.
- 15 4. The viral vector of claim 3, wherein the liver-specific promoter comprises a
hAAT promoter.
5. The viral vector of claim 1, wherein the PAH sequence or the variant thereof is
truncated.
6. The viral vector of claim 5, wherein the truncated portion of the PAH sequence or
20 the variant thereof is the 3' untranslated region (UTR) of the PAH sequence or the
variant thereof.
7. The viral vector of claim 1, wherein the therapeutic cargo portion further
comprises a beta globin intron.
8. The viral vector of claim 1, wherein the therapeutic cargo portion further
25 comprises at least one hepatocyte nuclear factor binding site.
9. The viral vector of claim 8, wherein the at least one hepatocyte nuclear factor
binding site is disposed upstream of the prothrombin enhancer.
10. The viral vector of claim 8, wherein the at least one hepatocyte nuclear factor
binding site is disposed downstream of the prothrombin enhancer.

11. The viral vector of claim 1, wherein the PAH sequence or the variant thereof comprises a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3; or SEQ ID NO: 4.
- 5 12. The viral vector of claim 11, wherein the PAH sequence or the variant thereof comprises SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3; or SEQ ID NO: 4.
13. The viral vector of claim 2, wherein the prothrombin enhancer comprises a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with SEQ ID NO: 5.
- 10 14. The viral vector of claim 2, wherein the sequence of prothrombin enhancer comprises SEQ ID NO: 5.
15. The viral vector of claim 4, wherein the sequence of the hAAT promoter comprises SEQ ID NO: 6.
16. The viral vector of claim 5, wherein the sequence of the beta globin intron comprises SEQ ID NOs: 7 or 8.
- 15 17. The viral vector of claim 6, wherein the sequence of the hepatocyte nuclear factor binding site comprises any one of SEQ ID NOs: 9-12.
18. The viral vector of claim 1, wherein the therapeutic cargo portion further comprises at least one small RNA sequence that is capable of binding to at least one pre-determined complementary mRNA sequence.
- 20 19. The viral vector of claim 18, wherein the at least one small RNA sequence targets a complementary mRNA sequence that contains a full-length UTR.
20. The viral vector of claim 18, wherein the at least one pre-determined complementary mRNA sequence is a PAH mRNA sequence.
- 25 21. The viral vector of claim 18, wherein the at least one small RNA sequence comprises a shRNA.
22. The viral vector of claim 18, wherein the at least one small RNA sequence is under the control of a first promoter and the PAH sequence or the variant thereof is under the control of a second promoter.
- 30 23. The viral vector of claim 20, wherein the first promoter comprises a H1 promoter.

