

CLAIMS

1. A method of large-scale production of a composition comprising full-length messenger RNA (mRNA), comprising synthesizing *in vitro* mRNA using an SP6 RNA polymerase, wherein the composition comprises less than 1% of double-stranded RNA (dsRNA) by weight, and wherein at least 100 mg of mRNA is synthesized in a single batch.
2. The method of claim 1, wherein the composition contains less than 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, 0.1%, 0.05%, 0.01% of the dsRNA by weight, optionally wherein the composition is substantially free of the dsRNA.
3. The method of any one of the preceding claims, wherein the dsRNA is detected by dot blot assay or ELISA.
4. The method of any one of the preceding claims, wherein:
 - (a) the dsRNA is detected prior to capping or tailing the synthesized mRNA; and/or
 - (b) the method further comprises a step of capping and/or tailing the synthesized mRNA.
5. The method of any one of claims 1-3, wherein the dsRNA is detected after capping and tailing of the synthesized mRNA.
6. The method of any one of the preceding claims, wherein at least 200 mg, 300 mg, 400 mg, 500 mg, 600 mg, 700 mg, 800 mg, 900 mg, 1 g, 5 g, 10 g, 25 g, 50 g, 75 g, 100 g, 150 g, 200 g, 250 g, 500 g, 750 g, 1 kg, 5 kg, or 10 kg, or more of mRNA is synthesized in a single batch.
7. The method of any one of the preceding claims, wherein the method does not:
 - (a) include a step of specifically removing the dsRNA; and/or
 - (b) comprise a chromatography step; and/or
 - (c) comprise a chaotropic agent.
8. The method of any one of the preceding claims, wherein:
 - (a) the SP6 RNA polymerase is a naturally occurring SP6 RNA polymerase; or
 - (b) the SP6 RNA polymerase is a recombinant SP6 RNA polymerase, optionally wherein the SP6 RNA polymerase comprises a tag, optionally a his-tag.

9. The method of any one of the preceding claims, wherein the mRNA is synthesized by the SP6 RNA polymerase based on a DNA template.
10. The method of claim 9, wherein the amount of SP6 RNA polymerase by weight in a reaction mixture is equal to or greater than the amount of DNA template by weight.
11. The method of claim 10, wherein the weight ratio of DNA template to SP6 RNA polymerase is between 1:1 and 1:3.
12. The method of any one of claims 9 to 11, wherein the DNA template comprises an SP6 promoter operably linked to a DNA sequence encoding the mRNA sequence to be synthesized, optionally wherein the DNA sequence is optimized, optionally wherein the DNA sequence is optimized to reduce the chance of a hairpin structure forming in the synthesized mRNA.
13. The method of any one of the preceding claims, wherein:
- (a) the *in vitro* synthesis of mRNA is performed at a pH between about 6 to 8.5, *e.g.*, from 6.5 to 8.0, from 7.0 to 7.5, optionally 7.5; and/or
 - (b) the mRNA is produced under non-denaturing conditions; and/or
 - (c) 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 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
 - (d) the mRNA is synthesized at a temperature ranging from 37 – 42°C; and/or
 - (e) the NTPs (i) are naturally-occurring NTPs or (ii) comprise modified NTPs, optionally wherein the modified NTPs comprise pseudouridine (“ψU”), optionally wherein the pseudouridine is 1-N-methyl-pseudouridine.
14. The method of any one of the preceding claims, wherein:
- (a) the full-length mRNA molecule is at least 100 bases, 200 bases, 300 bases, 400 bases, 500 bases, 600 bases, 700 bases, 800 bases, 900 bases, 1 kb, 1.5 kb, 2 kb, 2.5 kb, 3 kb, 3.5 kb, 4 kb, 4.5 kb, 5 kb, 8 kb, 10 kb, or 15 kb in length; and/or
 - (b) the mRNA is codon optimized.

15. A composition comprising mRNA produced by the method of any one of the preceding claims.