CLAIMS

What is claimed is:

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

a heterologous nucleic acid insert flanked by at least two adeno-associated virus (AAV) inverted terminal repeat (ITR) sequences, wherein the ITR sequences are asymmetric with respect to one another, each sequence having an operative terminal resolution site (trs) and a rolling circle replication protein binding element (RBE), wherein a first ITR sequence is interrupted by a cross-arm sequence forming two opposing, lengthwise-symmetric stem-loops, each of the opposing lengthwise-symmetric stem-loops having a stem portion in the range of 5 to 15 base pairs in length and a loop portion having 2 to 5 unpaired deoxyribonucleotides, wherein a second ITR sequence is interrupted by a truncated cross-arm sequence, wherein the truncated cross-arm sequence has one or more deletions in a B, B', C, and/or C' region.

2. The ceDNA of claim 1, wherein the ITR sequences are in the range of 40 to 1000 nucleotides in length.

3. The ceDNA of claim 1, wherein the cross-arm sequence has a Gibbs free energy (ΔG) of unfolding under physiological conditions in the range of -12 kcal/mol to -30 kcal/mol.

4. The ceDNA of claim 1, wherein each of the opposing lengthwise-symmetric stem-loops have a stem portion in the range of 3 to 15 base pairs in length; or wherein each loop portion has 2 to 5 unpaired deoxyribonucleotides.

5. The ceDNA of claim 1, wherein the rolling circle replication protein binding element is a Rep binding element (RBE), optionally wherein the RBE comprises the sequence 5'-GCTCGCTCGCTC-3'.

6. The ceDNA of claim 1, wherein the operative terminal resolution site comprises a sequence 5'-TT-3' or wherein the 3' end of the operative terminal resolution site is 15 to 25 nucleotides from the 5' end of the rolling circle replication protein binding element.

7. The ceDNA of claim 1, wherein the truncated cross-arm sequence forms two opposing, lengthwise-asymmetric stem-loops, optionally wherein one of the opposing, lengthwise-asymmetric stem-loops has a stem portion in the range of 8 to 10 base pairs in length and a loop portion having 2 to 5 unpaired deoxyribonucleotides or one lengthwise-asymmetric stem-loop has a stem portion less than 8 base pairs in length and a loop portion having 2 to 5 deoxyribonucleotides.

8. The ceDNA of claim 1, wherein the heterologous nucleic acid insert is engineered to express a protein or functional RNA.

9. The ceDNA of claim 1, wherein the heterologous nucleic acid insert is in the range of 500 to 50,000 nucleotides in length and is a promoterless construct as a substrate for gene editing selected from a substrate for TALENS, a substrate for zinc finger nucleases (ZFNs), a substrate for meganucleases, a substrate for Cas9, and a substrate for another gene editing protein.

10. The ceDNA of claim 1, contained within a vector.

11. The ceDNA of claim 1, wherein the ceDNA comprises a monomeric nucleic acid comprising a single subunit or at least one multimeric nucleic acid comprising two or more subunits.

12. A composition comprising a plurality of ceDNA as described in claim 1, optionally wherein the plurality of ceDNA are linked end-to-end.

13. A host cell comprising the ceDNA of claim 1, optionally wherein the host cell further comprises a rolling circle replication protein that selectively binds to the RBE of the ceDNA.

14. An in vitro method of delivering a heterologous nucleic acid to a cell, the method comprising delivering to the cell the ceDNA of claim 1.

15. The ceDNA of claim 1, wherein the ceDNA does not elicit an immune response in a subject, wherein the immune response is a humoral response or a cellular response.

16. The composition of claim 12 or the host cell of claim 13 for use in therapy.

17. Use of the composition of claim 12 or the host cell of claim 13 in the manufacture of a medicament for treating a genetic disease, wherein the medicament is to be administered to a subject once or multiple times, wherein the medicament does not elicit an immune response in the subject, wherein the immune response is a humoral response or a cellular response.

18. An in vitro method of preparing closed-ended linear duplex DNA (ceDNA), the method comprising:

(i) introducing into a permissive cell a ceDNA encoding a heterologous nucleic acid insert flanked by at least two adeno-associated virus (AAV) inverted terminal repeat (ITR) sequences, wherein the ITR sequences are asymmetric with respect to one another, each sequence having an operative terminal resolution site (trs) and a rolling circle replication protein binding element (RBE), wherein a first ITR sequence is interrupted by a cross-arm sequence forming two opposing, lengthwise-symmetric stem-loops, each of the opposing lengthwise-symmetric stem-loops having a stem portion in the range of 5 to 15 base pairs in length and a loop portion having 2 to 5 unpaired deoxyribonucleotides, wherein a second ITR sequence is interrupted by a truncated cross-arm sequence wherein the truncated cross-arm sequence has one or more deletions in a B, B', C, and/or C' region;

(ii) maintaining the permissive cell under conditions in which a rolling circle replication protein in the permissive cell initiates production of multiple copies of the nucleic acid, wherein the permissive cell does not express viral capsid proteins capable of packaging replicative copies of the nucleic acid into a viral particle, wherein the rolling circle replication protein is selected from the group consisting of AAV78, AAV52, AAV Rep68, and AAV Rep 40; and, optionally,

(iii) purifying the multiple copies of the nucleic acid, wherein the purifying optionally comprises contacting the nucleic acid with a silica gel resin.

19. The method of claim 18, wherein the rolling circle replication protein is encoded by an Autograph californica multiple nucleopolyhedrosis virus (AcMNPV) vector or a baculovirus expression vectors (BEV).

20. The method of claim 18, wherein the permissive cell is an insect cell line, yeast cell line, or bacterial cell line.