disease modifying and are only effective in a subset of patients, and still require strict dietary restrictions, and non-compliance can lead to neuronal damage. Second, there are no approved gene therapies for PKU, and AAV based therapies cannot be used by 25% to 40% of patients due to pre-existing antibodies. Further, AAV can only be administered once, and the resulting PAH levels might not be high enough to be efficacious, or may be supranormal, dose levels cannot be titrated.

[0015] Accordingly, there is need in the field for a technology that permits expression of a therapeutic PAH protein in a cell, tissue or subject for the treatment of PKU.

SUMMARY~~BRIEF DESCRIPTION~~

[0016] The technology described herein relates to methods and compositions for treatment of Phenylketonuria (PKU) by expression of enzyme phenylalanine hydroxylase (PAH) from a capsid-free (*e.g.,* non-viral) DNA vector with covalently-closed ends (referred to herein as a "closed-ended DNA vector" or a "ceDNA vector"), where the ceDNA vector comprises a PAH nucleic acid sequence that has been codon optimized and combined with particular cis-elements (*e.g.*, specific promoters, specific enhancers and specific promoter and enhancer combinations), and that have been tested for optimal correction of phenylalanine level (*e.g*., expression and duration) in a mouse model of PKU. According to some ~~embodiment~~examples, particular codon optimized PAH nucleic acid sequences perform better when combined with a specific promoter sequence and/or a specific enhancer sequence, compared to the same codon optimized PAH nucleic acid sequence combined with another promoter sequence and/or a specific enhancer sequence. As described by the present disclosure, the constructs comprising codon optimized sequences performed considerably better than native hPAH cDNA sequence, and certain constructs comprising codon optimized sequences and particular *cis*-acting elements showed extended correction through the duration of 28 days of the study, demonstrating durability of expression and efficacy. Surprisingly, it was found that constructs comprising certain promoters (*e.g.*, hAAT CpG minimized promoter) performed better with certain open reading frames (ORFs) (ceDNA412 codop 2 ORF) *in vivo* in the PAH^{enu2} mouse model, while hAAT promoter was generally outcompeted by VD Promoter (VD) or 3X VD *in vitro* or with other ORFs (*e.g.*, luciferase).

[0017] These ceDNA vectors can be used to produce PAH proteins for treatment, monitoring, and diagnosis. The application of ceDNA vectors expressing PAH to a subject for the treatment of PKU is useful to: (i) provide disease modifying levels of PAH enzyme, (ii) be minimally invasive in delivery, (iii) be repeatable and dosed-to-effect, (iv) have rapid onset of therapeutic effect, (v) result in sustained expression of corrective PAH enzyme in the liver, (vi) restoring urea cycle function, phenylalanine metabolism, and/or (vii) be titratable to achieve the appropriate pharmacologic levels of the defective enzyme.

[0018] Accordingly, the disclosure described herein relates to a capsid-free (*e.g.,* non-viral) DNA vector with covalently-closed ends (referred to herein as a "closed-ended DNA vector" or a "ceDNA

vector") comprising a PAH nucleic acid sequence that has been codon optimized and combined with particular cis-acting elements (*e.g.*, specific promoters, specific enhancers and specific promoter and enhancer combinations), to permit expression of the PAH therapeutic protein in a cell.

[0019] In one aspect, disclosed herein is closed-ended DNA (ceDNA) vector comprising: at least one nucleic acid sequence that encodes at least one phenylalanine hydroxylase (PAH) protein, wherein the at least one nucleic acid sequence is selected from a sequence having at least 90% identity a sequence selected from the group consisting of: SEQ ID NO: 425, 431, 387, 435, 437 429, 394, 403, 383, 384-387, 388-393, 395-402, 404-424, 426-428, 430, 432-434, 436, 438-440, and 1011-1015, wherein the at least one nucleic acid sequence is codon optimized, and wherein the at least one nucleic acid sequence is located between flanking inverted terminal repeats (ITRs); and a promoter operatively linked to the least one nucleotide sequence that encodes the at least one PAH protein, wherein the promoter is selected from the group consisting of a human alpha 1-antitrypsin (hAAT) promoter, and a transthyretin (TTR) liver specific promoter, and a promoter set comprising a serpin enhancer (SERP) and a TTR promoter. In one example, the present disclosure provides a closed-ended DNA (ceDNA) vector comprising at least one nucleic acid sequence that encodes at least one PAH protein, wherein the at least one nucleic acid sequence is selected from a sequence having at least 90% identity to any of the sequences listed in Table 1A, wherein the at least one nucleic acid sequence is codon optimized, and wherein the at least one nucleic acid sequence is located between flanking inverted terminal repeats (ITRs); a promoter operatively linked to the least one nucleic acid sequence that encodes the at least one PAH protein, wherein the promoter is selected from the group consisting of the VD promoter, the human alpha 1-antitrypsin (hAAT) promoter (including a sequence having at least 96%, 97%, 98%, 99% or 100% identity to the hAAT(979) promoter (hAAT_core_C10) or other CpG minimized (CpGmin)_hAAT promoters like hAAT_core_C06; hAAT_core_C07; hAAT_core_C08; and hAAT_core_C09) and the transthyretin (TTR) liver specific promoter.

[0020] In one ~~embodiment~~example, the at least one nucleic acid sequence encoding the at least one PAH protein is selected from a sequence having at least 95% identity to any one of the sequences set forth in Table 1A. In one ~~embodiment~~example, the at least one nucleic acid sequence encoding the at least one PAH protein is selected from a sequence having at least 96% identity to any one of the sequences set forth in Table 1A. In one ~~embodiment~~example, the at least one nucleic acid sequence encoding the at least one PAH protein is selected from a sequence having at least 97% identity to any one of the sequences set forth in Table 1A. In one ~~embodiment~~example, the at least one nucleic acid sequence encoding the at least one PAH protein is selected from a sequence having at least 98% identity to any one of the sequences set forth in Table 1A. In one ~~embodiment~~example, the at least one nucleic acid sequence encoding the at least one PAH protein is selected from a sequence having at least 99% identity to any one of the sequences set forth in Table 1A. In one ~~embodiment~~example, the at least one nucleic acid sequence encoding the at least one PAH protein is selected from a sequence comprising any one of the sequences set forth in Table 1A.

