## CLAIMS

What is claimed is:

1. A method for preparing an optimized DNA sequence encoding a protein as a template for *in vitro* transcription, said method comprising:
    a. providing a DNA sequence that comprises a protein coding sequence;
    b. determining the presence of a termination signal in the DNA sequence, wherein the termination signal has the following nucleic acid sequence: 5'-$X_1$ATCT$X_2$T$X_3$-3', wherein $X_1$, $X_2$ and $X_3$ are independently selected from A, C, T or G; and
    c. if one or more termination signal is present, modifying the DNA sequence by replacing one or more nucleic acids at any one of position 2, 3, 4, 5 and 7 of said termination signal(s) with any one of the other three nucleic acids to generate the optimized DNA sequence, wherein, if required, the one or more replacement nucleic acids are selected to preserve the amino acid sequence of the protein encoded by the protein coding sequence.
2. The method of claim 1, wherein steps b and c are carried out by a computer.
3. The method of claim 1 or 2, wherein the DNA sequence further comprises a first nucleic acid sequence encoding a 5' UTR and/or a second nucleic acid sequence encoding a 3' UTR.

~~4.~~ ~~The method of any one of claims 1-3,~~optionally wherein the 5 nucleotides immediately 3' of the termination signal in the DNA sequence do not comprise 3 or more T nucleotides.

~~5.~~4. The method of any one of claims 1-3~~4~~, wherein the method further comprises a step of modifying the DNA sequence relative to a wildtype DNA sequence encoding the same protein sequence to optimize:
    a. elements relevant to mRNA processing and stability; and/or
    b. elements relevant to translation or protein folding;

    wherein the modifications are made before the optimized DNA sequence is generated.

~~6.~~5. The method of claim 4~~5~~, wherein (a) the elements relevant to mRNA processing or stability include cryptic splice sites, mRNA secondary structure, stable free energy of mRNA, repetitive sequences, and RNA instability motifs; and or~~.~~

~~7.~~ ~~The method of claim 5, wherein~~(b) the elements relevant to translation or protein folding include codon usage bias, codon adaptability, internal chi sites, ribosomal binding

sites, premature polyA sites, Shine-Dalgarno sequences, codon context, codon-anticodon interactions, and translational pause sites.

~~8.~~6. The method of any one of the preceding claims, wherein the method further comprises a step of synthesizing the optimized DNA sequence,~~.~~

~~9. The method of claim 8,~~optionally wherein the method further comprises inserting the synthesized optimized DNA sequence in a nucleic acid vector for use *in vitro* transcription,~~.~~

~~10. The method of claim 9,~~optionally wherein (a) the nucleic acid vector comprises an RNA polymerase promoter operably linked to the optimized DNA sequence, optionally wherein the RNA polymerase is SP6 RNA polymerase or a T7 RNA polymerase; and/or~~.~~

~~11. The method of claim 9 or 10, wherein~~(b) the nucleic acid vector is a plasmid,~~.~~

~~12. The method of claim 11, wherein~~optionally the plasmid is linearized before *in vitro* transcription.

~~13.~~7. The method of ~~any one of~~ claim 6~~s 8-12~~, wherein the method further comprises using the synthesized optimized DNA sequence in *in vitro* transcription to synthesize mRNA, optionally wherein the mRNA is synthesized at a temperature ranging from 37-56 °C and/or by:~~.~~

~~14. The method of claim 13, wherein the mRNA is synthesized by~~(a) a SP6 RNA polymerase, optionally wherein~~.~~

~~15. The method of claim 14, wherein~~(i) the SP6 RNA polymerase is a naturally occurring SP6 RNA polymerase; or~~.~~

~~16. The method of claim 15, wherein~~(ii) the SP6 RNA polymerase is a recombinant SP6 RNA polymerase, optionally~~.~~

~~17. The method of claim 16,~~ wherein the SP6 RNA polymerase comprises a tag, optionally~~.~~

~~18. The method of claim 17,~~ wherein the tag is a his-tag; or~~.~~

~~19. The method of claim 18, wherein the mRNA is synthesized by~~(b) a T7 RNA polymerase.