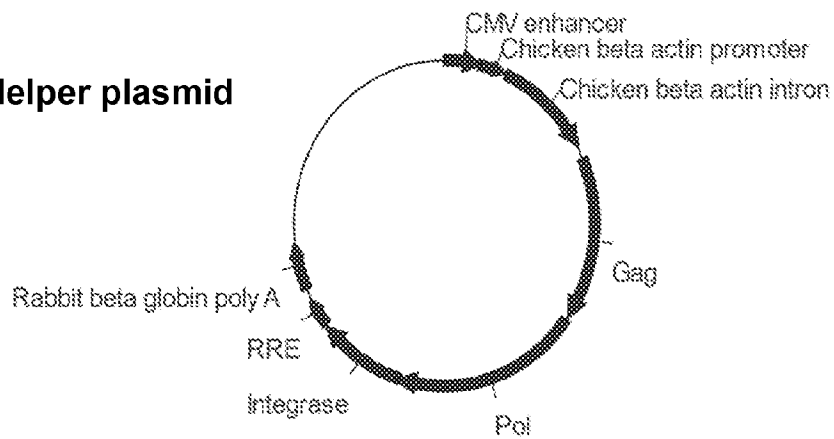
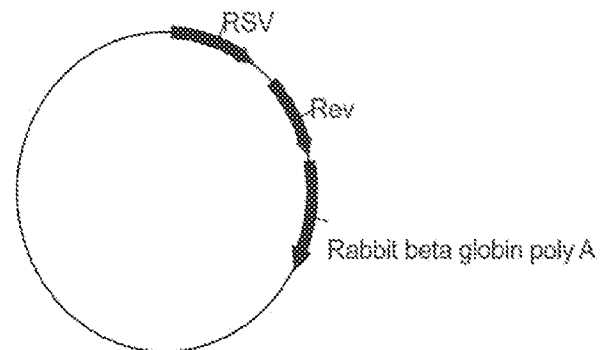
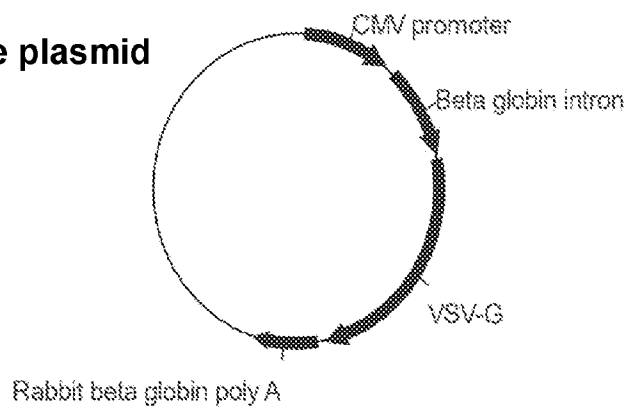
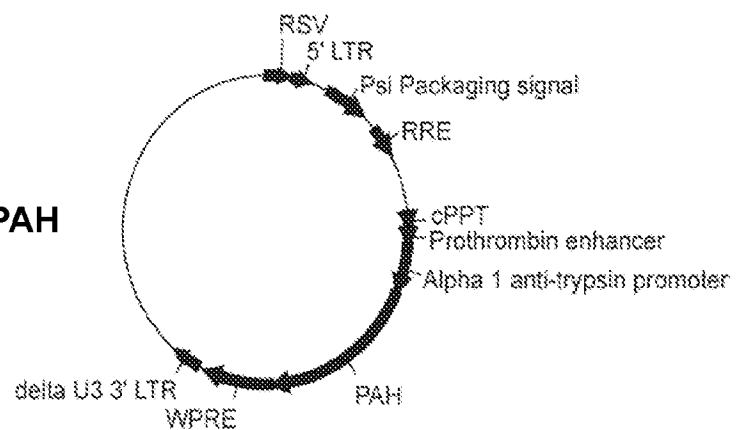
24. The viral vector of claim 20, wherein the second promoter comprises a liver-specific promoter.
25. The viral vector of claim 24, wherein the liver-specific promoter comprises a hAAT promoter.
- 5 26. The viral vector of claim 18, wherein the at least one small RNA sequence comprises a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with SEQ ID NO: 13 or SEQ ID NO: 14.
27. The viral vector of claim 21, wherein the at least one small RNA sequence comprises SEQ ID NO: 13 or SEQ ID NO: 14.
- 10 28. The viral vector of claim 1, wherein the viral vector is a lentiviral vector.
29. A lentiviral particle capable of infecting a target cell, the lentiviral particle comprising:
- an envelope protein optimized for infecting the target cell; and
- the viral vector according to claim 1.
- 15 30. The lentiviral particle of claim 29, wherein the target cell is a hepatic cell, a muscle cell, an epithelial cell, an endothelial cell, a neural cell, a neuroendocrine cell, an endocrine cell, a lymphocyte, a myeloid cell, a cell present within a solid organ, or cell of a hematopoietic lineage, a hematopoietic stem cell, or a precursor hematopoietic stem cell.
- 20 31. A method of treating PKU in a subject, the method comprising administering to the subject a therapeutically effective amount of the lentiviral particle of claim 29 or 30.
32. A method of preventing PKU in a subject, the method comprising administering to the subject a therapeutically effective amount of the lentiviral particle of claim
- 25 29 or 30.
33. The method of claim 31 or 32 further comprising diagnosing a PKU genotype in the subject that correlates with a PKU phenotype.
34. The method of claim 31 or 32, wherein the subject is *in utero*.
35. The method of claim 33, wherein the diagnosing occurs during prenatal screening
- 30 of the subject.

36. The method of claim 33, wherein the diagnosing occurs *in vitro*.
37. The method of claim 31 or 32, wherein the therapeutically effective amount of the lentiviral particle comprises a plurality of single doses of the lentiviral particle.
38. The method of claim 31 or 32, wherein the therapeutically effective amount of the
5 lentiviral particle comprises a single dose of the lentiviral particle.

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AGT Helper plus Rev plasmid**AGT Envelope plasmid****Lentiviral vector expressing PAH****Figure 1**

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AGT Helper plasmid**AGT Rev plasmid****AGT Envelope plasmid****Lentiviral vector expressing PAH****Figure 2**

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Vector A



Vector B



Vector C



Vector D



Vector E



Vector F



Vector G



Vector H

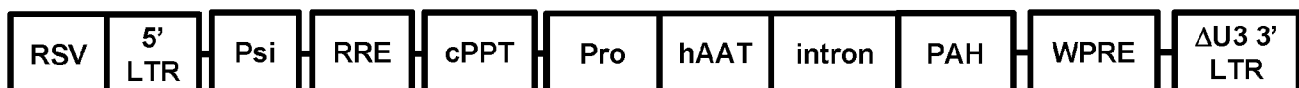


Figure 3

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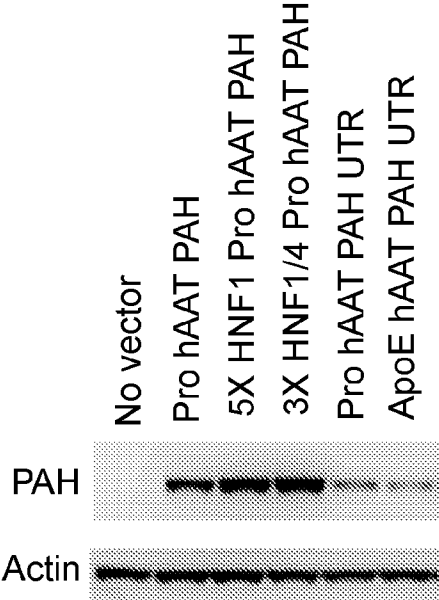