[0021] According to another ~~aspect~~example, the disclosure provides a closed-ended DNA (ceDNA) vector comprising a nucleic acid sequence that encodes at least one PAH protein, wherein the nucleic acid sequence is selected from a sequence having at least 95% identity to any of the sequences listed in Table 1A, wherein the at least one nucleic acid sequence is located between flanking inverted terminal repeats (ITRs); and a promoter operatively linked to the nucleic acid sequence that encodes the at least one PAH protein, wherein the promoter is selected from the group consisting of the VD promoter, the human alpha 1-antitrypsin (hAAT) promoter and the transthyretin (TTR) liver specific promoter.

[0022] According to another ~~aspect~~example, the disclosure provides a closed-ended DNA (ceDNA) vector comprising a nucleic acid sequence that encodes at least one PAH protein, wherein the nucleic acid

sequence is selected from a sequence having at least 96% identity to any of the sequences listed in Table 1A, wherein the at least one nucleic acid sequence is located between flanking inverted terminal repeats (ITRs); and a promoter operatively linked to the nucleic acid sequence that encodes the at least one PAH protein, wherein the promoter is selected from the group consisting of the VD promoter, the human alpha 1-antitrypsin (hAAT) promoter and the transthyretin (TTR) liver specific promoter.

[0023] According to another ~~aspect~~example, the disclosure provides a closed-ended DNA (ceDNA) vector comprising a nucleic acid sequence that encodes at least one PAH protein, wherein the nucleic acid sequence is selected from a sequence having at least 97% identity to any of the sequences listed in Table 1A, wherein the at least one nucleic acid sequence is located between flanking inverted terminal repeats (ITRs); and a promoter operatively linked to the nucleic acid sequence that encodes the at least one PAH protein, wherein the promoter is selected from the group consisting of the VD promoter, the human alpha 1-antitrypsin (hAAT) promoter and the transthyretin (TTR) liver specific promoter.

[0024] According to another ~~aspect~~example, the disclosure provides a closed-ended DNA (ceDNA) vector comprising a nucleic acid sequence that encodes at least one PAH protein, wherein the nucleic acid sequence is selected from a sequence having at least 98% identity to any of the sequences listed in Table 1A, wherein the at least one nucleic acid sequence is located between flanking inverted terminal repeats (ITRs); and a promoter operatively linked to the nucleic acid sequence that encodes the at least one PAH protein, wherein the promoter is selected from the group consisting of the VD promoter, the human alpha 1-antitrypsin (hAAT) promoter and the transthyretin (TTR) liver specific promoter.

[0025] According to another ~~aspect~~ example, the disclosure provides a closed-ended DNA (ceDNA) vector comprising a nucleic acid sequence that encodes at least one PAH protein, wherein the nucleic acid sequence is selected from a sequence having at least 99% identity to any of the sequences listed in Table 1A, wherein the at least one nucleic acid sequence is located between flanking inverted terminal repeats (ITRs); and a promoter operatively linked to the nucleic acid sequence that encodes the at least one PAH protein, wherein the promoter is selected from the group consisting of the VD promoter, the human alpha 1-antitrypsin (hAAT) promoter and the transthyretin (TTR) liver specific promoter.

[0026] In one ~~embodiment~~example, the at least one nucleic acid sequence encoding the at least one PAH protein is a sequence having at least 98% identity to the sequence set forth as SEQ ID NO:382. In one ~~embodiment~~example, the at least one nucleic acid sequence encoding the at least one PAH protein is a sequence having at least 99% identity to the sequence set forth as SEQ ID NO:382. In one ~~embodiment~~example, the at least one nucleic acid sequence encoding the at least one PAH protein comprises SEQ ID NO:382 or consists of SEQ ID NO:382.

[0027] In one ~~embodiment~~example, the at least one nucleic acid sequence encoding the at least one PAH protein is a sequence having at least 99% identity to the sequence set forth as SEQ ID NO:425. In one ~~embodiment~~example, the at least one nucleic acid sequence encoding the at least one PAH protein is set forth as SEQ ID NO:425. In one ~~embodiment~~example, the at least one nucleic acid sequence encoding the at least one PAH protein is a sequence having at least 99% identity to the sequence set forth as SEQ ID NO:431. In one ~~embodiment~~example, the at least one nucleic acid sequence encoding the at least one PAH protein is set forth as SEQ ID NO:431. In one ~~embodiment~~example, the at least one nucleic acid sequence encoding the at least one PAH protein is a sequence having at least 99% identity to the sequence set forth as SEQ ID NO:435. In one ~~embodiment~~example, the at least one nucleic acid sequence encoding the at least one PAH protein is set forth as SEQ ID NO:435.

[0028] In one ~~embodiment~~example, the promoter comprises a sequence that is at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to, comprises, or consists of any one of SEQ ID NOs:441-448 and/or a sequence set forth in **Table 7A**.

[0029] In one ~~embodiment~~example, the promoter comprises a nucleic acid sequence having at least 85% identity, at least 90% identity, at least 95% identity, at least 96% identity, at least 97% identity, at least 98% identity, at least 99% identity or comprises SEQ ID NO: 191.