~~20.~~8. The method of ~~any one of~~ claim 7~~s 13-19~~, wherein the method further comprises a separate step of capping and/or tailing the synthesized mRNA, optionally~~.~~

~~21. The method of any one of claims 13-19,~~ wherein capping and tailing occurs during *in vitro* transcription.

~~22.~~9. The method of ~~any one of~~ claims 7 or 8~~13-21~~, wherein the mRNA is synthesized in a reaction mixture comprising NTPs at a concentration ranging from 1-10 mM each NTP,

the DNA template at a concentration ranging from 0.01-0.5 mg/ml, and the SP6 RNA polymerase at a concentration ranging from 0.01-0.1 mg/ml, optionally wherein:

~~23. The method of claim 22, wherein~~(a) the reaction mixture comprises NTPs at a concentration of 5 mM each NTP, the DNA template at a concentration of 0.1 mg/ml, and the SP6 RNA polymerase at a concentration of 0.05 mg/ml; and/or

~~24. The method of any one of claims 13-23, wherein the mRNA is synthesized at a temperature ranging from 37-56 °C.~~

~~25. The method of any one of claims 13-23, wherein~~(b) the NTPs are naturally-occurring NTPs;

~~26. The method of any one of claims 13-24, wherein the NTPs~~or comprise modified NTPs.

~~27.~~10. A computer program comprising instructions which, when the program is executed by a computer, cause the computer to:

a. receive a DNA sequence that comprises a protein coding sequence, and

b. carry out steps b and c according to the any one of claims 1-5~~7~~.

~~28. A computer-readable data carrier having stored thereon the computer program of claim 27.~~

~~29. A data carrier signal carrying the computer program of claim 27.~~

~~30. A data processing system comprising means for carrying out the method of any one of claims 1-7.~~

~~31.~~11. A method for preparing an optimized DNA sequence encoding a protein as a template for *in vitro* transcription, said method comprising:

a. providing a DNA sequence encoding a protein; and

b. adding one or more termination signals at the 3' end of the DNA sequence to provide the optimized DNA sequence,

wherein the one or more termination signal(s) comprises the following nucleic acid sequence:

5'-$X_1$ATCT$X_2$T$X_3$-3', wherein $X_1$, $X_2$ and $X_3$ are independently selected from A, C, T or G.

~~32.~~12. The method of claim 11~~31~~, wherein the termination signal comprises the nucleic acid sequence 5'-$X_1$ATCTGTT-3', and/or wherein:

~~33. The method of claim 31 or 32, wherein~~(a) $X_1$ is T; or

~~34. The method of claim 31 or 32, wherein~~(b) $X_1$ is C.

~~35.~~ ~~The method of any one of claims 31-34,~~optionally wherein the termination signal is selected from 5'-TTTTATCTGTTTTTTT-3', 5'-TTTTATCTGTTTTTTTTT-3', 5'-CGTTTTATCTGTTTTTTT-3', 5'-CGTTCCATCTGTTTTTTT-3', 5'-CGTTTTATCTGTTTGTTT-3', 5'-CGTTTTATCTGTTTGTTT-3', or 5'-CGTTTTATCTGTTGTTTT-3'.

~~36. The method of any one of claims 31-35, wherein two or more, three or more, four or more termination signals are added to the 3' end of the DNA sequence.~~

~~37. The method of any one of claims 31-36, wherein the DNA sequence encoding the protein further comprises a first nucleic acid sequence encoding a 5' UTR and/or a second nucleic acid sequence encoding a 3' UTR.~~

~~38. The method of claim 37, wherein the DNA sequence encoding the protein further comprises a third nucleic acid sequence encoding a poly-A tail.~~

~~39. The method of claim 37, wherein the DNA sequence encoding the protein does not comprise a sequence encoding a poly-A tail.~~

~~40. The method of any one of claims 30-39, wherein the DNA sequence encoding the protein does not further comprise a DNA sequence encoding a ribozyme.~~

~~41.~~13. The method of ~~any one of~~ claim 11~~s 30-34 and 36-40~~, wherein the 5 nucleotides immediately 3' of the termination signal in the DNA sequence encoding the protein do not comprise 3 or more T nucleotides.

