CLAIMS

We claim:

1. A method for purifying messenger RNA (mRNA), the method comprising the steps of:
   I. precipitating mRNA from a solution comprising one or more protein and/or short abortive transcript contaminants from manufacturing the mRNA to provide a suspension comprising precipitated mRNA;
   II. loading the suspension comprising the precipitated mRNA into a filtering centrifuge comprising a filter wherein the precipitated mRNA is retained by the filter;
   III. washing the retained precipitated mRNA by adding a wash buffer to the filtering centrifuge; and
   IV. recovering the retained precipitated mRNA from the filter;

wherein the filtering centrifuge is operated during loading step (b) and washing step (c) at a centrifuge speed that exerts a gravitational (g) force of less than 1300 g.

2. The method of claim 1, wherein centrifuge speed exerts a gravitational (g) force of between about 300 g and about 1300 g, ~~for example,~~ between about 400 g and about 1100 g, between about 500 g and about 900 g, between about 700 g and about 900 g, between about 750 g and 850 g, between about 550 g and about 750 g, or between about 650 g and about 750.

~~3. The method of claim 2, wherein centrifuge speed exerts a gravitational (g) force of between about 500 g and about 900 g, for example, between about 700 g and about 900 g, for example, between about 750 g and 850 g (*e.g.* about 800 g).~~

~~4. The method of claim 2, wherein centrifuge speed exerts a gravitational (g) force of between about 550 g and about 750 g, for example, between about 650 g and about 750 g.~~

~~5.~~3. The method of any one of the preceding claims, wherein the filtering centrifuge is operated at the same centrifuge speed during loading step (b) and washing step (c).

~~6.~~4. The method of any one of the preceding claims, wherein the recovering the retained precipitated mRNA from the filter comprises the steps of:
   i. solubilising the retained precipitated mRNA; and
   ii. collecting the solubilised mRNA.

7. The method of any one of the preceding claims, wherein precipitating the mRNA comprises adding one or more agents that promote precipitation of mRNA, for example one or more of an alcohol, an amphiphilic polymer, a buffer, a salt, and/or a surfactant.

8. The method of claim 7, wherein the one or more agents that promote precipitation of the mRNA are:

i. a salt, and

ii. an alcohol or an amphiphilic polymer.

9. The method of claim 7 or 8, wherein the alcohol is ethanol.

10. The method of any one of claims 7-9, wherein the salt is a chaotropic salt.

11. The method of claim 10, wherein the salt is at a final concentration of 2-4 M, for example of 2.5-3 M.

12. The method of claim 11, wherein the salt is at a final concentration of about 2.7 M.

13. The method of any one of claims 10-12, wherein the chaotropic salt is guanidinium thiocyanate (GSCN).

14. The method of claim 7, 8, or 10-13, wherein the amphiphilic polymer is selected from pluronics, polyvinyl pyrrolidone, polyvinyl alcohol, polyethylene glycol (PEG), triethylene glycol monomethyl ether (MTEG), or combinations thereof.

15. The method of claim 14, wherein the molecular weight of PEG is about 200 to about 40,000 g/mol.

16. The method of claim 15, wherein the molecular weight of PEG is about 200-600 g/mol, about 2000-10000 g/mol, or about 4000-8000 g/mol.

17. The method of claim 16, wherein the molecular weight of PEG is about 6000 g/mol (for example, PEG-6000).

18. The method of any one of claims 14-17, wherein the PEG is at a final concentration of about 10% to about 100% weight/volume.

19. The method of claim 18, wherein the PEG is at a final concentration of about 50% weight/volume.

20. The method of claim 19, wherein the PEG is at a final concentration of less than 25% weight/volume.

21. The method of claim 20, wherein the PEG is at a final concentration of about 5% to 20% weight/volume.

22. The method of claim 21, wherein the PEG is at a final concentration of about 10% to 15% weight/volume.

23. The method of claims 7, 8 or 10-14, wherein the amphiphilic polymer is MTEG.

24. The method of claim 23, wherein the MTEG is at a final concentration of about 10% to about 100% weight/volume concentration.

25. The method of claim 24, wherein the MTEG is at a final concentration of about 15% to about 45% weight/volume, for example of about 20% to about 40% weight/volume.

26. The method of claim 25, wherein the MTEG is at a final concentration of about 20%, about 25%, about 30%, or about 35% weight/volume.