[0030] In one ~~embodiment~~example of the aspects and ~~embodiment~~examples herein, the promoter comprises a nucleic acid sequence having at least 98% identity to SEQ ID NO: 443. In one ~~embodiment~~example of the aspects and ~~embodiment~~examples herein, the promoter comprises a nucleic acid sequence having at least 99% identity to SEQ ID NO: 444. In one ~~embodiment~~example of the aspects and ~~embodiment~~examples herein, the promoter comprises a nucleic acid sequence having at least 99% identity to SEQ ID NO: 445. In one ~~embodiment~~example of the aspects and ~~embodiment~~examples herein, the promoter comprises a nucleic acid sequence having at least 99% identity to SEQ ID NO: 446. In one ~~embodiment~~example of the aspects and ~~embodiment~~examples herein, the promoter comprises a nucleic acid sequence having at least 96% identity to SEQ ID NO: 447. In one ~~embodiment~~example of the aspects and ~~embodiment~~examples herein, the promoter is a promoter set that comprises a nucleic acid sequence having at least 85% identity to SEQ ID NO: 462. In one ~~embodiment~~example of the aspects and ~~embodiment~~examples herein, the promoter is a promoter set that comprises a nucleic acid sequence having at least 85% identity to SEQ ID NO: 467. In one ~~embodiment~~example of the aspects and ~~embodiment~~examples herein, the promoter is a promoter set that comprises a nucleic acid sequence having at least 85% identity to SEQ ID NO: 470. In one ~~embodiment~~example of the aspects and ~~embodiment~~examples herein, the promoter is a promoter set that comprises a nucleic acid sequence having at least 90% identity to SEQ ID NO: 470. In one ~~embodiment~~example of the aspects and ~~embodiment~~examples herein, the promoter is a promoter set that comprises a nucleic acid sequence having at least 95% identity to SEQ ID NO: 470.

[0031] In one ~~embodiment~~example, the ceDNA vector further comprises an enhancer. In one ~~embodiment~~example, the enhancer comprises a sequence that is at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to, comprises, or consists of any one of SEQ ID NOs:449-461 and/or a sequence set forth in **Table**

8A. In one ~~embodiment~~example, the enhancer is selected from the group consisting of a serpin enhancer, a 3xHNF1-4_ProEnh_10mer, and a 5xHNF1_ProEnh_10mer. In one ~~embodiment~~example, the enhancer comprises a nucleic acid sequence having at least 85% identity to SEQ ID NO: 450. In one ~~embodiment~~examples, the enhancer comprises a nucleic acid sequence having at least 85% identity to SEQ ID NO: 586. In one ~~embodiment~~example, the enhancer comprises a nucleic acid sequence having at least 85% identity to SEQ ID NO: 587.

[0032] In one ~~embodiment~~example, the ceDNA vector further comprises one or more introns. In one ~~embodiment~~example, the one or more introns comprises a sequence that is at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to, comprises, or consists of any one of SEQ ID NOs:509-516 and 1000 and/or a sequence set forth in **Table 11A**. In one ~~embodiment~~example, the one or more introns is the minute virus of mice (MVM).

[0033] In one ~~embodiment~~example, the ceDNA vector comprises a 3' untranslated region (3' UTR). In one ~~embodiment~~example, the 3' UTR comprises a sequence that is at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to, comprises, or consists of any one of SEQ ID NOs:517-525 and/or a sequence set forth in **Table 12**.

[0034] In one ~~embodiment~~example, the ceDNA vector comprises a 5' untranslated region (5' UTR). In one ~~embodiment~~example, the 5' UTR comprises a sequence that is at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to, comprises, or consists of any one of SEQ ID NOs:482-508 and/or a sequence set forth in **Table 10**.

[0035] In one ~~embodiment~~example, the ceDNA vector comprises at least one polyA sequence.

[0036] In one ~~embodiment~~example, the VD promoter comprises a SERP enhancer. In one ~~embodiment~~example, the VD promoter comprises a 3xSERP enhancer.

[0037] In one ~~embodiment~~example, the promoter is the TTR liver promoter and the ceDNA further comprises an MVM intron.

[0038] In one ~~embodiment~~example, the ceDNA vector comprises a nucleic acid sequence that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to, comprises, or consists of a nucleic acid sequence selected from the group consisting of: SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197, SEQ ID NO: 198, SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 541, SEQ ID NO: 542, SEQ ID NO: 543, SEQ ID NO: 544, SEQ ID NO: 545, SEQ ID NO: 546, SEQ ID NO: 547, SEQ ID NO: 548, SEQ ID NO: 549, SEQ ID NO: 550, SEQ ID NO: 551, SEQ ID NO: 552, SEQ ID NO: 553, SEQ ID NO: 554, SEQ ID NO: 555, SEQ ID NO: 556, SEQ ID NO: 557, SEQ ID NO: 558, SEQ ID NO: 559, SEQ ID NO: 560, SEQ ID NO: 561, SEQ ID NO: 562, SEQ ID NO: 563, SEQ ID NO: 564, SEQ ID NO: 565, SEQ ID NO: 566, SEQ ID NO: 567, SEQ ID NO: 570, SEQ ID NO: 571, SEQ ID NO: 572, SEQ ID NO: 573, SEQ ID NO: 574, SEQ ID NO: 575, SEQ ID NO: 576, SEQ ID NO: 577, SEQ ID NO: 578, SEQ

ID NO: 579, SEQ ID NO: 580, SEQ ID NO: 581, SEQ ID NO: 582, SEQ ID NO: 583, and SEQ ID NO: 584.

[0039] In one ~~embodiment~~example, the at least one nucleic acid sequence is cDNA for PAH.

[0040] In one ~~embodiment~~example, at least one ITR comprises a functional terminal resolution site (TRS) and a Rep binding site. In one ~~embodiment~~example, one or both of the ITRs are from a virus selected from a parvovirus, a dependovirus, and an adeno-associated virus (AAV). In one ~~embodiment~~example, the flanking ITRs are symmetric or asymmetric. In one ~~embodiment~~example, the flanking ITRs are symmetrical or substantially symmetrical. In one ~~embodiment~~example, the flanking ITRs are asymmetric. In one ~~embodiment~~example, one or both of the ITRs are wild type, or wherein both of the ITRs are wild-type. In one ~~embodiment~~example, the flanking ITRs are from different viral serotypes. In one ~~embodiment~~example, the flanking ITRs are from a pair of viral serotypes shown in Table 2. In one ~~embodiment~~example, one or both of the ITRs comprises a sequence selected from the sequences in Table 3, Table 5A, Table 5B, or Table 6.