Figure 4A

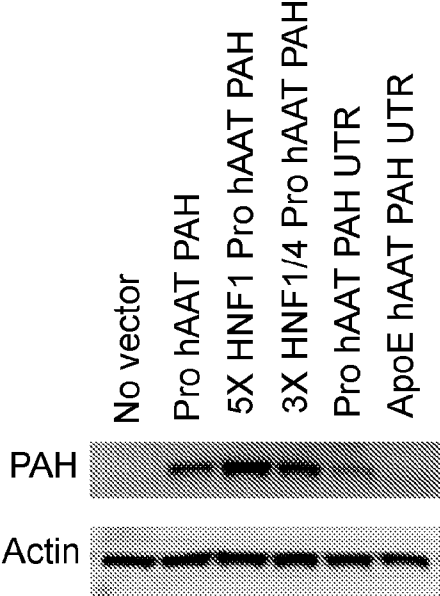


Figure 4B

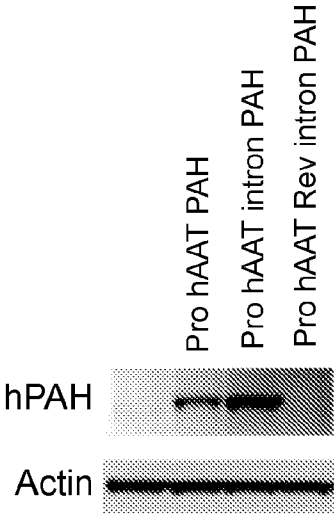


Figure 5A

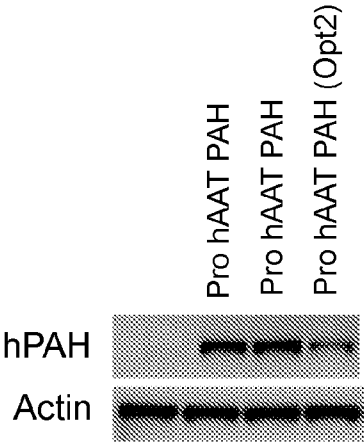


Figure 5B

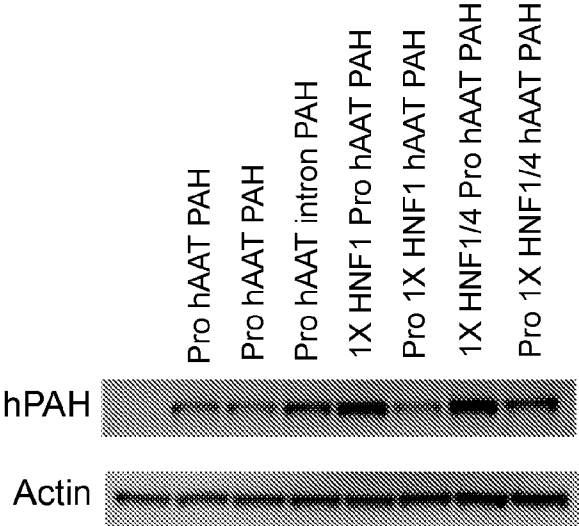


Figure 5C

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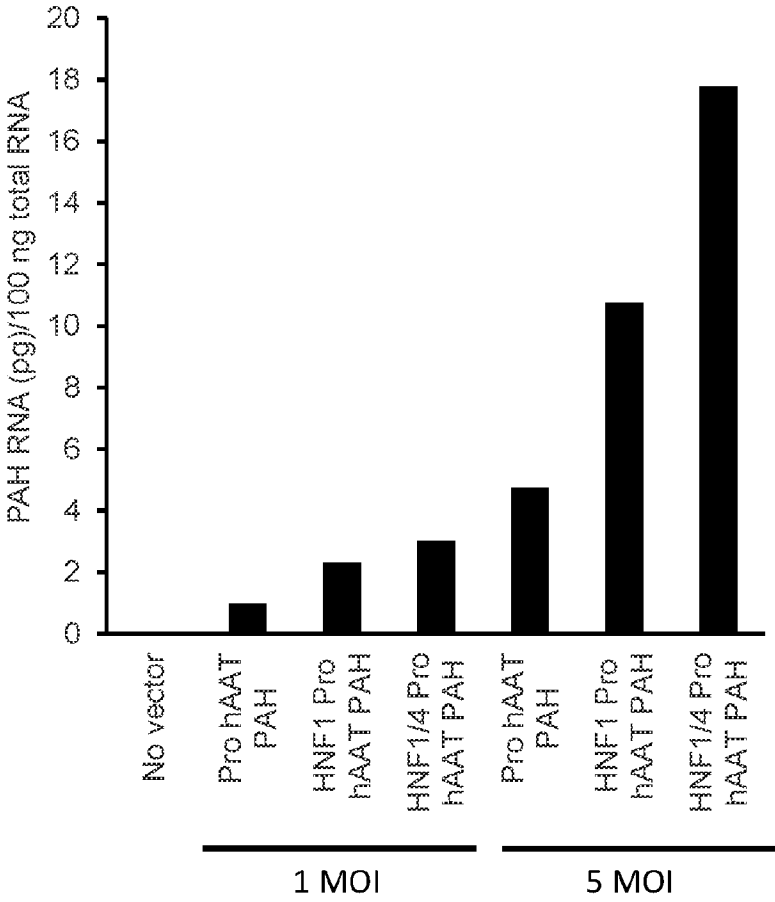


Figure 6