~~42.~~14. The method of any one of claims 11-13~~30-41~~, wherein the DNA sequence includes more than one termination signal (e.g., two or more, three or more, four or more termination signals), and optionally wherein said termination signals are separated by 10 base pairs or fewer, e.g. separated by 5-10 base pairs, optionally~~.~~

~~43.~~ ~~The method of any one of claims 30-42,~~ wherein the optimized DNA sequence comprises the following sequence: (a) 5'-$X_1$ATCTX$_2$TX$_3$-($Z_N$)- X$_4$ATCTX$_5$TX$_6$-3' or (b) 5'-$X_1$ATCTX$_2$TX$_3$-($Z_N$)- X$_4$ATCTX$_5$TX$_6$-($Z_M$)- X$_7$ATCTX$_8$TX$_9$-3', wherein $X_1$, $X_2$, $X_3$, $X_4$, $X_5$, $X_6$, $X_7$, $X_8$ and $X_9$ are independently selected from A, C, T or G, $Z_N$ represents a spacer sequence of N nucleotides, and $Z_M$ represents a spacer sequence of M nucleotides, each of which are independently selected from A, C, T of G, and wherein N and/or M are independently 10 or fewer, optionally wherein~~.~~

~~44.~~ ~~The method of claim 43, wherein~~(i) N is 5, 6, 7, 8, 9 or 10 and/or M is 5, 6, 7, 8, 9 or 10; and/or~~.~~

~~45.~~ ~~The method of any one of claims 43 and 44, wherein~~(ii) Z is T.

~~46.~~15. The method of any one of claims 11-14~~30-45~~, wherein the DNA sequence is optimized relative to another sequence encoding the protein regarding:

a. elements relevant to mRNA processing and stability,~~;~~ optionally wherein ~~and/or~~ the elements include cryptic splice sites, mRNA secondary structure, stable free energy of mRNA, repetitive sequences, and RNA instability motifs; and/or

b. elements relevant to translation or protein folding, optionally wherein the elements include codon usage bias, codon adaptability, internal chi sites, ribosomal binding sites, premature polyA sites, Shine-Dalgarno sequences, codon context, codon-anticodon interactions, and translational pause sites.~~.~~

~~47. The method of claim 46, wherein the elements relevant to mRNA processing or stability include cryptic splice sites, mRNA secondary structure, stable free energy of mRNA, repetitive sequences, and RNA instability motifs.~~

~~48. The method of claim 46, wherein the elements relevant to translation or protein folding include codon usage bias, codon adaptability, internal chi sites, ribosomal binding sites, premature polyA sites, Shine-Dalgarno sequences, codon context, codon-anticodon interactions, and translational pause sites.~~

~~49.~~16. The method of any one of claims 11-15~~30-48~~, wherein the DNA sequence provided in step a has been optimized by the method of any one of claims 1-5~~7~~.

~~50. The method of any one of claims 30-49, wherein the method further comprises inserting the optimized DNA sequence in a nucleic acid vector for use *in vitro* transcription.~~

~~51.~~17. A DNA sequence for use in *in vitro* transcription, comprising in 5’ to 3’ order:

a. a 5’UTR;

b. a protein coding sequence;

c. a 3’ UTR;

d. optionally a nucleic acid sequence encoding a polyA tail; and

e. a termination signal;

wherein the termination signal comprises the following nucleic acid sequence: 5’-$X_1$ATCT$X_2$T$X_3$-3’, wherein $X_1$, $X_2$ and $X_3$ are independently selected from A, C, T or G.