[0041] In one ~~embodiment~~example, at least one of the ITRs is altered from a wild-type AAV ITR sequence by a deletion, addition, or substitution that affects the overall three-dimensional conformation of the ITR. In one ~~embodiment~~example, one or both of the ITRs are derived from an AAV serotype selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, and AAV12.

[0042] In one ~~embodiment~~example, one or both of the ITRs are synthetic. In one ~~embodiment~~example, one or both of the ITRs is not a wild type ITR, or wherein both of the ITRs are not wild-type.

[0043] In one ~~embodiment~~example, one or both of the ITRs is modified by a deletion, insertion, and/or substitution in at least one of the ITR regions selected from A, A', B, B', C, C', D, and D'. In one ~~embodiment~~example, the deletion, insertion, and/or substitution results in the deletion of all or part of a stem-loop structure normally formed by the A, A', B, B' C, or C' regions.

[0044] In one ~~embodiment~~example, one or both of the ITRs are modified by a deletion, insertion, and/or substitution that results in the deletion of all or part of a stem-loop structure normally formed by the B and B' regions. In one ~~embodiment~~example, one or both of the ITRs are modified by a deletion, insertion, and/or substitution that results in the deletion of all or part of a stem-loop structure normally formed by the C and C' regions. In one ~~embodiment~~example, one or both of the ITRs are modified by a deletion, insertion, and/or substitution that results in the deletion of part of a stem-loop structure normally formed by the B and B' regions and/or part of a stem-loop structure normally formed by the C and C' regions. In one ~~embodiment~~example, one or both of the ITRs comprise a single stem-loop structure in the region that normally comprises a first stem-loop structure formed by the B and B' regions and a second stem-loop structure formed by the C and C' regions. In one ~~embodiment~~example, one or both of the ITRs comprise a single stem and two loops in the region that normally comprises a first stem-loop structure formed by the B and B' regions and a second stem-loop structure formed by the C and C' regions.

[0045] In one ~~embodiment~~example, one or both of the ITRs comprise a single stem and a single loop in the region that normally comprises a first stem-loop structure formed by the B and B' regions and a second stem-loop structure formed by the C and C' regions.

[0046] In one ~~embodiment~~example, both ITRs are altered in a manner that results in an overall three-dimensional symmetry when the ITRs are inverted relative to each other.

[0047] In one ~~embodiment~~example, the ceDNA vector comprises a nucleic acid sequence that is at least 90% identical to SEQ ID NOs: 382-440 or SEQ ID NOs: 1011-1015. In one ~~embodiment~~example, the ceDNA vector comprises a nucleic acid sequence that is at least 95% identical to SEQ ID NOs: 382-440, or SEQ ID NOs: 1011-1015. In one ~~embodiment~~example, the ceDNA vector comprises a nucleic acid sequence that is at least 96% identical to SEQ ID NOs: 382-440 or SEQ ID NOs: 1011-1015. In one ~~embodiment~~example, the ceDNA vector comprises a nucleic acid sequence that is at least 97% identical to SEQ ID NOs: 382-440 or SEQ ID NOs: 1011-1015. In one ~~embodiment~~example, the ceDNA vector comprises a nucleic acid sequence that is at least 98% identical to SEQ ID NOs: 382-440 or SEQ ID NOs: 1011-1015. In one ~~embodiment~~example, the ceDNA vector comprises a nucleic acid sequence that is at least 99% identical to SEQ ID NOs: 382-440 or SEQ ID NOs: 1011-1015.

[0048] In another aspect, disclosed herein is the use of the ceDNA vector as disclosed herein in the manufacture of a medicament for expressing a PAH protein in a cell. In one example, the present disclosure provides a method of expressing a PAH protein in a cell, the method comprising contacting the cell with a ceDNA vector disclosed herein. In one ~~embodiment~~example, the cell is a photoreceptor or a RPE cell. In one ~~embodiment~~example, the cell is contacted *in vitro* or *in vivo*.

[0049] In another aspect, disclosed herein is the use of the ceDNA vector as disclosed herein in the manufacture of a medicament for treating a subject with phenylketonuria (PKU). In one example, the present disclosure provides a method of treating a subject with phenylketonuria (PKU), the method comprising administering to the subject a ceDNA vector disclosed herein. In one ~~embodiment~~example, the at least one nucleic acid sequence that encodes at least one PAH protein is selected from a sequence having at least 90% identity with any of the sequences set forth in Table 1A. In one ~~embodiment~~example, the subject exhibits at least about a 50% decrease in level of serum phenylalanine as compared to a level of serum phenylalanine in the subject prior to administration. In one ~~embodiment~~example, the subject exhibits at least about a 10% increase in PAH activity after administration as compared to a level of PAH activity prior to administration.

[0050] In one ~~embodiment~~example, the ceDNA vector is formulated in lipid nanoparticles. In one ~~embodiment~~example, the ceDNA vector is administered intravenously. In one ~~embodiment~~example, the ceDNA vector is adnistered intramuscularly. In one ~~embodiment~~example, the ceDNA vector is administered by infusion.

[0051] In another aspect, disclosed herein is pharmaceutical composition comprising the ceDNA vector as disclosed herein. In one example, the present disclosure provides a pharmaceutical composition comprising a ceDNA vector.

[0052] In another aspect, disclosed herein is composition comprising a ceDNA vector as disclosed herein and a lipid, wherein the lipid is a lipid nanoparticle (LNP). In one example, the present disclosure provides a composition comprising a ceDNA vector and a lipid. In one ~~embodiment~~example, the lipid is a lipid nanoparticle (LNP).

[0053] In another aspect, disclosed herein is a kit comprising a ceDNA vector disclosed herein, a pharmaceutical composition disclosed herein, or a composition disclosed herein. In one ~~embodiment~~example, the kit comprises instructions for use.