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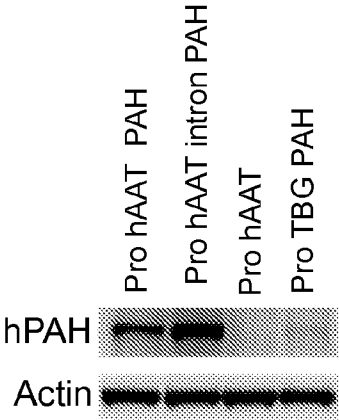


Figure 7

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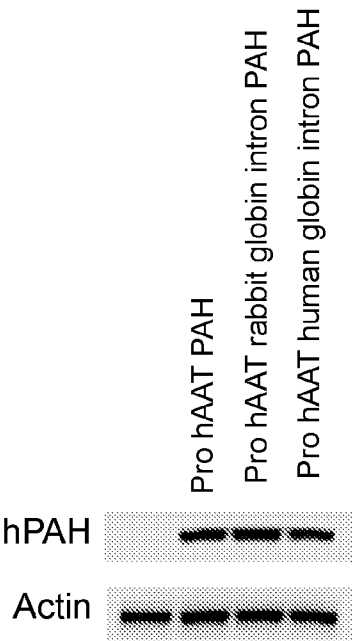


Figure 8A

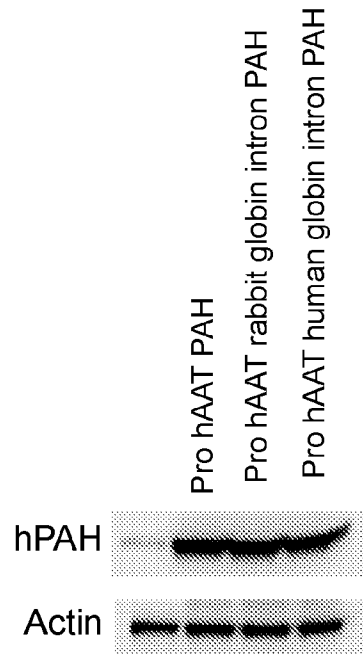


Figure 8B

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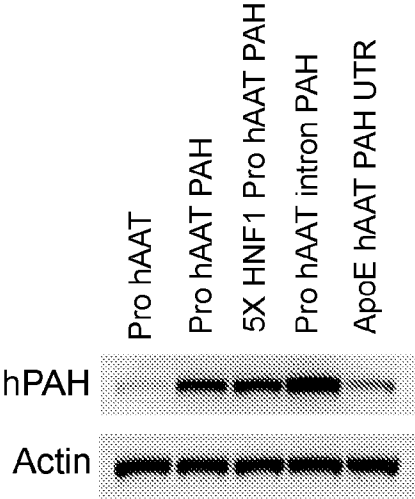