~~52.~~18. The DNA sequence of claim 17~~51~~, wherein the termination signal comprises the nucleic acid sequence 5’-$X_1$ATCTGTT-3’; and/or~~.~~

~~53. The DNA sequence of claim 51 or 52, wherein~~(i) $X_1$ is T; or~~.~~

~~54. The DNA sequence of claim 51 or 52, wherein~~(ii) $X_1$ is C,~~.~~

~~55.~~ ~~The DNA sequence of any one of claims 51-54,~~optionally wherein the termination signal is selected from 5'-TTTTATCTGTTTTTTT-3', 5'-TTTTATCTGTTTTTTTTT-3', 5'-CGTTTTATCTGTTTTTTT-3', 5'-CGTTCCATCTGTTTTTTT-3', 5'-CGTTTTATCTGTTTGTTT-3', 5'-CGTTTTATCTGTTTGTTT-3', or 5'-CGTTTTATCTGTTGTTTT-3'.

~~56.~~19. The DNA sequence of ~~any one of~~ claim 17 or 18~~s 51-55~~ comprising more than one termination signal~~,~~ (e.g. two or more, three or more, or four or more), and optionally~~.~~

~~57.~~ ~~The DNA sequence of claim 56,~~ wherein said~~the~~ termination signals are separated by 10 base pairs or fewer, e.g. separated by 5-10 base pairs, optionally~~.~~

~~58.~~ ~~The DNA sequence of any one of claims 51-57,~~ wherein the DNA sequence comprises the following sequence: (a) 5'-$X_1$ATCTX$_2$TX$_3$-($Z_N$)-X$_4$ATCTX$_5$TX$_6$-3' or (b) 5'-$X_1$ATCTX$_2$TX$_3$-($Z_N$)- X$_4$ATCTX$_5$TX$_6$-($Z_M$)- X$_7$ATCTX$_8$TX$_9$-3', wherein $X_1$, $X_2$, $X_3$, $X_4$, $X_5$, $X_6$, $X_7$, $X_8$ and $X_9$ are independently selected from A, C, T or G, $Z_N$ represents a spacer sequence of N nucleotides, and $Z_M$ represents a spacer sequence of M nucleotides, each of which are independently selected from A, C, T of G, and wherein N and/or M are independently 10 or fewer, optionally wherein~~.~~

~~59.~~ ~~The DNA sequence of claim 58, wherein~~(i) N is 5, 6, 7, 8, 9 or 10 and/or M is 5, 6, 7, 8, 9 or 10; and/or~~.~~

~~60.~~ ~~The DNA sequence of any one of claims 58 and 59, wherein~~(ii) Z is T.

~~61.~~20. The DNA sequence of any one of claims 17-19~~51-60~~, wherein the termination signal is absent from the 5' UTR, the protein coding sequence and the 3' UTR, and/or~~.~~

~~62.~~ ~~The DNA sequence of any one of claims 51-61,~~ wherein said DNA sequence does not comprise a DNA sequence encoding a ribozyme.

~~63.~~21. The DNA sequence of any one of claims 17-20~~51-62~~, wherein the DNA sequence is modified relative to a wildtype DNA sequence encoding the same protein sequence to optimize:

a. elements relevant to mRNA processing and stability; and/or
b. elements relevant to translation or protein folding.

~~64.~~22. A nucleic acid vector comprising the DNA sequence of any one of claims 17-21~~51-63~~.

~~65.~~23. The nucleic acid vector of claim 22~~64~~, wherein said vector comprises an RNA polymerase promoter operably linked to the optimized DNA sequence, optionally ~~,~~

~~66.~~ ~~The nucleic acid vector of claim 65,~~ wherein the RNA polymerase promotor is (a) a SP6 RNA polymerase promoter; or (b) a T7 RNA polymerase promoter optionally~~.~~

~~67. The nucleic acid vector of any one of claims 64-66,~~ wherein the nucleic acid vector is a plasmid.

~~68.~~24. A kit for use in *in vitro* transcription comprising the DNA sequence of any one of claims 17-21~~51-63~~ or the nucleic acid vector of ~~any one of~~ claim 22 or 23~~s 64-67~~, optionally ~~.~~

~~69. The kit of claim 68,~~ further comprising an RNA polymerase and NTPs.