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

CLAIMS

1. A closed-ended DNA (ceDNA) vector comprising:

at least one nucleic acid sequence that encodes at least one phenylalanine hydroxylase (PAH) protein, wherein the at least one nucleic acid sequence is selected from a sequence having at least 90% identity a sequence selected from the group consisting of: SEQ ID NO: 425, 431, 387, 435, 437 429, 394, 403, 383, 384-387, 388-393, 395-402, 404-424, 426-428, 430, 432-434, 436, 438-440, and 1011-1015~~to any of the sequences listed in Table 1A~~, wherein the at least one nucleic acid sequence is codon optimized, and wherein the at least one nucleic acid sequence is located between flanking inverted terminal repeats (ITRs); and

a promoter operatively linked to the least one nucleotide sequence that encodes the at least one PAH protein, wherein the promoter is selected from the group consisting of ~~the VD promoter, the~~ a human alpha 1-antitrypsin (hAAT) promoter, ~~(including the hAAT(979) promoter and the CpGmin_hAAT promoter)~~ and ~~the~~ a transthyretin (TTR) liver specific promoter, and a promoter set comprising a serpin enhancer (SERP) and a TTR promoter.

2. The ceDNA vector of claim 1, wherein the hAAT promoter is selected from the group consisting of an hAAT(979) promoter and a CpGmin_hAAT promoter.

~~2. The ceDNA vector of claim 1, wherein the at least one nucleic acid sequence encoding the at least one PAH protein is selected from a sequence having at least 95% identity to any one of the sequences set forth in Table 1A.~~

~~3. A closed-ended DNA (ceDNA) vector comprising:~~

~~a nucleic acid sequence that encodes at least one PAH protein, wherein the nucleic acid sequence is selected from a sequence having at least 95% identity to any of the sequences listed in Table 1A, wherein the at least one nucleic acid sequence is located between flanking inverted terminal repeats (ITRs); and~~

~~a promoter operatively linked to the nucleic acid sequence that encodes the at least one PAH protein, wherein the promoter is selected from the group consisting of the VD promoter, the human alpha 1-antitrypsin (hAAT) promoter and the transthyretin (TTR) liver specific promoter.~~

~~4. A closed-ended DNA (ceDNA) vector comprising:~~

~~a nucleic acid sequence that encodes at least one PAH protein, wherein the nucleic acid sequence is selected from a sequence having at least 96% identity to any of the sequences listed in Table 1A, wherein the at least one nucleic acid sequence is located between flanking~~

~~inverted terminal repeats (ITRs); and~~

~~a promoter operatively linked to the nucleic acid sequence that encodes the at least one PAH protein, wherein the promoter is selected from the group consisting of the VD promoter, the human alpha 1-antitrypsin (hAAT) promoter and the transthyretin (TTR) liver specific promoter.~~

~~5. A closed-ended DNA (ceDNA) vector comprising:~~

~~a nucleic acid sequence that encodes at least one PAH protein, wherein the nucleic acid sequence is selected from a sequence having at least 97% identity to any of the sequences listed in Table 1A, wherein the at least one nucleic acid sequence is located between flanking inverted terminal repeats (ITRs); and~~

~~a promoter operatively linked to the nucleic acid sequence that encodes the at least one PAH protein, wherein the promoter is selected from the group consisting of the VD promoter, the human alpha 1-antitrypsin (hAAT) promoter and the transthyretin (TTR) liver specific promoter.~~

~~6. A closed-ended DNA (ceDNA) vector comprising:~~

~~a nucleic acid sequence that encodes at least one PAH protein, wherein the nucleic acid sequence is selected from a sequence having at least 98% identity to any of the sequences listed in Table 1A, wherein the at least one nucleic acid sequence is located between flanking inverted terminal repeats (ITRs); and~~

~~a promoter operatively linked to the nucleic acid sequence that encodes the at least one PAH protein, wherein the promoter is selected from the group consisting of the VD promoter, the human alpha 1-antitrypsin (hAAT) promoter and the transthyretin (TTR) liver specific promoter.~~

~~7. A closed-ended DNA (ceDNA) vector comprising:~~

~~a nucleic acid sequence that encodes at least one PAH protein, wherein the nucleic acid sequence is selected from a sequence having at least 99% identity to any of the sequences listed in Table 1A, wherein the at least one nucleic acid sequence is located between flanking inverted terminal repeats (ITRs); and~~

~~a promoter operatively linked to the nucleic acid sequence that encodes the at least one PAH protein, wherein the promoter is selected from the group consisting of the VD promoter, the human alpha 1-antitrypsin (hAAT) promoter and the transthyretin (TTR) liver specific promoter.~~

~~8.~~3. The ceDNA vector of ~~any one of~~ claim~~s~~ 1~~-7~~ or 2, wherein the at least one nucleic acid

sequence encoding the at least one PAH protein ~~is a sequence having~~has at least 98% identity to the sequence set forth as SEQ ID NO:382.

~~9. The ceDNA vector of any one of claims 1-7, wherein the at least one nucleic acid sequence encoding the at least one PAH protein is a sequence having at least 99% identity to the sequence set forth as SEQ ID NO:382.~~

~~10.~~4. The ceDNA vector of any one of claims 1-~~7~~3, wherein the at least one nucleic acid sequence encoding the at least one PAH protein is set forth as SEQ ID NO:382.

~~11.~~5. The ceDNA vector of any one of claims 1-~~7~~3, wherein the at least one nucleic acid sequence encoding the at least one PAH protein ~~is a sequence having~~has at least 99% identity to the sequence set forth as SEQ ID NO:425.

~~12.~~6. The ceDNA vector of any one of claims 1-~~7~~3, wherein the at least one nucleic acid sequence encoding the at least one PAH protein is set forth as SEQ ID NO:425.

~~13.~~7. The ceDNA vector of any one of claims 1-~~7~~3, wherein the at least one nucleic acid sequence encoding the at least one PAH protein ~~is a sequence having~~has at least 99% identity to the sequence set forth as SEQ ID NO:431.

