CLAIMS

What is claimed is:

1. An isolated nucleic acid comprising a heterologous nucleic acid insert flanked by at least two interrupted self-complementary sequence, each self-complementary sequence having an operative terminal resolution site and a rolling circle replication protein binding element, wherein one self-complementary 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 interrupted self-complementary sequence is interrupted by a truncated cross-arm sequence.

2. The isolated nucleic acid of claim 1, wherein the interrupted self-complementary sequence(s) are in the range of 40 to 1000 nucleotides in length.

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

9. The isolated nucleic acid 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 isolated nucleic acid of claim 1, wherein the nucleic acid comprises a single closed-ended strand.

11. A composition comprising a plurality of isolated nucleic acids as described in claim 1, optionally wherein the plurality of isolated nucleic acids are linked end-to-end.

12. A host cell comprising the isolated nucleic acid of claim 1, optionally wherein the host cell further comprises a rolling circle replication protein that selectively binds to the rolling circle replication protein binding element of the nucleic acid.

13. A method of delivering a heterologous nucleic acid to a cell, the method comprising delivering to the cell the isolated nucleic acid of claim 1.

14. The isolated nucleic acid of claim 1 for use in delivery to a subject.

15. The isolated nucleic acid for use of claim 14, wherein the delivery of the isolated nucleic acid does not result in an immune response against the isolated nucleic acid in the subject, wherein the immune response is a humoral response or a cellular response, and wherein the delivery occurs once or multiple times.

16. The host cell of claim 12 for use in delivery to a subject.

17. The host cell for use of claim 16, wherein the delivery occurs once or multiple times.

18. A method of preparing nucleic acids, the method comprising:

(i) introducing into a permissive cell a nucleic acid encoding a heterologous nucleic acid insert flanked by at least one interrupted self-complementary sequence, each self-complementary sequence having an operative terminal resolution site and a rolling circle replication protein binding element, wherein the self-complementary 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;

(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; 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 selected from the group consisting of AAV Rep78, AAV Rep52, AAV Rep68, and AAV Rep 40, optionally 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.

21. A composition comprising: i) a monomeric nucleic acid comprising a single subunit and ii) at least one multimeric nucleic acid comprising two or more subunits, wherein each subunit of the monomeric nucleic acid and of the at least one multimeric nucleic acid comprises a heterologous nucleic acid insert flanked by interrupted self-complementary sequences, each self-complementary sequence having an operative terminal resolution site and a rolling circle replication protein binding element, wherein one self-complementary sequence is interrupted by a cross-arm sequence forming two opposing, lengthwise-symmetric stem-loops and the other of the self-complementary sequences is interrupted by a truncated cross-arm sequence.

22. A method of analyzing a nucleic acid, the method comprising:

i) obtaining a nucleic acid preparation comprising nucleic acid replication products isolated from a permissive cell, wherein the permissive cell comprises a nucleic acid comprising a heterologous nucleic acid insert flanked by interrupted self-complementary sequences, each self-complementary sequence having an operative terminal resolution site and a rolling circle replication protein binding element, wherein one self-complementary sequence is interrupted by a cross-arm sequence that forms two opposing, lengthwise-symmetric stem-loops, wherein the other of the self-complementary sequences is interrupted by a truncated cross-arm sequence, wherein the permissive cell expresses a rolling circle replication protein, but does not express viral capsid proteins capable of packaging replicative copies of the nucleic acid into a viral particle, and wherein the rolling circle replication protein binds to the rolling circle replication protein binding element of the nucleic acid and replicates the nucleic acid to produce nucleic acid replication products; and

ii) determining a physiochemical property of one or more replication products.

23. The method of claim 22, wherein the physiochemical property is:

(i) the nucleotide sequence of one or each self-complementary sequence;

(ii) the extent of multimerization of one or more replication products;

(iii) the stoichiometry of monomeric and/or multimeric forms of the replication product in the nucleic acid preparation;

(iv) the susceptibility of one or more replication products to digestion with a restriction endonuclease;

(v) the polarity of monomers in a dimeric form of the replication product, wherein the polarity is head-to-head, head-to-tail or tail-to-tail; or

(vi) the molecular weight of one or more replication products or of a fragment of a replication product.

24. The method of claim 23, wherein the molecular weight is of a fragment of the one or more replication products that comprises one or each self-complementary sequence, optionally wherein the molecular weight is determined based on electrophoretic mobility or based on mass spectroscopy, and optionally wherein the fragment is amplified prior to determining the molecular weight.

25. A method of preparing nucleic acids, the method comprising:

(i) introducing into a permissive cell a nucleic acid comprising a heterologous nucleic acid insert flanked by interrupted self-complementary sequences, each self-complementary sequence having an operative terminal resolution site and a rolling circle replication protein binding element, wherein one self-complementary sequence is interrupted by a cross-arm sequence that forms two opposing, lengthwise-symmetric stem-loops, wherein the other of the self-complementary sequences is interrupted by a truncated cross-arm sequence, wherein the permissive cell expresses a rolling circle replication protein, but does not express viral capsid proteins capable of packaging replicative copies of the nucleic acid into a viral particle; and

(ii) maintaining the permissive cell under conditions in which the rolling circle replication protein in the permissive cell replicates the nucleic acid.