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' (SEQ ID NO: 1), 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 °C 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.