~~14.~~8. The ceDNA vector of any one of claims 1-~~7~~3, wherein the at least one nucleic acid sequence encoding the at least one PAH protein is set forth as SEQ ID NO:431.

~~15.~~9. The ceDNA vector of any one of claims 1-~~7~~3, wherein the at least one nucleic acid sequence encoding the at least one PAH protein ~~is a sequence having~~has at least 99% identity to the sequence set forth as SEQ ID NO:435.

~~16.~~10. The ceDNA vector of any one of claims 1-~~7~~3, wherein the at least one nucleic acid sequence encoding the at least one PAH protein is set forth as SEQ ID NO:435.

~~17. The ceDNA vector of any one of claims 1-7, wherein the promoter comprises a nucleic acid sequence having at least 85% identity to SEQ ID NO: 191.~~

~~18.~~11. The ceDNA vector of any one of claims 1-~~7~~3, wherein the promoter comprises a nucleic acid sequence having at least 98% identity to SEQ ID NO: 443.

~~19.~~12. The ceDNA vector of any one of claims 1-~~7~~3, wherein the promoter comprises a nucleic

acid sequence having at least 99% identity to SEQ ID NO: 444.

~~20.~~13. The ceDNA vector of any one of claims 1-~~7~~3, wherein the promoter comprises a nucleic acid sequence having at least 99% identity to SEQ ID NO: 445.

~~21.~~14. The ceDNA vector of any one of claims 1-~~7~~3, wherein the promoter comprises a nucleic acid sequence having at least 99% identity to SEQ ID NO: 446.

~~22.~~15. The ceDNA vector of any one of claims 1-~~7~~3, wherein the promoter comprises a nucleic acid sequence having at least 96% identity to SEQ ID NO: 447.

16. The ceDNA vector of any one of claims 1-3, wherein the promoter comprises a nucleic acid sequence having at least 85% identity to SEQ ID NO: 191.

~~23.~~17. The ceDNA vector of any one of claims 1 to ~~22~~16, wherein the ceDNA vector further comprises an enhancer.

~~24.~~18. The ceDNA vector of claim ~~23~~17, wherein the enhancer is selected from the group consisting of a serpin enhancer, a 3xHNF1-4_ProEnh_10mer, and a 5xHNF1_ProEnh_10mer.

~~25.~~19. The ceDNA vector of claim 17~~23~~, wherein the enhancer comprises a nucleic acid sequence having at least 85% identity to a sequence selected from the group consisting of: SEQ ID NO: 450, SEQ ID NO: 586, and SEQ ID NO: 587.

~~26. The ceDNA vector of claim 23, wherein the enhancer comprises a nucleic acid sequence having at least 85% identity to SEQ ID NO: 586.~~

~~27. The ceDNA vector of claim 23, wherein the enhancer comprises a nucleic acid sequence having at least 85% identity to SEQ ID NO: 587.~~

~~28.~~20. The ceDNA vector of claim any one of claims 1-~~7~~3, wherein the promoter is a promoter set that comprises a nucleic acid sequence having at least 85% identity to a sequence selected from the group consisting of: SEQ ID NO: 462, SEQ ID NO: 467, and SEQ ID NO: 470.

~~29. The ceDNA vector of claim any one of claims 1-7, wherein the promoter is a promoter set~~

~~that comprises a nucleic acid sequence having at least 85% identity to SEQ ID NO: 467.~~

~~30. The ceDNA vector of claim any one of claims 1-7, wherein the promoter is a promoter set that comprises a nucleic acid sequence having at least 85% identity to SEQ ID NO: 470.~~

~~31. The ceDNA vector of claim any one of claims 1-7, wherein the promoter is a promoter set that comprises a nucleic acid sequence having at least 90% identity to SEQ ID NO: 470.~~

~~32. The ceDNA vector of claim any one of claims 1-7, wherein the promoter is a promoter set that comprises a nucleic acid sequence having at least 95% identity to SEQ ID NO: 470.~~

~~33.~~21. The ceDNA vector of any one of claims 1 to ~~32~~20, wherein the ceDNA vector further comprises one or more introns.

~~34.~~22. The ceDNA vector of claim ~~33~~21, wherein the one or more introns is the minute virus of mice (MVM) intron.

~~35.~~23. The ceDNA vector of any one of claims 1 to ~~34~~22, wherein the ceDNA vector comprises a 3' untranslated region (3' UTR).

~~36.~~24. The ceDNA vector of any one of claims 1 to ~~35~~23, wherein the ceDNA vector comprises at least one polyA sequence.

~~37. The ceDNA vector of any one of claims 1-7, wherein the VD promoter comprises a SERP enhancer.~~

~~38.~~25. The ceDNA vector of any one of claims 1-~~7~~3, wherein the ~~VD~~ promoter set comprising a SERP enhancer comprises a 3xSERP enhancer.

~~39.~~26. The ceDNA vector of any one of claims 1-~~7~~3, wherein the promoter is the TTR liver promoter and the ceDNA further comprises an MVM intron.

~~40.~~27. The ceDNA vector of any one of claims 1-~~39~~26, wherein the ceDNA vector comprises a nucleic acid sequence that is at least 90% identical to a nucleic acid sequence selected from the group consisting of: SEQ ID NO: 583, SEQ ID NO: 581, SEQ ID NO: 571, SEQ ID NO: 574, SEQ ID NO: 575, SEQ ID NO: 546, SEQ ID NO: 562, SEQ ID NO: 564, SEQ ID NO: 213, SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO:

