

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 has at least 90% identity to SEQ ID NO: 425, 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 nucleic acid 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 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 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 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 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 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 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 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 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

[0045] In one 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 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 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 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 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 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 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 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 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 example, the cell is a photoreceptor or a RPE cell. In one 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 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 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 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 example, the ceDNA vector is formulated in lipid nanoparticles. In one example, the ceDNA vector is administered intravenously. In one example, the ceDNA vector is administered intramuscularly. In one 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 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 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 has at least 90% identity to SEQ ID NO: 425, 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 nucleic acid 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.
2. The ceDNA vector of claim 1, wherein the hAAT promoter is selected from the group consisting of SEQ ID NOs 443, 444, 445, 446 and 447.
3. The ceDNA vector of any one of claims 1 or 2, wherein the at least one nucleic acid sequence encoding the at least one PAH protein has at least 95%, 96%, 97%, 98% or 99% identity to the sequence set forth as SEQ ID NO: 425.
4. The ceDNA vector of any one of claims 1- 3, wherein the at least one nucleic acid sequence encoding the at least one PAH protein has at least 99% identity to the sequence set forth as SEQ ID NO:425.
5. The ceDNA vector of any one of claims 1-3, wherein the at least one nucleic acid sequence encoding the at least one PAH protein is set forth as SEQ ID NO:425.
6. The ceDNA vector of any one of claims 1-3, wherein the promoter comprises a nucleic acid sequence having at least 98% identity to SEQ ID NO: 443.
7. The ceDNA vector of any one of claims 1-3, wherein the promoter comprises a nucleic acid sequence having at least 99% identity to SEQ ID NO: 444.
8. The ceDNA vector of any one of claims 1-3, wherein the promoter comprises a nucleic acid sequence having at least 99% identity to SEQ ID NO: 445.

9. The ceDNA vector of any one of claims 1-3, wherein the promoter comprises a nucleic acid sequence having at least 99% identity to SEQ ID NO: 446.
10. The ceDNA vector of any one of claims 1-3, wherein the promoter comprises a nucleic acid sequence having at least 96% identity to SEQ ID NO: 447.
11. The ceDNA vector of any one of claims 1 to 10, wherein the ceDNA vector further comprises an enhancer.
12. The ceDNA vector of claim 11, wherein the enhancer is selected from the group consisting of SEQ ID NOs: 449-465 and 586-601.
13. The ceDNA vector of claim 11, 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.
14. The ceDNA vector of claim any one of claims 1-3, 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.
15. The ceDNA vector of any one of claims 1 to 14, wherein the ceDNA vector further comprises one or more introns.
16. The ceDNA vector of claim 15, wherein the one or more introns is the minute virus of mice (MVM) intron.
17. The ceDNA vector of any one of claims 1 to 16, wherein the ceDNA vector comprises a 3' untranslated region (3' UTR).
18. The ceDNA vector of any one of claims 1 to 17, wherein the ceDNA vector comprises at least one polyA sequence.
19. The ceDNA vector of any one of claims 1-3, wherein the promoter set comprising a SERP enhancer comprises a 3xSERP enhancer.
20. The ceDNA vector of any one of claims 1-3, wherein the promoter is the TTR liver promoter and the ceDNA further comprises an MVM intron.

21. The ceDNA vector of any one of claims 1-20, 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: 197, SEQ ID NO: 570, SEQ ID NO: 572, SEQ ID NO: 573 and SEQ ID NO: 582.

22. The ceDNA vector of any one of claims 1-21, wherein the at least one nucleic acid sequence is a cDNA encoding PAH.

23. The ceDNA vector of any one of claims 1-22, wherein at least one flanking ITR comprises a functional terminal resolution site (TRS) and a Rep binding site.

24. The ceDNA vector of any one of claims 1-23, wherein one or both of the flanking ITRs are derived from a virus selected from the group consisting of: a *Parvovirus*, a *Dependovirus*, and an adeno-associated virus (AAV).

25. The ceDNA vector of any one of claims 1-24, wherein:
the flanking ITRs are symmetric;
the flanking ITRs are substantially symmetric;
the flanking ITRs are asymmetric;
one of the flanking ITRs is a wild-type ITR, or wherein both of the flanking ITRs are wild-type ITRs;
both of the flanking ITRs are derived from the same AAV serotype;
both of the flanking ITRs are derived from AAV2;
the flanking ITRs are derived from different viral serotypes;
the flanking ITRs are derived from a pair of viral serotypes selected from the group consisting of the pairs of viral serotypes set forth in Table 2; and/or
one or both of the flanking ITRs comprises a sequence selected from the group consisting of: the sequences set forth in Table 3, Table 5A, Table 5B, or Table 6.

26. The ceDNA vector of any one of claims 1-25, wherein at least one of the flanking ITRs is altered from a wild-type AAV ITR sequence by a deletion, an addition, and/or a substitution that affects the overall three-dimensional conformation of the ITR.

27. The ceDNA vector of any one of claims 1-26, 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.

28. The ceDNA vector of any one of claims 1-27, wherein one or both of the flanking ITRs are synthetic.
29. The ceDNA vector of any one of claims 1-28, wherein one of the flanking ITRs is not a wild-type ITR, or wherein both of the flanking ITRs are not wild-type ITRs.
30. The ceDNA vector of any one of claims 1-29, 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: an A, A', B, B', C, C', D, and/or D' region.
31. The ceDNA vector of claim 30, wherein the deletion, insertion, and/or substitution results in the deletion of all or part of a stem-loop structure formed by the A, A', B, B' C, and/or C' regions.
32. The ceDNA vector of any one of claims 1-31, 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 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.

33. Use of the ceDNA vector of any one of claims 1-32 in the manufacture of a medicament for treating a subject with phenylketonuria (PKU).
34. The use of claim 33, wherein the least one nucleic acid sequence that encodes at least one PAH protein comprises a sequence set forth as SEQ ID NO: 425.
35. The use of claim 33 or 34, wherein the subject exhibits at least a 50% decrease in level of serum phenylalanine as compared to a level of serum phenylalanine in the subject prior to administration or wherein the subject exhibits at least a 10% increase in PAH activity after administration as compared to a level of PAH activity prior to administration.
36. The use of any one of claims 33-35, wherein the ceDNA vector is formulated in lipid nanoparticles.
37. The use of any one of claims 33-36, wherein the ceDNA vector is to be administered by one or more routes selected from the group consisting of: intravenous injection, intramuscular injection, infusion, subretinal injection, suprachoroidal injection, and intravitreal injection.
38. A pharmaceutical composition comprising the ceDNA vector of any one of claims 1-32.
39. A composition comprising the ceDNA vector of any one of claims 1-32 and a lipid, wherein the lipid is a lipid nanoparticle (LNP).
40. A kit comprising the ceDNA vector of any one of claims 1-32, the pharmaceutical composition of claim 38, or the composition of claim 39.