## CLAIMS

WHAT IS CLAIMED IS:

1. A method of producing a transcribed RNA product comprising:

a. reacting a transcription reaction mixture comprising a buffer solution comprising $Mg^{2+}$, linear DNA (L.DNA) template, ribonucleoside tri-phosphate (rNTPs), optionally an RNA capping reagent, and RNA polymerase; wherein a molar concentration of $Mg^{2+}$ is 2 to 15 mM above the total molar concentration of all rNTPs plus the optional RNA capping reagent; and a RNA polymerase/L. DNA template mass ratio is between 0.25 and 3;

b. stopping the transcription reaction by digesting the L.DNA template with deoxyribonuclease (DNase) or quenching the enzyme with ethylenediaminetratracetic acid (EDTA);

wherein a yield of about 1 g to about 25 g of single stranded transcribed RNA per liter of liquid of the transcription reaction prior to the addition of DNase in step (b) is produced.

2. The method of claim 1, wherein the L.DNA template is characterized by one or more of the following:

wherein the L.DNA template is in a solution comprising 50 mM to 1200 mM NaCl to produce a salt-spiked L.DNA template prior to introduction to the transcription reaction mixture,

wherein the L.DNA template is from about 0.01 mg/mL to about 0.3 mg/mL in the transcription reaction mixture, and

wherein the L.DNA template comprises an open reading frame that encodes a vaccine antigen, enzyme, antibody, receptor, tRNA, and/ or a protein.

3. The method of claim 1 or 2, wherein the transcription reaction mixture of step (a) further comprises one or more of the group consisting of RNase inhibitor and inorganic pyrophosphatase.

4. The method of any one of claims 1 to 3, wherein the pH of the reaction mixture is a range from 6.5 to 8.0.

5. The method of any one of claims 1 to 4, wherein the temperature during step a) and b) is in a range from about 30 °C to about 40 °C.

6. The method of any one of claims 1 to 5, wherein the RNA polymerase is T7 polymerase, optionally wherein a T7 polymerase KU activity per mg of L.DNA is greater than or equal to 125 KU T7 polymerase activity.

7. The method of any one of claims 1 to 6, wherein the transcription reaction mixture of step (a) is allowed to react for at least 20 minutes prior to step (b) stopping the transcription reaction.

8. The method of any one of claims 1 to 7, wherein the transcription mixture of step (a) further comprises a transcription initiating RNA capping reagent.

9. The method of any one of claims 1 to 8, wherein the molar concentration of $Mg^{2+}$ is 5 to 15 mM above the total molar concentration of all rNTPS plus the molar concentration of any optional RNA capping reagent.

10. The method of any one of claims 1 to 9, wherein the RNA polymerase is 0.0125 to 0.15 µg/µL T7 polymerase.

11. The method of any one of claims 1 to 10, wherein the reaction produces 3 to 20 g/L of single stranded transcribed RNA.

12. The method of any one of claims 1 to 11, wherein the amount of single stranded transcribed RNA is measured after purification of the transcription mixture after step (b) via a silica column.

13. The method of any one of claims 1 to 12, wherein the amount of single stranded transcribed RNA is measured after purification of the transcription mixture after step (b) via affinity column chromatography.

14. The method of claim 13, wherein purification of the transcription mixture after step (b) via a silica column is performed before purification via affinity column chromatography.

15. The method of any one of claims 1 to 14, wherein the RNA transcription mixture of step (a) further comprises one or more solvents selected from the group consisting of EtOH in a concentration of 1 to 10 % v/v, i-PrOH in a concentration of 1 to 10% v/v, MeOH in a concentration of 1 to 10 % v/v, and acetonitrile of a concentration of 1 to 8 % v/v, with the

proviso that the total concentration of EtOH, i-PrOH, MeOH and acetonitrile does not exceed 10% v/v of the RNA transcription mixture of step (a).

16. The method of any one of claims 1 to 15, wherein the total concentration of rNTPs is at least 8 mM.

17. The method of any one of claims 1 to 16, wherein a yield of greater than about 5 g/ L of RNA transcript results in a reduced amount of dsRNA as compared to an otherwise identical transcription reaction wherein the yield is less than about 5 g/L.

18. A method of producing a transcribed RNA product comprising:

a. loading a reactor vessel with a buffer solution comprising $Mg^{2+}$ and nuclease free water;
b. adding a solution comprising L.DNA template spiked with 200-1000 mM NaCl;
c. adding rNTPs and optionally RNA cap;
d. adding RNA polymerase and mixing the resulting transcription solution wherein the RNA polymerase/DNA template mass ratio is between 0.25 and 3; and,
e. adding DNase to the transcription solution;

wherein the molar concentration of $Mg^{2+}$ is 2 to 15 mM above the total molar concentration of all rNTPs plus the optional RNA cap after steps (a)-(b) are completed, and wherein 1 to 25 g of single stranded transcribed RNA/ L of liquid of the transcription reaction prior to the addition of DNase in step (b) is produced, and

optionally wherein a yield of greater than about 5 g/L of RNA transcript results in a reduced amount of dsRNA as compared to an otherwise identical transcription reaction wherein the yield is less than about 5 g/L.

19. A method of producing a transcribed RNA product with reduced dsRNA comprising:

a. reacting a transcription reaction mixture comprising a buffer solution comprising $Mg^{2+}$, L.DNA template previously spiked with 50 to 1200 mM NaCl, rNTPs, optionally RNA cap, RNA polymerase wherein the RNA polymerase/L. DNA template mass ratio is between 0.25 and 3, and one or more solvents selected from the group consisting of EtOH in a concentration of 1 to 10 % v/v, i-PrOH in a concentration of 1 to 10% v/v, MeOH in a concentration of 1 to 10 % v/v, and acetonitrile of a concentration of 1 to 8 % v/v, with the proviso that the total concentration of EtOH, i-PrOH, MeOH and acetonitrile does not exceed 10 +/-1 % v/v of the RNA transcription mixture; and,
b. adding DNase to the transcription reaction mixture;

wherein the molar concentration of $Mg^{2+}$ in the transcription reaction mixture is 2 to 15 mM above the total molar concentration of all rNTPs plus the optional RNA cap.

20. The method of claim 19, wherein the amount of dsRNA is reduced compared to an otherwise identical transcription reaction wherein the transcription reaction mixture excludes added EtOH, i-PrOH, MeOH, and acetonitrile.