CLAIMS

1. A method of purifying messenger RNA (mRNA), comprising:

(a) subjecting a preparation comprising *in vitro* synthesized mRNA to an enzymatic digestion with a proteinase; and

(b) subjecting the preparation obtained from step (a) to an oligo dT affinity chromatography.

2. The method of claim 1, wherein:

i) the proteinase comprises a serine protease;

ii) the preparation comprising *in vitro* synthesized mRNA is incubated with the proteinase at about 37 °C for at least 30 minutes;

iii) the proteinase is inactivated with a reducing agent, and/or wherein the proteinase is inactivated with a reducing agent and the reducing agent is dithiothreitol (DTT); and/or

iv) the preparation obtained from step (a) is subjected to a tangential flow filtration (TFF) step prior to step (b), and/or the preparation obtained from step (a) is subjected to a tangential flow filtration (TFF) step prior to step (b) and the TFF uses about a 100 kDa to about a 300 kDa filter.

3. The method of of claim 1 or claim 2, wherein:

i) the preparation is subjected to a step of capping prior to step (b) to produce a capped mRNA;

ii) the preparation is subjected to a step of capping prior to step (b) to produce a capped mRNA and the capping is performed at about 37 °C for at least 30 minutes; and/or

iii) the preparation is subjected to a step of capping prior to step (b) to produce a capped mRNA and the capping is performed at about 37 °C for at least 30 minutes with stirring.

4. The method of any one of claims 1-3, wherein:

i) the preparation is subjected to a step of tailing with a poly(A) polymerase to produce a poly(A) tailed mRNA; and/or

ii) the preparation obtained from step (b) is subjected to a step of TFF, and/or the preparation obtained from step (b) is subjected to a step of TFF and the TFF uses about a 50 kDa to about a 300 kDa filter.

5. The method of claim 3 or claim 4, wherein the preparation is subjected to a step of frontal filtration after the capping step.

6. A method of purifying messenger RNA (mRNA), comprising:

(a) subjecting a preparation comprising *in vitro* synthesized mRNA to an enzymatic digestion with a proteinase;

(b) subjecting the preparation obtained from step (a) to a step of tangential flow filtration (TFF);

(c) subjecting the preparation obtained from step (b) to an oligo dT affinity chromatography; and

(d) subjecting the preparation obtained from step (c) to a step of TFF.

7. The method of claim 6, wherein the proteinase comprises a serine protease.

8. The method of claim 5 or 6, wherein the preparation comprising *in vitro* synthesized mRNA is incubated with the proteinase at about 37 °C for at least 30 minutes.

9. The method of any one of claims 6-8, wherein the proteinase is inactivated with a reducing agent, and/or wherein the proteinase is inactivated with a reducing agent wherein the reducing agent is dithiothreitol (DTT).

10. The method of any one of claims 6-9, wherein the TFF of step (b) uses about a 100 kDa to about a 300 kDa filter.

11. The method of any one of claims 6-10, wherein:

i) the preparation is subjected to a step of capping prior to step (c) to produce a capped mRNA, and/or the preparation is subjected to a step of capping prior to step (c) to produce a capped mRNA and wherein capping is performed at about 37 °C for at least 30 minutes, and/or wherein the preparation is subjected to a step of capping prior to step (c) to produce a capped mRNA and wherein capping is performed at about 37 °C for at least 30 minutes with stirring;

ii) the preparation is subjected to a step of tailing with a poly(A) polymerase to produce a poly(A) tailed mRNA, and/or the preparation is subjected to a step of tailing with a poly(A) polymerase to produce a poly(A) tailed mRNA wherein tailing is performed prior to step (c), and/or the preparation is subjected to a step of tailing with a poly(A)

polymerase to produce a poly(A) tailed mRNA wherein tailing is performed prior to step (c) concomitantly to capping or after capping; and/or

iii) the TFF of step (d) uses about a 50 kDa to about a 300 kDa filter.

12. The method of any one of claims 1-11, wherein:

i) no precipitation step is performed; and/or

ii) at least about 0.5 grams of mRNA is purified.

13. The method of claim 11 or claim 12, wherein the preparation is subjected to a step of frontal filtration after the capping step.

14. A method of purifying messenger RNA (mRNA), comprising:

(a) subjecting a preparation comprising *in vitro* synthesized mRNA to an enzymatic digestion with a proteinase;

(b) subjecting the preparation obtained from step (a) to a step of tangential flow filtration (TFF);

(c) subjecting the preparation obtained from step (b) to a step of capping;

(d) subjecting the preparation obtained from step (c) to a step of frontal filtration;

(e) subjecting the preparation obtained from step (d) to an oligo dT affinity chromatography; and

(f) subjecting the preparation obtained from step (e) to a step of TFF.

15. The method of claim 14, wherein the preparation is subjected to a step of frontal filtration after step (f).

16. The method of any one of claims 5, or 13-15, wherein:

i) the frontal filtration uses a 0.2 μm filter; and/or

ii) the frontal filtration reduces guanylyltransferase (GuaT) content.

17. The method of any one of the preceding claims, wherein the *in vitro* synthesized mRNA is purified at a scale of or greater than about 100 milligram, 1 gram, 10 gram, 20 gram, 50 gram, 100 gram or more per batch.

18. The method of any one of the preceding claims, wherein the *in vitro* synthesized mRNA is purified at a scale of or greater than about 10 gram per batch or at a scale of or greater than about 20 gram per batch, and:

(i) the residual plasmid DNA in the purified mRNA is equal to or less than about 0.2 pg/mg;

(ii) the purified mRNA contains less than about 0.1% of dsRNA;

(iii) wherein the purified mRNA contains less than about 1% of enzyme reagents used in in vitro synthesis; and/or

(iv) the purified mRNA has an integrity greater than about 80%.

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

i) the purified mRNA is suitable for therapeutic use; and/or

ii) the method does not include any additional affinity chromatography steps.

20. A method for manufacturing messenger RNA (mRNA) comprising:

synthesizing mRNA *in vitro*; and

purifying the *in vitro* synthesized mRNA using a method according to any one of the preceding claims.