27. The method of claim 26, wherein the MTEG is at a final concentration of about 25% weight/volume.

28. The method of any one of the preceding claims, wherein the suspension comprises precipitated mRNA, a salt and MTEG.

29. The method of any one of claims 26, wherein the salt is guanidinium thiocyanate (GSCN).

30. The method of any one of claims 1-8 and 10-29, wherein the suspension is free of alcohol, for example ethanol.

~~31.~~5. The method of any one of the preceding claims, wherein step (a) further comprises adding at least one filtration aid to the suspension comprising precipitated mRNA.

~~32. The method of claim 31, wherein the precipitated mRNA and the at least one filtration aid are at a mass ratio of about 1:2; about 1:5; about 1:10 or about 1:15.~~

~~33. The method of claim 32, wherein the precipitated mRNA and the at least one filtration aid are at a mass ratio of about 1:10.~~

~~34. The method of any one of claims 31-33, wherein the filtration aid is a dispersant.~~

~~35. The method of claim 34, wherein the dispersant is one or more of ash, clay, diatomaceous earth, glass beads, plastic beads, polymers, polymer beads (e.g., polypropylene beads, polystyrene beads), salts (e.g., cellulose salts), sand, and sugars.~~

~~36. The method of claim 35, wherein the polymer is a naturally occurring polymer, e.g. cellulose (for example, powdered cellulose fibre).~~

~~37.~~6. The method of any one of the preceding claims, wherein the suspension comprises at least 100mg, 1g, 10g, 100g, 250g, 500g, 1kg, 10kg, or 100kg~~, one metric ton, or ten metric tons,~~ of mRNA ~~or any amount there between~~.

~~38. The method of claim 37, wherein the suspension comprises greater than 1kg of mRNA.~~

~~39.~~7. The method of any one of the preceding claims, wherein the filter (i) comprises a porous substrate and/or a microfiltration membrane or ultrafiltration membrane.

~~40. The method of claim 39, wherein the porous substrate is a filter cloth, a filter paper, a screen and a wire mesh.~~

~~41. The method of any one of the preceding claims, wherein the filter is a microfiltration membrane or ultrafiltration membrane.~~

42.8. The method of any one of the preceding claims, wherein the filter has an average pore size is of about 0.5 micron or greater, about 0.75 micron or greater, about 1 micron or greater, about 2 microns or greater, about 3 microns or greater, about 4 microns or greater, or about 5 microns or greater, or about 0.01 micron to about 200 microns, about 1 micron to about 2000 microns, about 0.2 microns to about 5 microns, or about 1 micron to about 3 microns.

43. The method of claim 42, wherein the filter has an average pore size of about 0.01 micron to about 200 microns, about 1 micron to about 2000 microns, about 0.2 microns to about 5 micron, or about one micron to about 3 microns, e.g. about 1 micron.

44. The method of any one of claims 40, 42 and 43, wherein the filter cloth is a polypropylene cloth having an average pore size of about 1 micron.

45.9. The method of any one of the preceding claims, wherein the volume of wash buffer for washing the retained precipitated mRNA is between about 0.5 L/g mRNA and about 8 L/g mRNA.

46. The method of claim 45, wherein the volume of wash buffer for washing the retained precipitated mRNA is less than 2 L/g mRNA.

47. The method of any one of the preceding claims, wherein the volume of wash buffer for washing the retained precipitated mRNA is between about 0.5 L/g mRNA and about 1.5 L/g mRNA.

48. The method of any one of the preceding claims, wherein the volume of wash buffer for washing the retained precipitated mRNA is about 0.5 L/g mRNA.

49.10. The method of any one of claims 45-489, wherein the wash buffer is loaded into the filtering centrifuge at a rate of about 5 liter/min/m^2 to about 25 liter/min/m^2 (with respect to the surface area of the filter of the filtering centrifuge), for example at a rate of about 10 liter/min/m^2 to about 20 liter/min/m^2, *e.g.* at a rate of about 15 liter/min/m^2.

50. The method of claim 49, wherein the total volume of wash buffer is loaded into the filtering centrifuge in between about 0.5 hours to about 4 hours, for example in less than about 90 minutes.

51. The method of claim 50, wherein the retained precipitated mRNA is washed to a purity of between about 50% to about 100% in between about 0.5 hours to about 4 hours.

52. The method of claim 51, wherein the retained precipitated mRNA is washed to a purity of at least 95%, for example about 99%, in less than about 90 minutes.