Figure 9

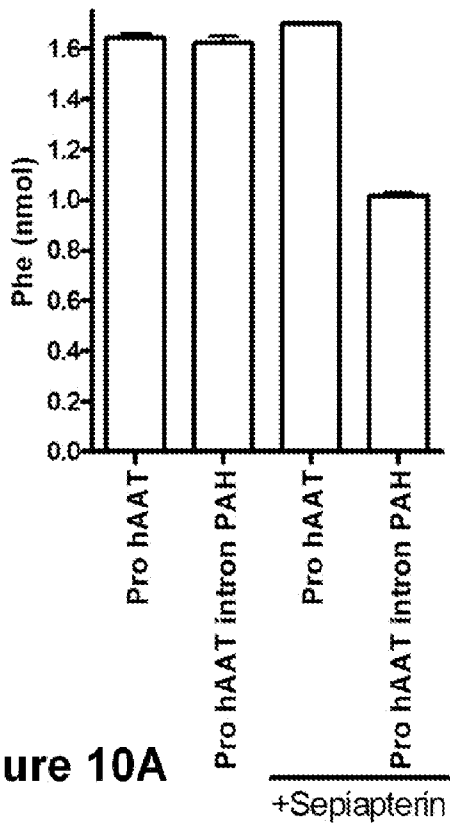


Figure 10A

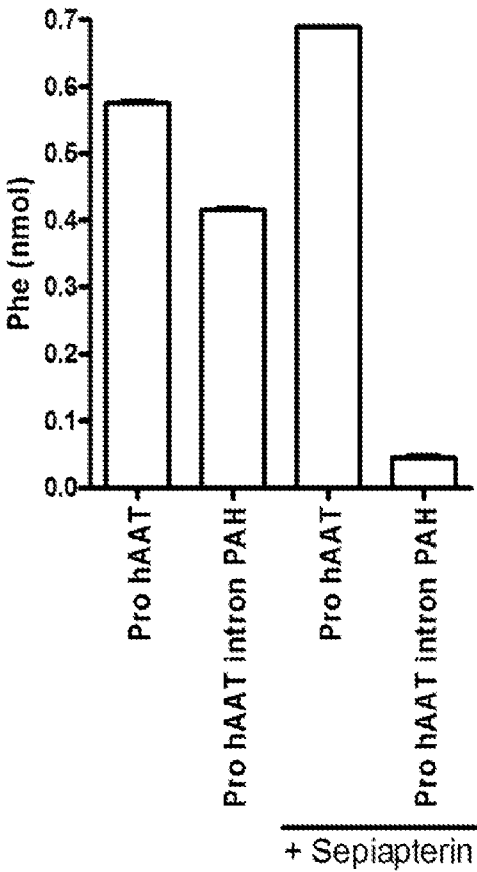


Figure 10B

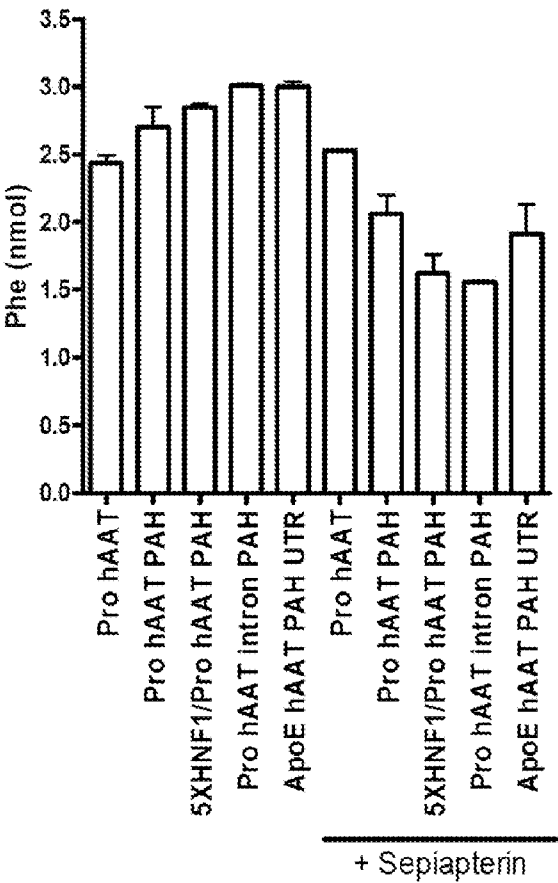


Figure 10C

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