197, SEQ ID NO: 198, SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, SEQ ID NO: 205, SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, SEQ ID NO: 209, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 212, ~~SEQ ID NO: 213,~~ SEQ ID NO: 541, SEQ ID NO: 542, SEQ ID NO: 543, SEQ ID NO: 544, SEQ ID NO: 545, ~~SEQ ID NO: 546,~~ SEQ ID NO: 547, SEQ ID NO: 548, SEQ ID NO: 549, SEQ ID NO: 550, SEQ ID NO: 551, SEQ ID NO: 552, SEQ ID NO: 553, SEQ ID NO: 554, SEQ ID NO: 555, SEQ ID NO: 556, SEQ ID NO: 557, SEQ ID NO: 558, SEQ ID NO: 559, SEQ ID NO: 560, SEQ ID NO: 561, ~~SEQ ID NO: 562,~~ SEQ ID NO: 563, ~~SEQ ID NO: 564,~~ SEQ ID NO: 565, SEQ ID NO: 566, SEQ ID NO: 567, SEQ ID NO: 570, ~~SEQ ID NO: 571,~~ SEQ ID NO: 572, SEQ ID NO:573, ~~SEQ ID NO: 574, SEQ ID NO: 575,~~ SEQ ID NO: 576, SEQ ID NO: 577, SEQ ID NO: 578, SEQ ID NO: 579, SEQ ID NO:580, ~~SEQ ID NO: 581,~~ SEQ ID NO: 582, ~~SEQ ID NO:583,~~ and SEQ ID NO:584.

~~41.~~28. The ceDNA vector of any one of claims 1-~~40~~27, wherein the at least one nucleic acid sequence is a cDNA ~~for~~ encoding PAH.

~~42.~~29. The ceDNA vector of any one of claims 1-~~41~~28, wherein at least one flanking ITR comprises a functional terminal resolution site (TRS) and a Rep binding site.

~~43.~~30. The ceDNA vector of any one of claims 1-~~42~~29, wherein one or both of the flanking ITRs are derived from a virus selected from the group consisting of: a ~~parvovirus~~*Parvovirus*, a ~~dependovirus~~*Dependovirus*, and an adeno-associated virus (AAV).

31. The ceDNA vector of any one of claims 1-~~43~~30, wherein:

~~44.~~ the flanking ITRs are symmetric or asymmetric;~~.~~

~~45. The ceDNA vector of claim 44, wherein~~ the flanking ITRs are ~~symmetrical or~~ substantially symmetric~~al.~~;

~~46. The ceDNA vector of claim 45, wherein~~ the flanking ITRs are asymmetric;~~.~~

~~47. The ceDNA vector of any one of claims 1-46, wherein~~ one ~~or both~~ of the flanking ITRs ~~are~~ is a wild type ITR, or wherein both of the flanking ITRs are wild-type ITRs;~~.~~

~~48. The ceDNA vector of any one of claims 1-47, wherein~~ both of the flanking ITRs are

derived from ~~wild-type of~~ the same AAV serotype.

~~49. The ceDNA vector of claim 48, wherein~~ both of the flanking ITRs are derived from ~~wild-type of~~ AAV2~~.~~.

~~50. The ceDNA vector of any one of claims 1-49, wherein~~ the flanking ITRs are derived from different viral serotypes~~.~~.

~~51. The ceDNA vector of any one of claims 1-50, wherein~~ the flanking ITRs are derived from a pair of viral serotypes selected from the group consisting of the pairs of viral serotypes set forth ~~shown~~ in Table 2; and/or~~.~~

~~52. The ceDNA vector of any one of claims 1-51, wherein~~ one or both of the flanking ITRs comprises a sequence selected from the the group consisting of: the sequences set forth in Table 3, Table 5A, Table 5B, or Table 6.

~~53.~~32. The ceDNA vector of any one of claims 1-~~52~~31, wherein at least one of the flanking ITRs is altered from a wild-type AAV ITR sequence by a deletion, an addition, or a substitution that affects the overall three-dimensional conformation of the ITR.

~~54.~~33. The ceDNA vector of any one of claims 1-~~53~~32, wherein one or both of the flanking ITRs are derived from an AAV serotype selected from the group consisting of: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, and AAV12.

~~55.~~34. The ceDNA vector of any one of claims 1-~~54~~33, wherein one or both of the flanking ITRs are synthetic.

~~56.~~35. The ceDNA vector of any one of claims 1-~~55~~34, wherein one ~~or both~~ of the flanking ITRs is not a wild~~-~~type ITR, or wherein both of the flanking ITRs are not wild-type ITRs.

~~57.~~36. The ceDNA vector of any one of claims 1-~~56~~35, wherein one or both of the flanking ITRs is modified by a deletion, an insertion, and/or a substitution in at least one of the ITR regions selected from the group consisting of: the A, A', B, B', C, C', D, and D' regions.

~~58.~~37. The ceDNA vector of claim ~~57~~36, wherein the deletion, insertion, and/or substitution results in the deletion of all or part of a stem-loop structure ~~normally~~ formed by the A, A', B, B' C, or C' regions.

~~59.~~38. The ceDNA vector of any one of claims 1-~~58~~37, wherein one or both of the flanking ITRs are modified by a deletion, an insertion, and/or a substitution that results in the deletion of all or part of a stem-loop structure ~~normally~~ formed by the B and B' regions;~~.~~

one or both of the flanking ITRs are modified by a deletion, an insertion, and/or a substitution that results in the deletion of all or part of a stem-loop structure formed by the C and C' regions;

one or both of the flanking ITRs are modified by a deletion, an insertion, and/or a substitution that results in the deletion of all or part of a stem-loop structure formed by the B and B' regions and/or all or part of a stem-loop structure formed by the C and C' regions;

one or both of the flanking ITRs comprise a single stem-loop structure in the region that, in a wild-type ITR, would comprise a first stem-loop structure formed by the B and B' regions and a second stem-loop structure formed by the C and C' regions;

one or both of the flanking ITRs comprise a single stem and two loops in the region that, in a wild-type ITR, would comprise a first stem-loop structure formed by the B and B' regions and a second stem-loop structure formed by the C and C' regions;

one or both of the flanking ITRs comprise a single stem and a single loop in the region that, in a wild-type ITR, would comprise a first stem-loop structure formed by the B and B' regions and a second stem-loop structure formed by the C and C' regions; or

both flanking ITRs are altered in a manner that results in an overall three-dimensional symmetry when the flanking ITRs are inverted relative to each other.