53. The method of any of the preceding claims, wherein the wash buffer comprises one or more of an alcohol, an amphiphilic polymer, a buffer, a salt, and/or a surfactant.

54. The method of claim 53 wherein the wash buffer comprises an alcohol or an amphiphilic polymer.

55. The method of claim 53 or 54, wherein the wash buffer comprises ethanol, optionally wherein the ethanol is at about 80% weight/volume concentration.

56. The method of claim 53 or 54, wherein the wash buffer comprises an amphiphilic polymer selected from pluronics, polyvinyl pyrrolidone, polyvinyl alcohol, polyethylene glycol (PEG), triethylene glycol monomethyl ether (MTEG), or combinations thereof.

57. The method of claim 56, wherein the amphiphilic polymer is PEG.

58. The method of claim 57, wherein the PEG is present in the wash solution at about 10% to about 100% weight/volume concentration.

59. The method of claim 58, wherein the PEG is present in the wash solution at about 50% to about 95% weight/volume concentration.

60. The method of claim 59, wherein the PEG is present in the wash solution at about 90% weight/volume concentration.

61. The method of any one of claims 57-60, wherein the molecular weight of the PEG is about 100 to about 1,000 g/mol.

62. The method of claim 61, wherein the molecular weight of PEG is about 200-600 g/mol.

~~63. The method of claim 62, wherein the molecular weight of PEG is about 400 g/mol (for example PEG-400).~~

~~64. The method of claim 56, wherein the amphiphilic polymer is MTEG.~~

~~65. The method of claim 64, wherein the MTEG is present in the wash solution at about 75%, about 80%, about 85%, about 90% or about 95% weight/volume concentration.~~

~~66. The method of claim 65, wherein the MTEG is present in the wash solution at about 90% weight/volume concentration or about 95% weight/volume concentration.~~

~~67. The method of claim 66, wherein the MTEG is present in the wash solution at about 95% weight/volume concentration.~~

~~68. The method of any one of claims 53, 54 and 56-67, wherein the wash buffer is free of alcohol, for example ethanol.~~

~~69.~~11. The method of any one of the proceeding claims, wherein the recovering the retained mRNA occurs (i) while the filtering centrifuge is in operation, or (ii) while the filtering centrifuge is not in operation.

~~70. The method of claim 69, wherein the recovering the retained mRNA occurs via a blade that removes the retained precipitated mRNA from the filter of the filtering centrifuge.~~

~~71. The method of any one of claims 1-70, wherein the recovering the retained mRNA occurs while the filtering centrifuge is not in operation.~~

~~72. The method of any one of claims 1-6, 10-29, 31-52 and 56-71, wherein the method is free of alcohol, for example ethanol.~~

~~73.~~12. The method of any one of claims ~~34-72~~11, wherein the solubilising the retained mRNA comprises dissolving the mRNA in an aqueous medium.

~~74. The method of claim 73, wherein the aqueous medium comprises water, a buffer (e.g., Tris-EDTA (TE) buffer or sodium citrate buffer), a sugar solution (e.g., a sucrose or trehalose solution), or combinations thereof.~~

~~75. The method of claim 74, wherein the aqueous medium is water for injection.~~

~~76. The method of claim 74, wherein the aqueous medium is TE buffer.~~

~~77. The method of claim 74, wherein the aqueous medium is a 10% trehalose solution.~~

~~78. The method of any one of claims 73-77, wherein the solubilising occurs within the filtering centrifuge.~~

~~79. The method of any one of claims 73-77, wherein the solubilising occurs outside the filtering centrifuge.~~

~~80.~~13. The method of any one of claims ~~3~~15~~-79~~12, wherein the collecting of the solubilised mRNA comprises one or more steps of separating the filtration aid from the solubilised mRNA.

~~81. The method of claim 80, wherein the one or more steps for separating the filtration aid from the solubilised mRNA comprise applying the solution comprising the solubilised mRNA and filtration aid to a filter, wherein the filtration aid is retained by the filter, yielding a solution of purified mRNA.~~

~~82. The method of claim 81, wherein the solution comprising the solubilised mRNA and filtration aid is applied to a filter of a filtering centrifuge by centrifugation.~~

~~83. The method of claim 82, wherein the centrifuge speed exerts a gravitational (g) force of less than 3100 g, e.g., between about 1000 g and about 3000 g.~~

~~84.~~14. The method of any one of the preceding claims, wherein the filtering centrifuge is (i) a continuous centrifuge and/or the filtering centrifuge is orientated vertically or horizontally or the centrifuge is an inverted horizontal centrifuge, and/or (ii) comprises a sample feed port and/or a sample discharge port.