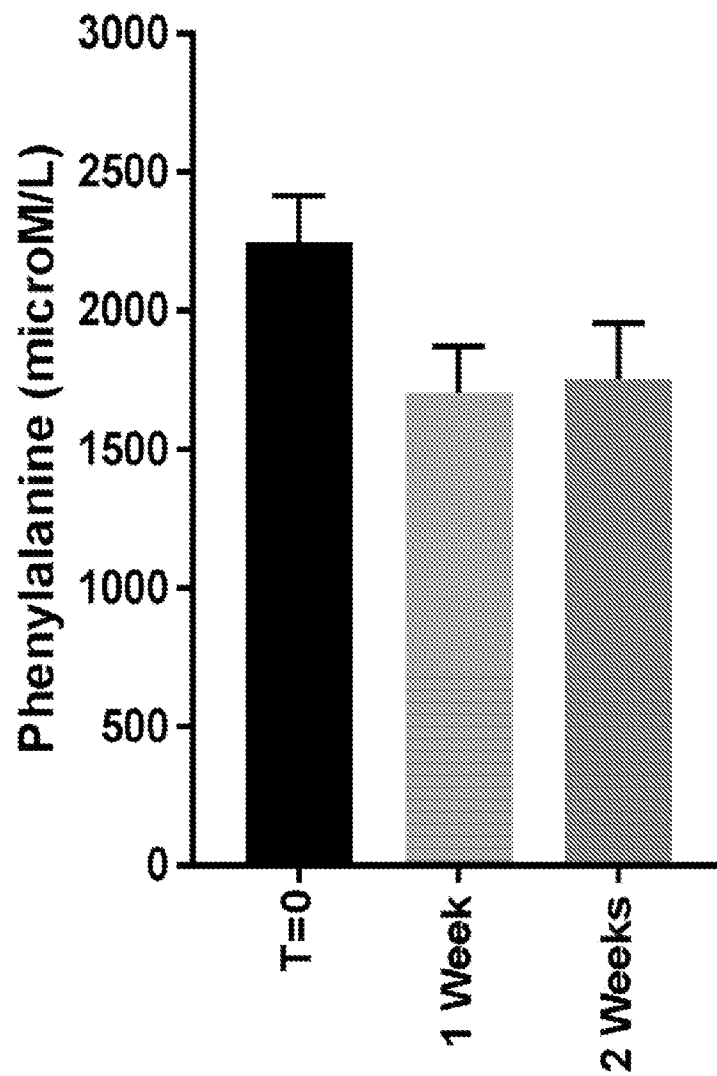
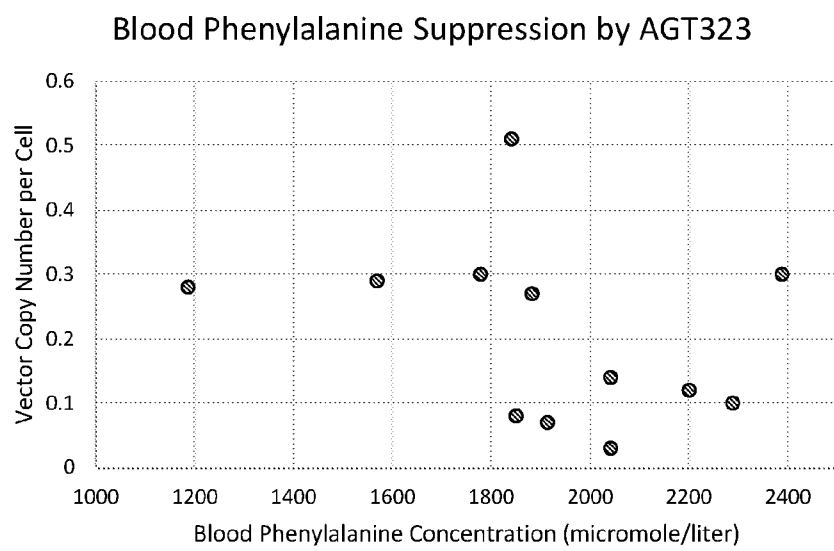


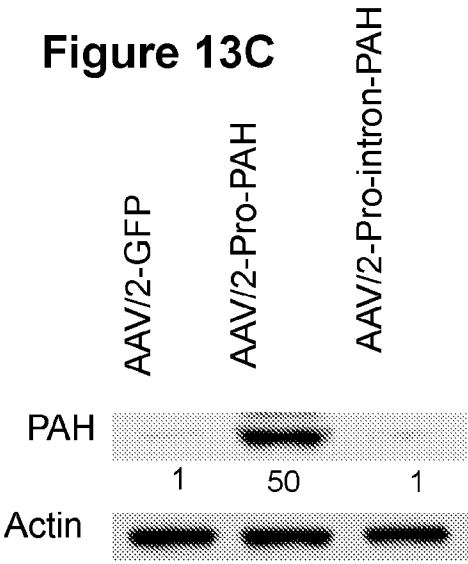
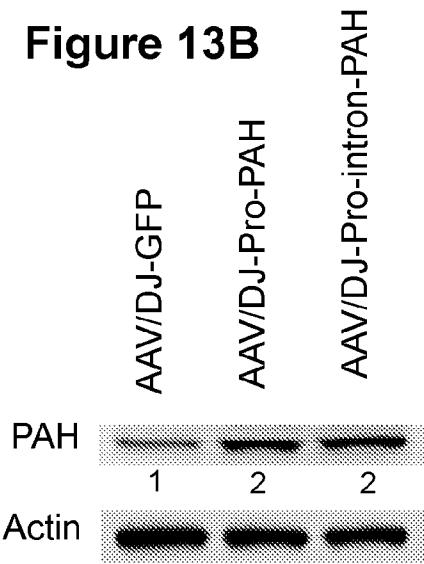
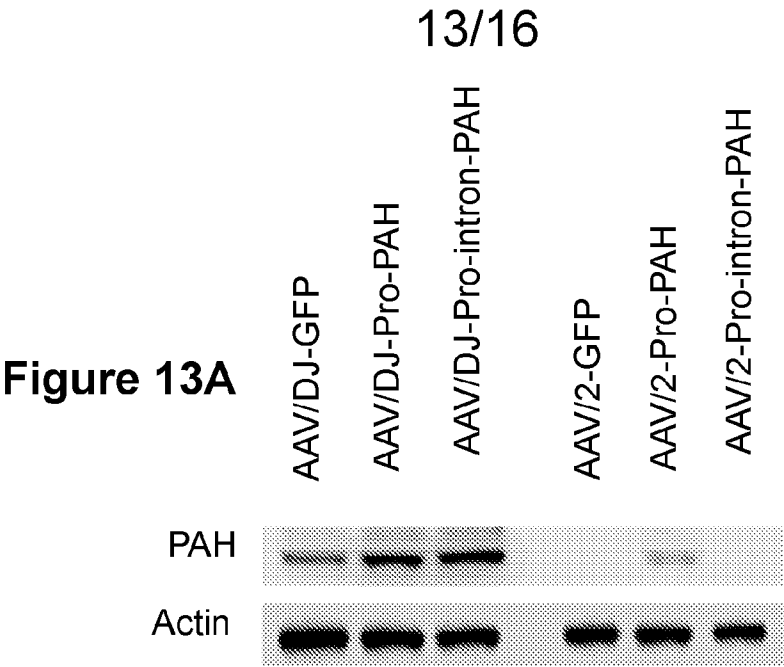
Figure 11