~~60. The ceDNA vector of any one of claims 1-59, wherein one or both of the ITRs are modified by a deletion, insertion, and/or substitution that results in the deletion of all or part of a stem-loop structure normally formed by the C and C' regions.~~

~~61. The ceDNA vector of any one of claims 1-60, wherein one or both of the ITRs are modified by a deletion, insertion, and/or substitution that results in the deletion of part of a stem-loop structure normally formed by the B and B' regions and/or part of a stem-loop structure normally formed by the C and C' regions.~~

~~62. The ceDNA vector of any one of claims 1-61, wherein one or both of the ITRs comprise a single stem-loop structure in the region that normally comprises a first stem-loop structure formed by the B and B' regions and a second stem-loop structure formed by the C and C' regions.~~

~~63. The ceDNA vector of any one of claims 1-62, wherein one or both of the ITRs comprise a single stem and two loops in the region that normally comprises a first stem-loop structure~~

~~formed by the B and B' regions and a second stem-loop structure formed by the C and C' regions.~~

~~64. The ceDNA vector of any one of claims 1-63, wherein one or both of the ITRs comprise a single stem and a single loop in the region that normally comprises a first stem-loop structure formed by the B and B' regions and a second stem-loop structure formed by the C and C' regions.~~

~~65. The ceDNA vector of any one of claims 1-64, wherein both ITRs are altered in a manner that results in an overall three-dimensional symmetry when the ITRs are inverted relative to each other.~~

~~66.~~39. The ceDNA vector of any one of claims 1-~~65~~38, wherein the ceDNA vector comprises a nucleic acid sequence that is at least 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 382, SEQ ID NO:383, SEQ ID NO: 384, SEQ ID NO: 385 or SEQ ID NO:386.

~~67. The ceDNA vector of any one of claims 1-65, wherein the ceDNA vector comprises a nucleic acid sequence that is at least 95% identical to SEQ ID NO: 382, SEQ ID NO:383, SEQ ID NO: 384, SEQ ID NO: 385 or SEQ ID NO:386.~~

~~68. The ceDNA vector of any one of claims 1-65, wherein the ceDNA vector comprises a nucleic acid sequence that is at least 96% identical to SEQ ID NO: 382, SEQ ID NO:383, SEQ ID NO: 384, SEQ ID NO: 385 or SEQ ID NO:386.~~

~~69. The ceDNA vector of any one of claims 1-65, wherein the ceDNA vector comprises a nucleic acid sequence that is at least 97% identical to SEQ ID NO: 382, SEQ ID NO:383, SEQ ID NO: 384, SEQ ID NO: 385 or SEQ ID NO:386.~~

~~70. The ceDNA vector of any one of claims 1-65, wherein the ceDNA vector comprises a nucleic acid sequence that is at least 98% identical to SEQ ID NO: 382, SEQ ID NO:383, SEQ ID NO: 384, SEQ ID NO: 385 or SEQ ID NO:386.~~

~~71. The ceDNA vector of any one of claims 1-65, wherein the ceDNA vector comprises a nucleic acid sequence that is at least 99% identical to SEQ ID NO: 382, SEQ ID NO:383, SEQ ID NO: 384, SEQ ID NO: 385 or SEQ ID NO:386.~~

~~72.~~40. Use of the ceDNA vector of any one of claims 1-39 in the manufacture of a medicament for ~~A~~

~~method of~~ expressing a PAH protein in a cell~~, the method comprising contacting the cell with the ceDNA vector of any one of claims 1-71~~.

~~73.~~41. The ~~method~~ use of claim ~~72~~40, wherein the cell is a liver cell, a photoreceptor cell, or a retinal pigment epithelial (RPE) cell.

~~74.~~42. The ~~method~~ use of claim ~~72-~~40 or ~~73~~41, wherein the cell is~~in~~ *in vitro* or *in vivo*.

~~75.~~43. Use of the ceDNA vector of any one of claims 1-39 in the manufacture of a medicament for ~~A method of~~ treating a subject with phenylketonuria (PKU)~~, the method comprising administering to the subject a ceDNA vector of any one of claims 1-71~~.

~~76.~~44. The ~~method~~ use of claim ~~75~~43, wherein the least one nucleic acid sequence that encodes at least one PAH protein is selected from a sequence selected from the group consisting of: SEQ ID NO: 425, 431, 387, 435, 437 429, 394, 403, 383, 384-387, 388-393, 395-402, 404-424, 426-428, 430, 432-434, 436, 438-440, and 1011-1015~~having at least 90% identity with any of the sequences set forth in Table 1A~~.

~~77.~~45. The ~~method~~ use of any one of claims ~~72-76~~40-44, wherein the subject exhibits at least about a 50% decrease in level of serum phenylalanine as compared to a level of serum phenylalanine in the subject prior to administration~~.~~

~~78. The method of any one of claims 72-77,~~ or wherein the subject exhibits at least about a 10% increase in PAH activity after administration as compared to a level of PAH activity prior to administration.

~~79.~~46. The ~~method~~ use of any one of claims ~~72-78~~40-45, wherein the ceDNA vector is formulated in lipid nanoparticles.

~~80.~~47. The ~~method~~ use of any one of claims ~~72-79~~40-46, wherein the ceDNA vector is to be administered intravenously, intramuscularly or by infusion.

~~81. The method of any one of claims 72-79, wherein the ceDNA vector is administered intramuscularly.~~

~~82. The method of any one of claims 72-79, wherein the ceDNA vector is administered by infusion.~~

~~83.~~48. A pharmaceutical composition comprising the ceDNA vector of any one of claims 1-~~71~~39.

~~84.~~49. A composition comprising a ceDNA vector of any one of claims 1-~~71~~ 39 and a lipid.~~,~~

~~85. The composition of claim 84,~~ wherein the lipid is a lipid nanoparticle (LNP).

~~86.~~50. A kit comprising the ceDNA vector of any one of claims 1-~~71~~39, the pharmaceutical composition of claim ~~83~~48, or the composition of claim ~~84 or 85~~49.