~~85. The method of any one of the preceding claims, wherein the filtering centrifuge comprises a sample feed port and/or a sample discharge port.~~

~~86.~~15. The method of any one of the preceding claims, ~~method of any one of the preceding claims,~~ wherein the mRNA suspension is loaded into the filtering centrifuge at a rate of about 1 liter/min to about 60 liter/min~~, e.g., at a rate of about 5 liter/min to about 45 liter/min~~.

~~87. The method of claim 86, wherein the total mRNA suspension is loaded into the filtering centrifuge in between about 0.5 hours to about 8 hours, for example in between about 2 hours to about 6 hours.~~

~~88. The method of any one of the preceding claims, wherein the manufacturing the mRNA comprises *in vitro* transcription (IVT) synthesis of the mRNA.~~

~~89. The method of claim 88, wherein the manufacturing the mRNA comprises a separate step of 3'-tailing of the mRNA.~~

~~90. The method of claim 89, wherein the separate step of 3'-tailing of the mRNA further comprising 5' capping of the mRNA.~~

~~91. The method of claim 88, wherein IVT synthesis of the mRNA comprises 5'-capping and optionally 3'-tailing of the mRNA.~~

~~92. The method of any one of claims 88-91, wherein steps (a) through (d) are performed after IVT synthesis of the mRNA.~~

~~93. The method of claim 92, wherein the volume of wash buffer for washing the retained precipitated mRNA after IVT synthesis is less than 8 L/g mRNA, e.g., less than 6 L/g mRNA or less than 5 L/g mRNA.~~

~~94. The method of claim 93, wherein the volume of wash buffer for washing the retained precipitated mRNA after IVT synthesis is between about 0.5 L/g mRNA and about 4 L/g mRNA.~~

95. The method of claim 94, wherein the volume of wash buffer for washing the retained precipitated mRNA after IVT synthesis is between about 0.5 L/g mRNA and about 1.5 L/g mRNA.

96. The method of any one of claims 87-91, wherein steps (a) through (d) are performed after IVT synthesis of the mRNA and again after the separate step of 3’-tailing of the mRNA.

97. The method of claim 96, wherein the total volume of wash buffer for washing the retained precipitated mRNA after IVT synthesis and/or after the separate step of 3’-tailing of the mRNA is less than 8 L/g mRNA, e.g., less than 6 L/g mRNA or less than 5 L/g mRNA.

98. The method of claim 97, wherein the total volume of wash buffer for washing the retained precipitated mRNA after IVT synthesis and/or after the separate step of 3’-tailing of the mRNA is between about 0.5 L/g mRNA and about 4 L/g mRNA.

99. The method of claim 98, wherein the total volume of wash buffer for washing the retained precipitated mRNA after IVT synthesis and/or after the separate step of 3’-tailing of the mRNA is between about 0.5 L/g mRNA and about 1.5 L/g mRNA, for example about 1 L/g mRNA.

100. The method of any one of the preceding claims, wherein the mRNA is or greater than about 1 kb, 1.5 kb, 2 kb, 2.5 kb, 3 kb, 3.5 kb, 4 kb, 4.5 kb, 5 kb, 6 kb, 7 kb, 8 kb, 9 kb, 10 kb, 11 kb, 12 kb, 13 kb, 14 kb, 15 kb, or 20 kb in length.

101. The method of any one of the preceding claims, wherein the mRNA comprises one or more nucleotide modifications.

102. The method of claim 101, wherein the one or more nucleotide modifications comprises modified sugars, modified bases, and/or modified sugar phosphate backbones.

103. The method of any one of claims 1-100, wherein the mRNA is comprises no nucleotide modifications.

104. The method of any one of the preceding claims, wherein the recovery of purified mRNA is at least 10g, 20g, 50g, 100g, 250g, 500g, 1kg, 5kg, 10kg, 50kg, or 100kg per single batch.