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Pearson product correlation coefficient = -0.326

Figure 12



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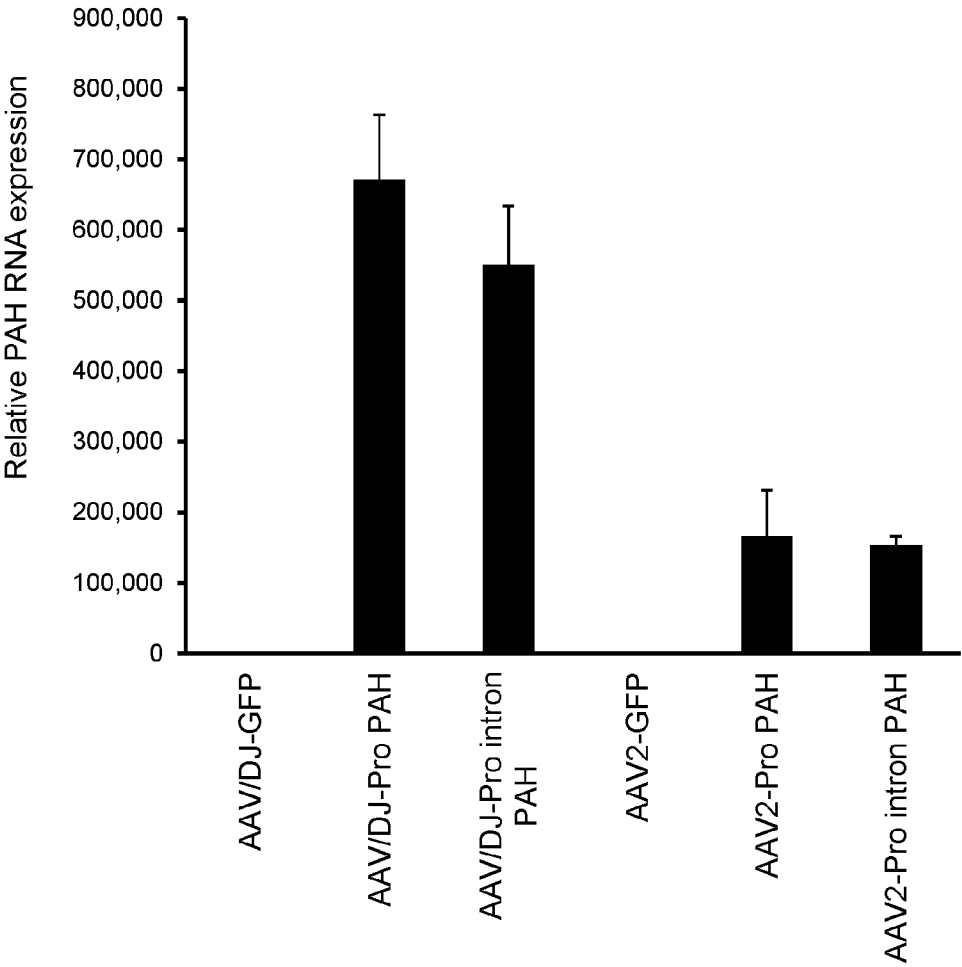


