nucleotide and/or modified sugar phosphate backbones. In some embodiments, the mRNA is unmodified.

[0023] In some embodiments, the purified mRNA has an integrity of or greater than about 95% (e.g., of or greater than about 96%, 97%, 98%, or 99%). In some embodiments, the purified mRNA has an integrity of or greater than about 98%. In some embodiments, the purified mRNA has an integrity of or greater than about 99%.

[0024] In some embodiments, the present invention provides a method of purifying messenger RNA (mRNA), comprising (a) precipitating mRNA from an impure preparation; (b) subjecting the impure preparation comprising precipitated mRNA to tangential flow filtration such that the precipitated mRNA is captured by a filtration membrane while impurities are discarded through permeation; and (c) eluting the captured precipitated mRNA by re-solubilizing the precipitated mRNA, resulting in a purified mRNA solution.

[0025] In another aspect, the present invention provides a method for manufacturing messenger RNA (mRNA) comprising synthesizing mRNA *in vitro*; and purifying the *in vitro* synthesized mRNA using a method described herein.

[0026] Among other things, the present invention also provides a messenger RNA (mRNA) purified using a method described herein.

[0027] In another aspect, the present invention also provides a batch of purified mRNA comprising 5 gram or more of a single mRNA species suitable for administration to a human subject. In another aspect, the present invention also provides a batch of purified mRNA comprising 5, 10 or 25 gram or more of a single mRNA species suitable for administration to a human subject. In some embodiments, the batch comprises 10 gram of more of a single mRNA species. In some embodiments, the batch comprises 25 gram of more of a single mRNA species. In some embodiments, the batch is substantially free of impurities from an mRNA synthesis process. In some embodiments, the batch is substantially free of prematurely aborted RNA sequences, DNA templates, and/or enzyme reagents used in in vitro synthesis of the single mRNA species. In some embodiments, the purified mRNA contains less than about 5% of enzyme reagents used in in vitro synthesis. In some embodiments, the purified mRNA has an integrity greater than about 95%.

[0028] In another aspect, the present invention also provides a composition comprising messenger RNA purified using a method as described herein.

[0028a] In another aspect, the present invention also provides composition comprising messenger RNA purified using a method according to any one of the preceding claims,

optionally wherein

(a) the messenger RNA is *in vitro* synthesized and

(b) (i) the composition contains less than 1% of prematurely aborted RNA sequences and/or enzyme reagents used in *in vitro* synthesis; or

(ii) the mRNA has an integrity of or greater than 95%.

[0029] In another aspect, the present invention also provides a composition comprising *in vitro* synthesized messenger RNA, wherein the composition contains less than 1% of prematurely aborted RNA sequences and/or enzyme reagents used in in vitro synthesis.

[0030] In another aspect, the present invention also provides a composition comprising in vitro synthesized messenger RNA (mRNA), wherein the mRNA has an integrity of or greater than 95%.

[0031] As used in this application, the terms "about" and "approximately" are used as equivalents. Any numerals used in this application with or without about/approximately are meant to cover any normal fluctuations appreciated by one of ordinary skill in the relevant art.

[0032] Other features, objects, and advantages of the present invention are apparent in the detailed description that follows. It should be understood, however, that the detailed description, while indicating embodiments of the present invention, is given by way of illustration only, not limitation. Various changes and modifications within the scope of the invention will become apparent to those skilled in the art from the detailed description.

BRIEF DESCRIPTION OF THE DRAWING

[0033] The following figures are for illustration purposes only and not for limitation.

[0034] **Figure 1** depicts an exemplary process for large-scale purification of mRNA involving load, wash and elute steps. For example, the precipitated mRNA can be loaded to membranes such that the precipitated mRNA may be captured as retentate while soluble impurities as well as insoluble ones less than 0.22 um are discarded through the permeate. After capture, the solid precipitate is washed with various buffers followed by re-solubilization and elution for a pure messenger RNA product.

[0035] **Figure 2** shows an exemplary silver-stained protein gel of modified cystic fibrosis transmembrane conductance regulator (CFTR) mRNA from an initial 1 gram *in vitro* transcription (IVT) reaction according to provided methods (with three elutions) and control enzymes present in the IVT reaction.

CLAIMS

We claim:

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

(a) precipitating mRNA from an impure preparation; and

(b) subjecting the impure preparation comprising precipitated mRNA to a purification process involving membrane filtration such that the precipitated mRNA is captured by a membrane; and

(c) eluting the captured precipitated mRNA from the membrane by re-solubilizing the mRNA, thereby resulting in a purified mRNA solution.

2. The method of claim 1, wherein the purification process involving membrane filtration is

(i) tangential flow filtration,

(ii) direct flow filtration.

