

CLAIMSWhat is claimed is:

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 at least 1 g of mRNA is synthesized in a single batch and wherein the composition contains less than 1% of double stranded RNA (dsRNA) by weight.~~
2. ~~— The method of claim 1, wherein the method does not comprise a chromatography step.~~
3. ~~— The method of claim 1 or 2, wherein the mRNA is synthesized at a non denaturing condition.~~
4. ~~— A method of producing a composition comprising full length messenger RNA (mRNA), comprising synthesizing *in vitro* mRNA, wherein the composition contains less than 1% of double stranded RNA (dsRNA) by weight, and wherein the method does not comprise a chromatography step.~~
5. ~~— A method of producing a composition comprising full length messenger RNA (mRNA), comprising synthesizing *in vitro* mRNA, wherein the composition contains less than 1% of double stranded RNA (dsRNA) by weight, and wherein the mRNA is synthesized at a non denaturing condition.~~
6. ~~— The method of claim 4 or 5, wherein at least 1 g of mRNA is synthesized in a single batch.~~
7. ~~— The method of any one of the preceding claims, wherein at least 10 g, 100 g, 250 g, 500 g, 1 kg, or 10 kg of mRNA is synthesized in the single batch.~~
8. ~~— The method of any one of the preceding claims, 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.~~
9. ~~— The method of claim 8, wherein the composition is substantially free of the dsRNA~~
- 10.1. ~~— The method of any one of the preceding claims, wherein the *in vitro* synthesis of mRNA is performed at pH between about 6 to 8.5.~~

~~11. The method of any one of the preceding claims, wherein the *in vitro* synthesis of mRNA is performed in a buffer comprising 25mM tris HCl, 2mM spermidine, 25mM MgCl, 0.5mM NaCl, and pH 7.5.~~

~~12.1. The method of any one of the preceding claims, wherein the method does not comprise a chaotropic agent.~~

~~13.1. 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.~~

~~14. The method of claim ~~11~~13, 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:~~

~~15.2. The method of claim 14, optionally wherein the composition is substantially free of the dsRNA.~~

~~16.3. 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:~~

~~17.(a) the dsRNA is detected prior to capping or tailing the synthesized mRNA; and/or~~

~~18.(b) The method of any one of the preceding claims, wherein the method further comprises a step of capping and/or tailing the synthesized mRNA.~~

~~19.5. The method of any one of claims 1-~~3~~16, wherein the dsRNA is detected after capping and tailing of the synthesized mRNA.~~

~~20.6. The method of any one of the preceding claims ~~13-19~~, 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 ~~13-20~~, wherein the method does not:~~

~~21.(a) -include a step of specifically removing the dsRNA; and/or~~

~~22.(b) The method of any one of claims 13-21, wherein the method does not comprise a chromatography step; and/or~~

~~(c) The method of any one of the preceding claims, wherein the method does not comprise a chaotropic agent.~~

8. The method of any one of the preceding claims, wherein:

~~23.(a) the SP6 RNA polymerase is a naturally occurring SP6 RNA polymerase; or~~

~~24. The method of any one of the preceding claims, wherein the SP6 RNA polymerase is a recombinant SP6 RNA polymerase.~~

~~25. The method of claim 24, optionally wherein the SP6 RNA polymerase comprises a tag.~~

~~26.(b) The method of claim 25, optionally wherein the tag is a his-tag.~~

~~27.9. The method of any one of the preceding claims, wherein the mRNA is synthesized by the SP6 RNA polymerase based on a DNA template.~~

~~28.10. The method of claim 927, 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.~~

~~29.11. The method of claim 1028, wherein the weight ratio of DNA template to SP6 RNA polymerase is between 1:1 and 1:3.~~

~~30. The method of any one of claims 927 to 1129, wherein the DNA template comprises an SP6 promoter operably linked to a DNA sequence encoding the mRNA sequence to be synthesized.~~

~~31. The method of claim 30, optionally wherein the DNA sequence is optimized.~~

~~32.12. The method of claim 31, 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

~~33. The method of any one of the preceding claims, 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.~~

~~34.(c) The method of claim 33, 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~~

~~35.(d) The method of any one of the preceding claims, wherein the mRNA is synthesized at a temperature ranging from 37 – 42°C; and/or~~

~~36. The method of any one of claims 33-35, wherein the NTPs (i) are naturally-occurring NTPs; or (ii)~~

~~37. The method of any one of claims 33-35, wherein the NTPs comprise modified NTPs;~~

~~38. The method of claim 37, optionally wherein the modified NTPs comprise pseudouridine (“ψU”).~~

~~39.(e) The method of claim 38, optionally wherein the pseudouridine is 1-N-methyl-pseudouridine.~~

14. The method of any one of the preceding claims, wherein:

~~40.(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~~

~~41.(b) The method of any one of the preceding claims, wherein the mRNA is codon optimized.~~

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

~~43. A composition comprising *in vitro* synthesized messenger RNA (mRNA) produced in a single batch using an SP6 RNA polymerase without purification by a chromatography step, wherein the composition contains less than 1% of double-stranded RNA (dsRNA).~~

~~44. The composition of claim 43, wherein the composition comprises at least 100mg, 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, 10 kg, or more of mRNA.~~

~~45. The composition of any one of claims 43-44, wherein the composition comprises 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.~~

~~46. The composition of claim 45, wherein the composition is substantially free of the dsRNA.~~

~~47. The composition of any one of claims 43-46, wherein the dsRNA is detected by dot blot assay.~~

~~48. The composition of any one of claims 43-47, wherein the mRNA is substantially free of shortmer contamination.~~

~~49. The composition of claim 48, wherein the mRNA is substantially free of both dsRNA and shortmer contamination.~~

~~50. The composition of any one of claims 43-49, wherein the mRNA is codon optimized.~~

~~51. The composition of any one of claims 43-50, wherein the mRNA 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.~~

~~52. The composition of any one of claims 42-51, wherein the mRNA is encapsulated within a liposome.~~

~~53. The composition of claim 42, wherein the liposome comprises one or more cationic lipids, one or more non-cationic lipids, and one or more the PEG-modified lipids.~~

~~54. The composition of claim 53, wherein the liposome further comprises cholesterol-based lipids.~~

~~55. The composition of any one of claims 43-54, wherein the mRNA is unmodified.~~

~~56. The composition of any one of claims 43-54, wherein the mRNA is modified.~~

~~57. The composition of claim 56, wherein the modified mRNA comprises pseudouridine ("ψU").~~

~~58. The composition of claim 57, wherein the pseudouridine is 1 N-methyl pseudouridine.~~

59.—~~A method of treating a disease or disorder comprising use of the composition of any one claims 42-54.~~