Figure 13D

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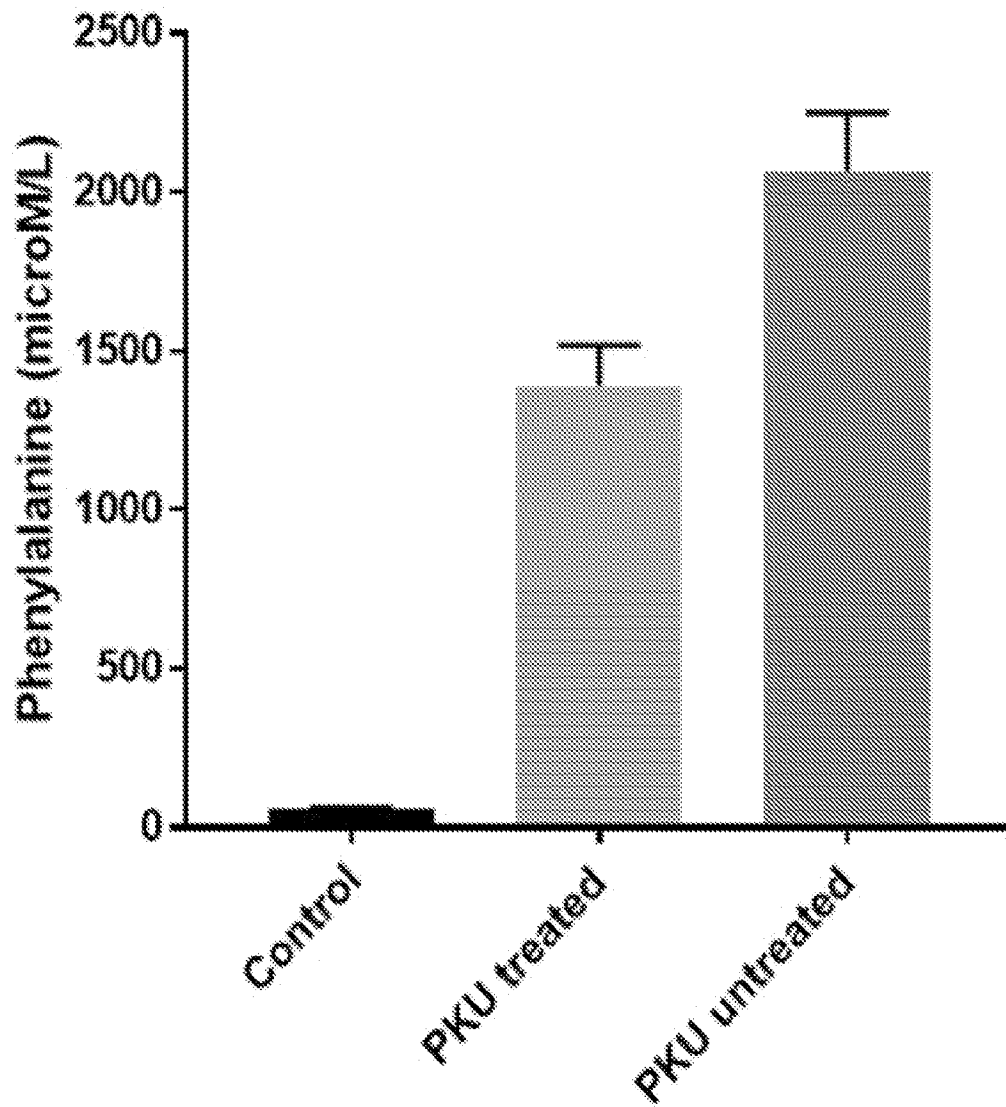


Figure 14

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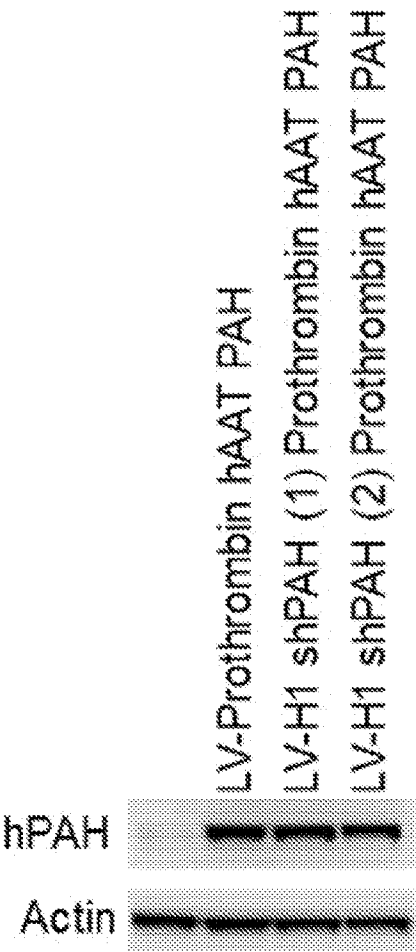


Figure 15