3. The method of claim 2, wherein the step of precipitating mRNA comprises:

(a) treating the impure preparation with a solution comprising a reagent selected from the group consisting of lithium chloride, potassium chloride, guanidinium chloride, guanidinium thiocyanate, guanidinium isothiocyanate, ammonium acetate and combinations thereof,

optionally wherein the reagent is guanidinium thiocyanate,

optionally wherein the solution comprises:

(i) 4M guanidinium thiocyanate, 0.5% sodium lauryl sarcosyl, and 25 mM sodium citrate;

(ii) 4M guanidinium thiocyanate, and 0.5% sodium lauryl sarcosyl; or

(iii) 4M guanidinium thiocyanate, and 25 mM sodium citrate,

and/or

(b) further comprises a step of treating the impure preparation with absolute ethanol.

4. The method of any one of the preceding claims, wherein the membrane is selected from the group consisting of polyethersulfone (mPES) (not modified), polyethersulfone (mPES) hollow fiber membrane, polyvinylidene fluoride (PVDF), cellulose acetate, nitrocellulose, MCE (mixed cellulose esters), ultra-high MW polyethylene (UPE), polyfluorotetraethylene (PTFE), nylon, and combination thereof.

5. The method of any one of the preceding claims, wherein the method further comprises washing the captured precipitated mRNA before eluting,

optionally wherein the washing step comprises multiple rinse cycles using a wash solution comprising a guanidinium buffer and ethanol, followed by about 70-80% ethanol,

optionally wherein the multiple rinse cycles are more than 5 cycles.

6. The method of any one of the preceding claims, wherein the eluting step comprises re-solubilizing the captured precipitated mRNA with RNAse-free water,

optionally wherein the RNAse-free water is re-circulated for 5-10 minutes.

7. The method of any one of the preceding claims, wherein the method further comprises a step of dialyzing the purified mRNA solution,

optionally wherein the purified mRNA solution is dialyzed with 1mM sodium citrate using a 100 kDa molecular weight cut-off (MWCO) membrane.

8. The method of any one of the preceding claims, wherein the mRNA is *in vitro* synthesized and the impure preparation comprises an *in vitro* mRNA synthesis reaction mixture,

optionally wherein the impure preparation comprises prematurely aborted RNA sequences and/or enzyme reagents used in *in vitro* synthesis

optionally wherein the purified mRNA solution contains less than 1% , less than 0.5%, less than 0.1% of prematurely aborted RNA sequences and/or enzyme reagents used in *in vitro* synthesis or

is substantially free of prematurely aborted RNA sequences and/or enzyme reagents used in *in vitro* synthesis,

optionally wherein (i) the prematurely aborted RNA sequences and/or enzyme reagents used in *in vitro* synthesis are measured via silver stain, gel electrophoresis, HPLC, UPLC, and/or capillary electrophoresis,

optionally wherein the prematurely aborted RNA sequences comprise less than 15 bases,

e.g. about 8-12 bases,

or (ii) the enzyme reagents used in *in vitro* synthesis comprise T7 RNA polymerase, DNAse I, pyrophosphatase, and/or RNAse inhibitor.

9. The method of any one of the preceding claims, wherein the mRNA is purified:

(a) at a scale of or greater than 1 gram, 10 gram, 100 gram, 1 kg, 10 kg, or 100 kg per batch, and/or

(b) (i) before a cap and tail are added to the mRNA,

(ii) after a cap and tail are added to the mRNA,

(iii) after a cap is added, or

(iv) both before and after a cap and/or tail are added to the mRNA.

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

(a) is, or is 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, and/or

(b) comprises one or more modifications to enhance stability,

optionally wherein the one or more modifications comprises modified nucleotide and/or modified sugar phosphate backbones, or

is unmodified.

11. The method of any one of the preceding claims, wherein the purified mRNA has an integrity of or greater than 95%, 98% or 99%.

12. 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.

13. A messenger RNA (mRNA) purified using a method according to any one of the preceding claims.

14. A batch of purified mRNA comprising 5, 10 or 25 gram or more of a single mRNA species suitable for administration to a human subject.

15. The batch according to claim 14, wherein;

(a) the batch is substantially free of impurities from an mRNA synthesis process,

optionally wherein the batch is substantially free of prematurely aborted RNA sequences, DNA templates, and/or enzyme reagents used in in vitro synthesis of the single mRNA species,

(b) the purified mRNA contains less than about 5% of enzyme reagents used in in vitro synthesis, and/or

(c) the purified mRNA has an integrity greater than about 95%.

16. A composition comprising messenger RNA purified using a method according to any one of the preceding claims,

optionally wherein

(a) the messenger RNA is *in vitro* synthesized and

(b) (i) the composition contains less than 1% of prematurely aborted RNA sequences and/or enzyme reagents used in *in vitro* synthesis; or

(ii) the mRNA has an integrity of or greater than 95%.