

CLAIMS

What is claimed is:

- 5 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 a promoter operably linked to the ITS.
- 10 3. The engineered nucleic acid of claim 1 or 2 comprising the nucleotide sequence of any one of SEQ ID NO: 10-13 or 42-45.
4. The engineered nucleic acid of any one of claims 1-3, further comprising a sequence
15 of interest downstream of the nucleotide sequence of the ITS.
5. The engineered nucleic acid of any one of claims 1-4, further comprising one or more terminator sequences downstream of the sequence of interest.
- 20 6. The engineered nucleic acid of claim 5, wherein the terminator sequence comprises a rrnBT1, rrnBT2, TT7, pET-T7, T7U, TT3, and/or PTH terminator sequence.
7. The engineered nucleic acid of claim 5 or 6, wherein the terminator sequence comprises a nucleotide sequence of any one of SEQ ID NOs: 19-30.
- 25 8. The engineered nucleic acid of any one of claims 1-7, wherein the promoter is a bacteriophage T7 promoter.
9. The engineered nucleic acid of any one of claims 1-8, wherein the promoter
30 comprises a nucleotide sequence of SEQ ID NO: 9 or 18.
10. The engineered nucleic acid of any one of claims 1-9, wherein the engineered nucleic acid is double-stranded.

11. The engineered nucleic acid of any one of claims 1-10, wherein the engineered nucleic acid is circular.
12. A construct comprising:
- 5 a first expression cassette comprising a promoter operably linked to an initial transcription sequence (ITS) upstream of a nucleotide sequence encoding a sense strand of a double-stranded RNA (dsRNA); and
- a second expression cassette comprising a promoter operably linked to an initial transcription sequence (ITS) upstream of a nucleotide sequence encoding an antisense strand
- 10 of the dsRNA,
- wherein the sense strand of the dsRNA is complementary to the antisense strand of the dsRNA.
13. The construct of claim 12, wherein either or both of the first and second expression
- 15 cassette further comprise a terminator sequence downstream of the nucleotide sequence encoding a strand of the dsRNA.
14. The construct of claim 12 or 13, wherein either or both of the first and second expression cassette further comprise a restriction endonuclease recognition site.
- 20 15. The construct of any one of claims 12-14, wherein the initial transcription sequences have a length of 1-15 nucleotides.
16. The construct of any one of claims 12-15, wherein the initial transcription sequences
- 25 comprise the nucleotide sequence of any one of SEQ ID NOs: 1-8 or 38-41.
17. The construct of claim 16, wherein the initial transcription sequences comprise the nucleotide sequence of any one of SEQ ID NOs: 1-4 or 38-41.
- 30 18. The construct of any one of claims 12-17, wherein the first expression cassette and the second expression cassette are located within a single DNA molecule and are oriented in the same direction.

19. The construct of any one of claims 12-17, wherein the first expression cassette and the second expression cassette are located within a single DNA molecule and are oriented in opposition directions.
- 5 20. The construct of any one of claims 12-19, wherein the nucleotide sequence encoding the sense strand of the first expression cassette is flanked by the ITS and a reverse complement of the ITS and the antisense strand of the second expression cassette is flanked by the ITS and a reverse complement of the ITS.
- 10 21. The construct of any one of claims 12-20, 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.
- 15 22. The construct of any one of claims 13-21, wherein the terminator sequence comprises a rrnBT1, rrnBT2, TT7, pET-T7, T7U, TT3, and/or PTH terminator sequence.
23. The construct of any one of claims 13-21, wherein the terminator sequence comprises a nucleotide sequence of any one of SEQ ID NOs: 19-30.
- 20 24. The construct of any one of claims 11-22 further comprising a selection marker.
25. The construct of claim 24, wherein the selection marker is located between the first expression cassette and the second expression cassette.
- 25 26. The construct of claim 24 or 25, wherein the selection marker is an antibiotic resistance selection marker or an antibiotic-free selection marker.
27. The construct of any one of claims 12-26, wherein either the promoter of the first
30 expression cassette, the promoter of the second expression cassette, or both the promoter of the first expression cassettes and the promoter of the second expression cassettes is a bacteriophage T7 promoter.

