

CLAIMS

1. A capsid-free closed-ended DNA (ceDNA) vector comprising:
at least one nucleic acid sequence positioned between flanking inverted terminal repeat sequences (ITRs), wherein the at least one nucleic acid sequence encodes at least one beta glucocerebrosidase (GBA) protein.
2. The ceDNA vector of claim 1, wherein the least one nucleic acid sequence that encodes at least one GBA protein is selected from the group consisting of: any of the sequences set forth in Table 1 and any of the open reading frame sequences (ORFs) set forth in Table 13.
3. The ceDNA vector of claims 1 or 2, wherein:
the ceDNA vector comprises a promoter selected from any of the sequences set forth in in Table 7 operatively linked to the least one nucleic acid sequence that encodes the at least one GBA protein;
the ceDNA vector comprises an enhancer selected from the group consisting: of any of the sequences set forth in Table 8;
the ceDNA vector comprises a 5' UTR and/or intron sequence selected the group consisting: of any of the sequences set forth fromin Table 9A;
the ceDNA vector comprises a 3' UTR sequence selected from the group consisting: of any of the sequences set forth in Table 9B;
the ceDNA vector comprises at least one poly A sequence selected from the group consisting: of any of the sequences set forth in Table 10; and/or
the ceDNA vector comprises at least one promoter sequence operably linked to at least one nucleic acid sequence.
4. The ceDNA vector of any one of claims 1-3, wherein the at least one nucleic acid sequence is cDNA.
5. The ceDNA vector of any one of claims 1-4, wherein the at least one ITR comprises a functional terminal resolution site and a Rep binding site.
6. The ceDNA vector of any one of claims 1-5, 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).
7. The ceDNA vector of any one of claims 1-6, wherein:
the flanking ITRs are symmetric or asymmetric with respect to each other;
the flanking ITRs are symmetric or substantially symmetric; or
the flanking ITRs are asymmetric.
8. The ceDNA vector of any one of claims 1-7, wherein:
one of the flanking ITRs is wild-type, or wherein both of the flanking ITRs are wild-type ITRs;

one of the flanking ITRs is not a wild-type ITR, or wherein both of the flanking ITRs are not wild-type ITRs;

the flanking ITRs are derived from different viral serotypes;

one of 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;

the flanking ITRs are selected from the group consisting of: any pair of viral serotypes shown in Table 2; and/or

one or both of the flanking ITRs comprises a sequence selected from the group consisting of: one or more of the sequences set forth in Table 3.

9. The ceDNA vector of any one of claims 1-8, 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.

10. The ceDNA vector of any one of claims 1-9, wherein one or both of the ITRs are synthetic.

11. The ceDNA vector of any one of claims 1-10, wherein:

one or both of the flanking ITRs are 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;

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 part of a stem-loop structure formed by the B and B' regions and/or part of a stem-loop structure normally 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; or

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.

12. The ceDNA vector of claim 11, wherein the deletion, the insertion, and/or the substitution results in the deletion of all or part of a stem-loop structure formed by the A, A', B, B', C, or C' regions.
13. The ceDNA vector of any one of claims 1-12, wherein 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.
14. The ceDNA vector of any one of claims 1-13, wherein one or both of the flanking ITRs comprise a sequence selected from the group consisting of one or more of the sequences set forth in Tables 3, 5A, 5B, and 6.
15. The ceDNA vector of any one of claims 1-14, wherein the ceDNA vector comprises at least one regulatory switch.
16. A capsid-free close-ended DNA (ceDNA) vector comprising a nucleic acid sequence selected from the group consisting of: any of the sequences set forth in Table 13.
17. A capsid-free close-ended DNA (ceDNA) vector comprising a nucleic acid sequence at least 85% identical to a nucleic acid selected from SEQ ID NO: 385, SEQ ID NO: 386, SEQ ID NO: 387, SEQ ID NO: 388, SEQ ID NO: 389 or SEQ ID NO: 390.
18. A capsid-free close-ended DNA (ceDNA) vector consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NO: 385, SEQ ID NO: 386, SEQ ID NO: 387, SEQ ID NO: 388, SEQ ID NO: 389 or SEQ ID NO: 390.
19. Use of the ceDNA vector of any one of claims 1 to 18 in the manufacture of a medicament for expressing a beta glucocerebrosidase (GBA) protein in a cell.
20. A GBA protein produced by translation of the ORF nucleic acid sequence selected from the group consisting of: any one of SEQ ID NO: 385, SEQ ID NO: 386, SEQ ID NO: 387, SEQ ID NO: 388, SEQ ID NO: 389 and SEQ ID NO: 390.
21. The use of claim 19 or 20, wherein the at least one nucleic acid sequence is codon optimized for expression in a eukaryotic cell.
22. The use of claim 19 or 20, wherein the at least one nucleic acid sequence is selected from the group consisting of any of the sequences set forth in Table 5.
23. Use of the ceDNA vector of any one of claims 1-18 or the GBA protein of claim 20 in the manufacture of a medicament for treating a subject with Gaucher's disease.
24. The use of claim 20, wherein the least one nucleic acid sequence that encodes at least one GBA protein is selected from the group consisting of: any of the sequences set forth in Table 1 or any of the ORFs set forth in Table 13.
25. The use claims 23 or 24, wherein the ceDNA vector expresses the GBA protein in a liver cell, or in a muscle cell, or both.
26. A pharmaceutical composition comprising the ceDNA vector of any one of claims 1-18.
27. A cell containing the ceDNA vector of any of claims 1-18.

28. A composition comprising the ceDNA vector of any of claims 1-18 and a lipid, wherein the lipid is a lipid nanoparticle (LNP).
29. A kit comprising the ceDNA vector of any one of claims 1-18 or the composition of claim 28 or the cell of claim 27 and instructions for use.