CLAIMS

1. A lyophilized composition comprising a capsid-free closed ended non-viral DNA (ceDNA) vector comprising at least one nucleic acid sequence between flanking inverted terminal repeats (ITRs), wherein the at least one nucleic acid sequence encodes a therapeutic protein; and wherein the ceDNA is snap frozen in a sucrose solution prior to lyophilization.

2. The lyophilized composition of claim 1, wherein the sucrose solution is between about 2.5% to about 5% sucrose.

3. The lyophilized composition of claim 1 or 2, wherein the therapeutic protein is an antigen, or an immunogenic peptide.

4. The lyophilized composition of claim 3, wherein:
the antigen, or the immunogenic peptide, is derived from a bacterial, a viral, a fungal or a parasitic infectious agent;
the antigen, or the immunogenic peptide, is a tumor associated antigen;
the antigen, or the immunogenic peptide, is associated with an autoimmune condition; and/or
the antigen, or the immunogenic peptide, is selected from one or more of those set forth in Tables 1-7.

5. The lyophilized composition of any one of claims 1-4, wherein:
the composition is stable at ambient temperature for at least one week or more
the composition is stable at ambient temperature for at least four weeks or more;
the composition is stable at 4°C for at least one month or more; and/or
the composition is stable at 4°C for at least six months or more.

6. The lyophilized composition of claim 5, wherein ambient temperature is about 20 to 40 °C, or about 25 to 35°C, or about 25 to 30 °C.

7. The lyophilized composition of any one of claims 1-6, wherein:
the composition comprises less than 10% impurities over one week or more;
the composition comprises less than 10% impurities over one month or more;
the composition retains at least about 90% of its activity over one week or more; and/or
the composition retains at least about 90% of its activity over one month or more.

8. The lyophilized composition of any one of claims 1-7, wherein:

the composition comprises at least 90% monomer and dimer purity over one week or more;
the composition comprises at least 95% monomer and dimer purity over one week or more;
the composition comprises at least 90% monomer and dimer purity over one month or more;
the composition comprises at least 95% monomer and dimer purity over one month or more and/or
the ceDNA vector is encapsulated in a lipid nanoparticle (LNP).

9. The lyophilized composition of any one of claims 1-8, for use in a vaccine.

10. The lyophilized composition of any one of claims 1-9, wherein:
the lyophilized composition further comprises a promoter sequence operatively linked to the at least one nucleic acid sequence;
the ceDNA vector comprises at least one poly A sequence;
the ceDNA vector comprises a 5' UTR and/or intron sequence;
the ceDNA vector comprises a 3' UTR sequence; and/or
the ceDNA vector comprises an enhancer sequence;
at least one ITR comprises a functional terminal resolution site and a Rep binding site;
one or both of the ITRs are from a virus selected from a Parvovirus, a Dependovirus, and an adeno-associated virus (AAV);
the flanking ITRs are symmetric or asymmetric with respect to one another;
one or both of the ITRs are wild type, or wherein both of the ITRs are wild-type ITRs;
the flanking ITRs are from different viral serotypes;
the flanking ITRs are selected from any pair of viral serotypes shown in Table 8;
one or both of the ITRs comprises a sequence selected from one or more of the sequences in Table 9;
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;
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;
one or both of the ITRs are synthetic; and/or
one or both of the ITRs are not a wild type ITR, or wherein both of the ITRs are not wild-type ITRs.

11. The lyophilized composition of claim 10, wherein:
the flanking ITRs are symmetrical or substantially symmetrical;
the flanking ITRs are asymmetric.

12. The lyophilized composition of any one of claims 1-11, wherein:
one or both of the ITRs are modified by a deletion, an insertion, and/or a substitution in at least one of

the ITR regions selected from A, A', B, B', C, C', D, and D';
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;
one or both of the 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 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 C and C' regions;
one or both of the ITRs are modified by a deletion, an insertion, and/or a 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;
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;
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;
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; and/or
both ITRs are altered in a manner that results in an overall three-dimensional symmetry when the ITRs are inverted relative to each other.

13. A method of expressing a therapeutic protein in a cell comprising contacting the cell with the lyophilized composition of any one of claims 1-12.

14. The method of claim 13, wherein the at least one nucleic acid sequence is codon optimized for expression in the cell.

15. Use of the lyophilized composition of any one of claims 1-12 in the manufacture of a medicament for expressing a therapeutic protein in a cell, wherein the medicament is to be contacted with the cell.

16. The use of claim 15, wherein the at least one nucleic acid sequence is codon optimized for expression in the cell.

17. Use of the lyophilized composition of any one of claims 1-12 in the manufacture of a

medicament for treating and/or preventing a bacterial, a viral, a parasitic or a fungal infection in a subject, wherein the medicament is to be administered to the subject.

18. Use of the lyophilized composition of any one of claims 1-12 in the manufacture of a medicament for treating and/or preventing a cancer in a subject, wherein the medicament is to be administered to the subject.

19. Use of the lyophilized composition of any one of claims 1-12 in the manufacture of a medicament for treating and/or preventing an autoimmune disease or disorder in a subject, wherein the medicament is to be administered to the subject.

20. The use of any one of claims 13-19, wherein:
the lyophilized composition is reconstituted before it is to be administered;
the composition is to be administered with one or more additional therapeutic agents to the subject; and/or
the reconstituted lyophilized composition is to be administered by intravenous, subcutaneous, intratumoral, or intramuscular injection.

21. A pharmaceutical composition comprising the lyophilized composition of any one of claims 1-13.

22. The pharmaceutical composition of claim 21, further comprising:
a diluent, wherein the diluent is used to reconstitute the lyophilized composition; and/or one or more additional therapeutic agents.

23. A kit comprising the lyophilized composition of any one of claims 1-12, the pharmaceutical composition of claim 21 or claim 22.

24. The lyophilized composition of any one of claims 1-12 for use in therapy.