28. The construct of any one of claims 12-27, wherein the promoter of the first expression cassette or the promoter of the second expression cassette, or both 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.

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29. The construct of any one of claims 12-28, wherein the construct is selected from plasmids, cosmids, bacterial artificial chromosomes, yeast artificial chromosomes, natural chromosomes, bacteriophages, and viruses.

10 30. The construct of claim 29, wherein the construct is a high, medium, or low copy number plasmid.

31. The construct of claim 30, wherein the plasmid comprises the ColE1 replicon or the pUC replicon or a replicon derived from the ColE1, pBR322, pUC, R6K, p15a or pSC101
15 replicon.

32. The construct of any one of claims 12-31, wherein the dsRNA targets a genomic sequence of an insect, a plant, a fungus, or a virus.

20 33. 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
25 (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.

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34. The construct of claim 33, wherein the first expression cassette and the second expression cassette are located within a single DNA molecule and are oriented in the same direction.

35. The construct of claim 33, 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.
- 5 36. The construct of any one of claims 33-35, 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.
- 10 37. The construct of any one of claims 33-36, 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.
- 15 38. The construct of any one of claims 33-37, 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.
39. The construct of any one of claims 33-38, wherein the terminator sequence comprises
20 a rrnBT1, rrnBT2, TT7, pET-T7, T7U, TT3, and/or PTH terminator sequence.
40. The construct of any one of claims 33-39, wherein the terminator sequence comprises a nucleotide sequence of any one of SEQ ID NOs: 19-30.
- 25 41. The construct of any one of claims 33-30, 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.
- 30 42. The construct of any one of claims 33-41 further comprising a selection marker.
43. The construct of claim 42, wherein the selection marker is located between the first expression cassette and the second expression cassette.

44. The construct of claim 42 or 43, wherein the selection marker is an antibiotic resistance selection marker or an antibiotic-free selection marker.
- 5 45. The construct of any one of claims 33-44, 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.
- 10 46. The construct of any one of claims 33-45, 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.
- 15 47. The construct of any one of claims 33-46, wherein the construct is selected from plasmids, cosmids, bacterial artificial chromosomes, yeast artificial chromosomes, natural chromosomes, bacteriophages, and viruses.
48. The construct of claim 47, wherein the construct is a high, medium, or low copy number plasmid.
- 20 49. The construct of claim 48, wherein the plasmid comprises the ColE1 replicon or the pUC replicon or a replicon derived from the ColE1, pBR322, pUC, R6K, p15a or pSC101 replicon.
- 25 50. The construct of any one of claims 33-49, wherein the dsRNA targets a genomic sequence of an insect, a plant, a fungus, or a virus.
- 30 51. A method, comprising combining in a transcription reaction the engineered nucleic acid of any one of claims 1-11 or the construct of any one of claims 12-50 and a polymerase, and producing an RNA transcript.
52. The method of claim 50, wherein the transcript is produced in an amount that is at least 20%, at least 30%, or at least 40% greater than a control.

53. 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.

54. A construct comprising:
a first expression cassette comprising a promoter operably linked to a nucleotide sequence encoding a sense strand of a double-stranded RNA (dsRNA), and a terminator sequence; and
a second expression cassette comprising a promoter operably linked to a nucleotide sequence encoding an antisense strand of the dsRNA (dsRNA), and a terminator sequence, wherein the first and second expression cassette are oriented in the same direction or opposite directions within the same DNA molecule,
wherein the sense strand of the dsRNA is complementary to the antisense strand of the dsRNA.

55. A kit comprising:
an engineered nucleic acid comprising the nucleotide sequence of any one of SEQ ID NO: 1-4 or 38-41; and
a polymerase.

56. The kit of claim 55, further comprising nucleoside triphosphates and/or nucleoside monophosphates.