~~70.~~25. A method for the production of mRNA, said method comprising adding the nucleic acid vector of ~~any one of~~ claim 22 or 23~~s 64-67~~ to a reaction mixture comprising NTPs and an RNA polymerase, wherein the RNA polymerase transcribes the DNA sequence into mRNA transcripts.

~~71. The method of claim 70, wherein the nucleic acid vector is a plasmid.~~

~~72. The method of claim 72, wherein the plasmid is linearized before *in vitro* transcription.~~

~~73. The method of claim 72, wherein the plasmid is not linearized before *in vitro* transcription.~~

~~74. The method of any one of claim 70-73, wherein the RNA polymerase is a SP6 RNA polymerase.~~

~~75. The method of claim 74, wherein the SP6 RNA polymerase is a naturally occurring SP6 RNA polymerase.~~

~~76. The method of claim 74, wherein the SP6 RNA polymerase is a recombinant SP6 RNA polymerase.~~

~~77. The method of claim 76, wherein the recombinant SP6 RNA polymerase comprises a tag.~~

~~78. The method of claim 77, wherein the tag is a his-tag.~~

~~79. The method of any one of claims 70-73, wherein the RNA polymerase is a T7 RNA polymerase.~~

~~80. The method of any one of claims 70-79, wherein the method further comprises a separate step of capping and/or tailing the synthesized mRNA.~~

~~81. The method of any one of claims 70-79, wherein capping and tailing occurs during *in vitro* transcription.~~

~~82. The method of any one of claims 70-81, wherein the mRNA is synthesized in a reaction mixture comprising NTPs at a concentration ranging from 1-10 mM each NTP, the DNA template at a concentration ranging from 0.01-0.5 mg/ml, and the SP6 RNA polymerase at a concentration ranging from 0.01-0.1 mg/ml.~~

~~83. The method of claim 82, wherein the reaction mixture comprises NTPs at a concentration of 5 mM each NTP, the DNA template at a concentration of 0.1 mg/ml, and the SP6 RNA polymerase at a concentration of 0.05 mg/ml.~~

~~84. The method of any one of claims 70-83, wherein the mRNA is synthesized at a temperature ranging from 37-56 °C, e.g. at 50-52 °C.~~

~~85. The method of any one of claims 70-84, wherein the NTPs are naturally-occurring NTPs.~~

~~86. The method of any one of claims 70-84, wherein the NTPs comprise modified NTPs.~~

~~87. The method of any one of claims 70-86, wherein at least 75% of the mRNA transcripts are terminated at the termination signal.~~

~~88. The method of a claim 87, wherein at least 80% of the mRNA transcripts are terminated at the termination signal.~~

~~89. The method of a claim 87, wherein at least 85% of the mRNA transcripts are terminated at the termination signal.~~

~~90. The method of a claim 87, wherein at least 90% of the mRNA transcripts are terminated at the termination signal.~~

~~91. The method of a claim 87, wherein at least 95% of the mRNA transcripts are terminated at the termination signal.~~

~~92. The method of a claim 87, wherein at least 99% of the mRNA transcripts are terminated at the termination signal.~~

~~93. The method of any one of claims 87-92, wherein the site of termination is determined by (i) digestion of the mRNA to produce 3' end fragments less than 100 nucleotides in size, and (ii) analysis of said 3' end fragments by liquid chromatography.~~

~~94. The method of any one of claims 87-92, wherein the site of termination is determined by RNA-sequencing.~~

~~95. The method of any one of claims 70-94, wherein the RNA polymerase is a T7 RNA polymerase and wherein the mRNA transcripts are substantially free of RNA duplexes.~~

~~96. The method of claim 95, wherein the mRNA transcripts contain undetectable levels of RNA duplexes relative to a control.~~

~~97. The method of claim 96, wherein RNA duplexes are detected with an antibody that specifically binds to dsRNA.~~