~~105. The method of any one of the preceding claims, wherein the total purified mRNA is recovered in an amount that results in a yield of at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or about 100%.~~

~~106. The method of claim 105, wherein the total purified mRNA is recovered in an amount that results in a yield of about 80% to about 100%.~~

~~107. The method of claim 106, wherein the total purified mRNA is recovered in an amount that results in a yield of about 90% to about 99%.~~

~~108. The method of claim 107, wherein the total purified mRNA is recovered in an amount that results in a yield of at least about 90%.~~

~~109. The method of any one of the preceding claims, wherein the purity of the purified mRNA is between about 60% and about 100%.~~

~~110. The method of claim 109, wherein the purity of the purified mRNA is between about 80% and 99%.~~

~~111. The method of claim 110, wherein the purity of the purified mRNA is between about 90% and about 99%.~~

~~112. The method of any one of the preceding claims, wherein the purified mRNA has an integrity of at least about 80%, 85%, 90%, 92%, 94%, 95%, 96%, 97%, 98%, or 99%.~~

~~113. The method of claim 112, wherein the purified mRNA has an integrity of or greater than about 95%.~~

~~114. The method of claim 113, wherein the purified mRNA has an integrity of or greater than about 98%.~~

~~115. The method of claim 114, wherein the purified mRNA has an integrity of or greater than about 99%.~~

116. The method of any one of the preceding claims, wherein the purified mRNA has a clinical grade purity without further purification.

117. The method of claim 116, wherein the purified mRNA comprises 5% or less, 4% or less, 3% or less, 2% or less, 1 % or less or is substantially free of protein contaminants as determined by capillary electrophoresis.

118. The method of claim 116 or 117, wherein the purified mRNA comprises less than 5%, less than 4%, less than 3%, less than 2%, less than 1 %, or is substantially free of salt contaminants determined by high performance liquid chromatography (HPLC).16

119. The method of any one of claims 116-118, wherein the purified mRNA comprises 5% or less, 4% or less, 3% or less, 2% or less, 1 % or less or is substantially free of short abortive transcript contaminants determined by high performance liquid chromatography (HPLC).

120. The method of any one of the preceding claims, wherein the purified mRNA has integrity of 95% or greater, 96% or greater, 97% or greater, 98% or greater, or 99% or greater as determined by capillary electrophoresis.

121. The method of any one of claims 116-120, wherein the clinical grade purity is achieved without the further purification selected from high performance liquid chromatography (HPLC) purification, ligand or binding based purification, tangential flow filtration (TFF) purification, and/or ion exchange chromatography.

122. The method of any one of the preceding claims, wherein one or more protein and/or short abortive transcript contaminants include enzyme reagents used in IVT mRNA synthesis.

123. The method of claim 122, wherein the enzyme reagents include a polymerase enzyme (e.g., T7 RNA polymerase or SP6 RNA polymerase), DNAse I, pyrophosphatase and a capping enzyme.

124. The method of any one of the preceding claims, wherein the method also removes long abortive RNA species, double-stranded RNA (dsRNA), residual plasmid DNA residual solvent and/or residual salt.

~~125. The method of any one of the preceding claims, wherein the short abortive transcript contaminants comprise less than 15 bases.~~

~~126. The method of any one of preceding claims, wherein the short abortive transcript contaminants comprise about 8-12 bases.~~

~~127. The method of the preceding claims, wherein the method also removes RNAse inhibitor.~~

~~128.~~16. A purified mRNA obtained by the method of any one of claims 1-~~127~~15.

~~129.~~17. A composition comprising the purified mRNA of claim ~~128~~16.

~~130. The composition of claim 129, further comprising at least one pharmaceutically acceptable excipient.~~

~~131. A method for treating a disease or disorder comprising administering to a subject in need thereof the purified mRNA of claim 128 or the composition of claim 129 or 130.~~

~~132.~~18. The purified mRNA of claim ~~128~~ 16 or the composition of claim ~~129 or 130~~ 17 for use in therapy.

~~133.~~19. A process for purifying mRNA, the process comprising the steps of:

I. providing a suspension comprising precipitated mRNA in a first vessel, wherein the precipitated mRNA comprises one or more protein and/or short abortive transcript contaminants from manufacturing the mRNA;
II. providing a wash buffer in a second vessel;
III. transferring the content of the first vessel into a filtering centrifuge comprising a filter, wherein the transferring occurs at a rate of about 5 liter/min/m^2 to about 25 liter/min/m^2 (with respect to the surface area of the filter of the filtering centrifuge) while the filtering centrifuge is in operation at a first centrifuge speed such that the precipitated mRNA is retained on the filter of said filtering centrifuge;
IV. transferring the content of the second vessel into the filtering centrifuge, wherein the transferring occurs at a rate of about 5 liter/min/m^2 to about 25 liter/min/m^2 (with respect to the surface area of the filter of the filtering centrifuge)while the filtering

centrifuge remains in operation at the first centrifuge speed, thereby washing the precipitated mRNA retained on the filter of said filtering centrifuge with the wash buffer; and

