

CLAIMS

1. An engineered nucleic acid comprising an initial transcription sequence (ITS) comprising the nucleotide sequence of any one of SEQ ID NO: 1-4 or 38-41.
2. The engineered nucleic acid of claim 1, comprising the ITS of SEQ ID NO: 1 or SEQ ID NO: 38.
3. The engineered nucleic acid of claim 1 or 2, comprising a promoter operably linked to the ITS.
4. The engineered nucleic acid of claim 3 comprising the nucleotide sequence of any one of SEQ ID NO: 10-13 or 42-45.
5. The engineered nucleic acid of claim 4, comprising the nucleic sequence of SEQ ID NO: 10 or 42.
6. The engineered nucleic acid of any one of claims 1 to 5, further comprising a sequence of interest downstream of the nucleotide sequence of the ITS.
7. The engineered nucleic acid of claim 6, wherein the sequence of interest encodes an RNA product, which is optionally a dsRNA or a mRNA.
8. The engineered nucleic acid of any one of claims 1 to 7, further comprising one or more terminator sequences downstream of the sequence of interest.
9. The engineered nucleic acid of claim 8, wherein the terminator sequence comprises a rmBT1, rrnBT2, TT7, pET-T7, T7U, TT3, and/or PTH terminator sequence.
10. The engineered nucleic acid of claim 8 or 9, wherein the terminator sequence comprises a nucleotide sequence of any one of SEQ ID NOs: 19-30.
11. The engineered nucleic acid of any one of claims 1 to 10, wherein the promoter is a bacteriophage T7 promoter.

12. The engineered nucleic acid of claim 11, wherein the promoter comprises a nucleotide sequence of SEQ ID NO: 9 or 18.
13. The engineered nucleic acid of any one of claims 1 to 12, wherein the engineered nucleic acid is double- stranded.
14. The engineered nucleic acid of any one of claims 1 to 13, wherein the engineered nucleic acid is circular.
15. A construct comprising:
 - (a) a first expression cassette comprising
 - a promoter operably linked to an initial transcription sequence (ITS) comprising the nucleotide sequence of any one of SEQ ID NO: 1-4 or 38-41, a nucleotide sequence encoding a sense strand of a double-stranded RNA (dsRNA), and a terminator sequence; and
 - (b) a second expression cassette comprising
 - a promoter operably linked to an ITS comprising the nucleotide sequence of any one of SEQ ID NO: 1-4 or 38-41, a nucleotide sequence encoding an antisense strand of the dsRNA, and a terminator sequence, wherein the sense strand of the dsRNA is complementary to the antisense strand of the dsRNA.
16. The construct of claim 15, wherein the ITS of the first and second expression cassettes comprises the nucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 38.
17. The construct of claim 15 or 16, wherein the first expression cassette and the second expression cassette are located within a single DNA molecule and are oriented in the same direction.
18. The construct of claim 15 or 16, wherein the first and second expression cassettes are located within a single DNA molecule; and optionally wherein the first and second expression cassettes are oriented in opposite directions.
19. The construct of any one of 15 to 18, wherein the first expression cassette includes a reverse complement of the ITS downstream of the nucleotide sequence encoding the sense strand and the

second expression cassette includes a reverse complement of the ITS downstream of the nucleotide sequence encoding the antisense strand of the dsRNA product.

20. The construct of any one of claims 15 to 19, wherein the first expression cassette further comprises one or more terminator sequences downstream of the nucleotide sequence encoding the sense strand and the second expression cassette further comprises one or more terminator sequences downstream of the nucleotide sequence encoding the antisense strand.

21. The construct of any one of claims 15 to 20, wherein the first expression cassette is downstream of a reverse complement of the ITS and/or the second expression cassette is downstream of a reverse complement of the ITS.

22. The construct of any one of claims 15 to 21, wherein the terminator sequence comprises a rmBT1, rmBT2, TT7, pET-T7, T7U, TT3, and/or PTH terminator sequence.

23. The construct of any one of claims 15 to 22, wherein the terminator sequence comprises a nucleotide sequence of any one of SEQ ID NOs: 19-30.

24. The construct of any one of claims 15 to 23, further comprising a restriction endonuclease recognition site downstream of the nucleotide sequence encoding the sense strand and/or the nucleotide sequence encoding the antisense strand, optionally downstream of either of the reverse complements of the ITS and/or downstream of either of the terminator sequences.

25. The construct of any one of claims 15 to 24 further comprising a selection marker.

26. The construct of claim 25, wherein the selection marker is located between the first expression cassette and the second expression cassette.

27. The construct of claim 25 or 26, wherein the selection marker is an antibiotic resistance selection marker or an antibiotic-free selection marker.

28. The construct of any one of claims 15 to 27, wherein either or both of the promoter of the first expression cassette and the promoter of the second expression cassette is a bacteriophage T7 promoter.

29. The construct of any one of claims 15 to 28, wherein either or both of the promoter of the first expression cassette and the promoter of the second expression cassette comprises a nucleotide sequence of SEQ ID NO: 9 or 18.
30. The construct of any one of claims 15 to 29, wherein the construct is selected from plasmids, cosmids, bacterial artificial chromosomes, yeast artificial chromosomes, natural chromosomes, bacteriophages, and viruses.
31. The construct of claim 30, wherein the construct is a high, medium, or low copy number plasmid.
32. The construct of claim 31, wherein the plasmid comprises the ColEI replicon or the pUC replicon or a replicon derived from the ColEI, pBR322, pUC, R6K, p15a or pSC101 replicon.
33. The construct of any one of claims 15 to 32, wherein the dsRNA targets a genomic sequence of an insect, a plant, a fungus, or a virus.
34. A method, comprising combining in a transcription reaction the engineered nucleic acid of any one of claims 1 to 14 or the construct of any one of claims 15 to 33 and a polymerase, and producing an RNA transcript.
35. An RNA product produced by the method of claim 34.
36. The method of claim 34, wherein the transcript is produced in an amount that is at least 20%, at least 30%, or at least 40% greater than a control.
37. An expression cassette comprising a promoter operably linked to an initial transcription sequence (ITS) upstream of a nucleotide sequence encoding a product of interest and optionally followed by an ITS-RC and/or a restriction endonuclease site and/or two tandem terminator sequences, wherein the ITS comprises the nucleotide sequence of any one of SEQ ID NOs: 1-8 or 38-41.