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_1ATCTX_2TX_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, optionally wherein the 5 nucleotides immediately 3' of the termination signal in the DNA sequence do not comprise 3 or more T nucleotides.
4. The method of any one of claims 1-3, 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.
5. The method of claim 4, 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

    (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.

6. The method of any one of the preceding claims, wherein the method further comprises a step of synthesizing the optimized DNA sequence, optionally wherein the method further comprises inserting the synthesized optimized DNA sequence in a nucleic acid vector for use *in vitro* transcription, 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

   (b) the nucleic acid vector is a plasmid, optionally the plasmid is linearized before *in vitro* transcription.

7. The method of claim 6, 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 ℃ and/or by:

   (a) a SP6 RNA polymerase, optionally wherein

      (i) the SP6 RNA polymerase is a naturally occurring SP6 RNA polymerase; or

      (ii) the SP6 RNA polymerase is a recombinant SP6 RNA polymerase, optionally wherein the SP6 RNA polymerase comprises a tag, optionally wherein the tag is a his-tag; or

   (b) a T7 RNA polymerase.

8. The method of claim 7, wherein the method further comprises a separate step of capping and/or tailing the synthesized mRNA, optionally wherein capping and tailing occurs during *in vitro* transcription.

9. The method of claims 7 or 8, 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:

   (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

   (b) the NTPs are naturally-occurring NTPs; or comprise modified NTPs.

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.

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_1ATCTX$_2$TX$_3$-3', wherein X_1, X_2 and X_3 are independently selected from A, C, T or G.
12. The method of claim 11, wherein the termination signal comprises the nucleic acid sequence 5'-X_1ATCTGTT-3', and/or wherein:
    (a) X_1 is T; or
    (b) X_1 is C,
    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'.
13. The method of claim 11, 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.
14. The method of any one of claims 11-13, 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 wherein the optimized DNA sequence comprises the following sequence: (a) 5'-X_1ATCTX$_2$TX$_3$-(Z_N)- X$_4$ATCTX$_5$TX$_6$-3' or (b) 5'-X_1ATCTX$_2$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
    (i) N is 5, 6, 7, 8, 9 or 10 and/or M is 5, 6, 7, 8, 9 or 10; and/or
    (ii) Z is T.
15. The method of any one of claims 11-14, wherein the DNA sequence is optimized relative to another sequence encoding the protein regarding:

a. elements relevant to mRNA processing and stability, optionally wherein 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.

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

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_1ATCTX$_2$TX$_3$-3', wherein X_1, X_2 and X_3 are independently selected from A, C, T or G.

18. The DNA sequence of claim 17, wherein the termination signal comprises the nucleic acid sequence 5'-X_1ATCTGTT-3'; and/or

(i) X_1 is T; or

(ii) X_1 is C,

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'.

19. The DNA sequence of claim 17 or 18 comprising more than one termination signal (e.g. two or more, three or more, or four or more), and optionally wherein said termination signals are separated by 10 base pairs or fewer, e.g. separated by 5-10 base pairs, optionally wherein the DNA sequence comprises the following sequence: (a) 5'-X_1ATCTX$_2$TX$_3$-(Z_N)-X_4ATCTX$_5$TX$_6$-3' or (b) 5'-X_1ATCTX$_2$TX$_3$-(Z_N)- X_4ATCTX$_5$TX$_6$-(Z_M)- X_7ATCTX$_8$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

(i) N is 5, 6, 7, 8, 9 or 10 and/or M is 5, 6, 7, 8, 9 or 10; and/or

(ii) Z is T.

20. The DNA sequence of any one of claims 17-19, wherein the termination signal is absent from the 5' UTR, the protein coding sequence and the 3' UTR, and/or wherein said DNA sequence does not comprise a DNA sequence encoding a ribozyme.
21. The DNA sequence of any one of claims 17-20, 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.
22. A nucleic acid vector comprising the DNA sequence of any one of claims 17-21.
23. The nucleic acid vector of claim 22, wherein said vector comprises an RNA polymerase promoter operably linked to the optimized DNA sequence, optionally wherein the RNA polymerase promotor is

    (a) a SP6 RNA polymerase promoter; or

    (b) a T7 RNA polymerase promoter

    optionally wherein the nucleic acid vector is a plasmid.
24. A kit for use in *in vitro* transcription comprising the DNA sequence of any one of claims 17-21 or the nucleic acid vector of claim 22 or 23, optionally further comprising an RNA polymerase and NTPs.
25. A method for the production of mRNA, said method comprising adding the nucleic acid vector of claim 22 or 23 to a reaction mixture comprising NTPs and an RNA polymerase, wherein the RNA polymerase transcribes the DNA sequence into mRNA transcripts.