V. recovering the washed precipitated mRNA from the filter of said filtering centrifuge.

~~134. The process of claim 133, wherein the transferring in steps (III) and (IV) is by pumping.~~

~~135. The process of claim 134, wherein the pumping in steps (III) and (IV) is by a single pump operably linked to the first and second vessels.~~

~~136. The process of claim 135, wherein one or more valves control the transferring from the first vessel and the second vessel.~~

~~137. The process of any one of claims 133-136, wherein the content of the first vessel and the content of the second vessel are transferred to the filtering centrifuge via a sample feed port.~~

~~138. The process of claim 133, wherein the filter of the filtering centrifuge is rinsed with water for injection comprising 1% 10N NaOH after step (V).~~

~~139. The process of any one of claims 133-138, wherein the suspension comprising precipitated mRNA includes a filtration aid.~~

~~140. The process of claim 139, further comprising:~~

~~i. solubilising the washed precipitated mRNA comprising the filtration aid, which was recovered in step (V);~~

~~ii. transferring the solubilised mRNA from step (i) into a or said filtering centrifuge at a rate of about 5 liter/min/m^2 to about 25 liter/min/m^2 with respect to the surface area of the filter of the filtering centrifuge (*e.g.* about 15 liter/min/m^2), wherein the filtering centrifuge comprises a filter for retaining the filtration aid; and~~

~~iii. collecting the solubilised purified mRNA from the filtering centrifuge by centrifugation.~~

~~141. The process of claim 140, wherein the transferring is done through a sample feed port of the filtering centrifuge.~~

~~142. The process of claim 140 or 141, wherein step (iii) comprises collecting the solubilised purified mRNA via a sample discharge port of the filtering centrifuge.~~

~~143. A system for purifying mRNA, wherein the system comprises:~~

~~I. a first vessel for receiving precipitated mRNA;~~

~~II. a second vessel for receiving wash buffer;~~

~~III. a third vessel for receiving the washed precipitated mRNA and/or an aqueous medium for solubilising precipitated mRNA;~~

~~IV. a filtering centrifuge comprising:~~

~~i. a filter, wherein the filter is arranged and dimensioned to retain precipitated mRNA and/or a filtration aid, and to let pass solubilised mRNA;~~

~~ii. a sample feed port; and~~

~~iii. a sample discharge port;~~

~~V. a fourth vessel for receiving purified mRNA, wherein said vessel is connected to the sample discharge port of the filtering centrifuge;~~

~~VI. a pump configured to direct flow through the system at a rate of about 5 liter/min/m^2 to about 25 liter/min/m^2 with respect to the surface area of the filter of the filtering centrifuge (*e.g.* about 15 liter/min/m^2); wherein the first vessel, the second vessel and the third vessel are operably linked to an input of the pump, and wherein the sample feed port of the filtering centrifuge is connected to an output of the pump; and~~

~~VII. one or more valves configured to preclude simultaneous flow from the first, second and third vessels.~~

~~144. The system according to claim 143, wherein the first centrifuge speed exerts a gravitational (g) force of less than 1300 g.~~

~~145. The system according to claim 144, wherein the system further comprises a data processing apparatus comprising means for controlling the system to carry out the method of claim 137.~~

~~146. The system according to claim 145, wherein the data processing apparatus is (a) a computer program comprising instructions or (b) a computer-readable storage medium comprising instructions.~~

~~147.~~ 20. A composition comprising mRNA, amphiphilic polymer and a filtration aid at relative concentrations of about 1:1:10 in a sterile, RNase-free container.

~~148. The composition of claim 147, wherein the composition comprises 10 g, 50 g, 100 g, 200 g, 300 g, 400 g, 500 g, 600 g, 700 g, 800 g, 900 g, 1 kg, 5 kg, 10 kg, 50 kg, 100 kg, one metric ton, ten metric ton or more of mRNA.~~

~~149. The composition of claim 147 or 148, wherein the amphiphilic polymer comprises PEG having a molecular weight of about 2000-10000 g/mol; 4000-8000 g/mol or about 6000 g/mol (for example PEG-6000).~~

~~150. The composition of claim 147 or 148, wherein the amphiphilic polymer comprises MTEG.~~

~~151. The composition of any one of claims 147-150, wherein the filtration aid is cellulose-based.~~