

CLAIMS

What is claimed is:

1. An engineered expression construct (EEC) having a coding sequence operably linked to a 5' untranslated region (UTR) consisting of the sequence as set forth in SEQ ID NO: 38 and a 3' UTR consisting of the sequence as set forth in SEQ ID NO: 13, 14, or 15.
2. An engineered expression construct (EEC) having a 5' untranslated region (UTR) operably linked to a coding sequence, wherein the 5' UTR has the sequence as set forth in CAUACUCA in between a minimal promoter and a Kozak sequence.
3. The EEC of claim 2, wherein the minimal promoter is a T7 promoter.
4. The EEC of claim 3, wherein the T7 promoter has the sequence as set forth in GGGAGA.
5. The EEC of claim 2, wherein the Kozak sequence has the sequence as set forth in GCCRCCAUG, wherein R is A or G.
6. The EEC of claim 2, wherein the 5' UTR has
 - (i) the sequence as set forth in SEQ ID NO: 2 operably linked to a start codon or
 - (ii) the sequence as set forth in SEQ ID NO: 3 operably linked to a start codon.
7. The EEC of claim 6, wherein the sequence as set forth in SEQ ID NO: 2 operably linked to a start codon has the sequence as set forth in SEQ ID NO: 38.
8. The EEC of claim 6, wherein the sequence as set forth in SEQ ID NO: 3 operably linked to a start codon has the sequence as set forth in SEQ ID NO: 39.
9. The EEC of claim 2, wherein the 5' UTR is less than 30 nucleotides.
10. The EEC of claim 2, further comprising a 3' UTR.
11. The EEC of claim 10, wherein the 3' UTR comprises a spacer, and a stem loop structure operably linked to a stop codon.
12. The EEC of claim 11, wherein the stop codon has the sequence UAA, UGA, or UAG.
13. The EEC of claim 11, wherein the spacer has the sequence [N₁₋₃]AUA or [N₁₋₃]AAA.
14. The EEC of claim 11, wherein the spacer has the sequence UGCAUA or UGCAAA.
15. The EEC of claim 11, wherein the stem loop structure has hybridizing sequences as set forth in CCUC and GAGG.
16. The EEC of claim 11, wherein the stem loop structure has hybridizing sequences as set forth in AAACCUC and GAGG or as set forth in AAAGAGG and CCUC.
17. The EEC of claim 11, wherein the stem loop structure has a loop segment having at least 7 nucleotides.
18. The EEC of claim 11, wherein the stem loop structure has a loop segment having 7 – 15

nucleotides.

19. The EEC of claim 11, wherein the stem loop structure has a loop segment having the sequence as set forth in UAACGGUCUU (SEQ ID NO: 34).

20. The EEC of claim 10, wherein the 3' UTR is less than 30 nucleotides.

21. The EEC of claim 10, wherein the 3' UTR further comprises a polyadenine (polyA) tail.

22. The EEC of claim 21, wherein the polyA tail has 60 residues or less.

23. The EEC of claim 21, wherein the polyA tail has 40 residues.

24. The EEC of claim 10, wherein the 3' UTR has the sequence as set forth in SEQ ID NO: 4, 5, 6, 7, 8, or 9.

25. The EEC of claim 10, wherein the 3' UTR has the sequence as set forth in SEQ ID NO: 10, 11, or 12.

26. The EEC of claim 10, wherein the 3' UTR has the sequence as set forth in SEQ ID NO: 13, 14, 15, 16, 17, 18, 19, 20, or 21.

27. The EEC of claim 2, wherein the EEC comprises in vitro-synthesized messenger RNA (mRNA).

28. The EEC of claim 2, wherein the coding sequence encodes Green Fluorescent Protein (GFP), Human Interleukin-2 (IL2) or Human POU5F1 (or OCT3/4).

29. The EEC of claim 2, having the sequence as set forth in SEQ ID NO: 56, 58, or 60.

30. The EEC of claim 2, wherein the coding sequence encodes a therapeutic protein.

31. The EEC of claim 30, wherein the therapeutic protein comprises an antibody or binding fragment thereof.

32. The EEC of claim 31, wherein the antibody or binding fragment thereof comprises an anti-SARS-Cov2 antibody or binding fragment thereof, an anti-SARS antibody or binding fragment thereof, an anti-RSV antibody or binding fragment thereof, an anti-HIV antibody or binding fragment thereof, an anti-Dengue virus antibody or binding fragment thereof, an anti-Bordatella pertussis antibody or binding fragment thereof, an anti-hepatitis C antibody or binding fragment thereof, an anti-influenza virus antibody or binding fragment thereof, an anti-parainfluenza virus antibody or binding fragment thereof, an anti-metapneumovirus (MPV) antibody or binding fragment thereof, an anti-cytomegalovirus antibody or binding fragment thereof, an anti-Epstein Barr virus antibody; anti-herpes simplex virus antibody or binding fragment thereof, an anti-Clostridium difficile bacterial toxin antibody or binding fragment thereof, or an anti-tumor necrosis factor (TNF) antibody or binding fragment thereof.

33. The EEC of claim 2, wherein the coding sequence encodes a vaccine antigen.

34. The EEC of claim 33, wherein the vaccine antigen comprises a SARS-CoV-2 vaccine

antigen, a CMV vaccine antigen, an EBV vaccine antigen, a hepatitis vaccine antigen, a herpes simplex vaccine antigen, a human immunodeficiency virus (HIV), vaccine antigen, a human papillomavirus virus (HPV) viral antigen, an influenza vaccine antigen, a Japanese encephalitis vaccine antigen, a malaria vaccine antigen, a measles vaccine antigen, a rabies vaccine antigen, a respiratory syncytial vaccine antigen, a rotaviral vaccine antigen, a varicella zoster vaccine antigen, or a zika vaccine antigen.

35. The EEC of claim 2, wherein the coding sequence encodes a cytokine.

36. The EEC of claim 2, wherein the coding sequence encodes a cell-penetrating protein.

37. The EEC of claim 36, wherein the cell-penetrating protein comprises penetratin, the minimal domain of TAT, VP22, ZEBRA, melittin, mastoporan, maurocalcin, crotamine, buforin, poly-arginine, or transportan.

38. The EEC of claim 2, wherein the EEC does not comprise modified nucleosides.

39. The EEC of claim 2, wherein the EEC does not comprise microRNA binding sites.

40. The EEC of claim 2, wherein the coding sequence encodes an immune evading factor.

41. The EEC of claim 41, wherein the immune evading factor comprises B18R, E3, K3, NS1, or ORF8.

42. The EEC of claim 2, wherein the EEC does not comprise immune evading factors.

43. The EEC of claim 2, formulated for administration to a subject.

44. An enhancer sequence consisting of the sequence as set forth in CAUACUCA.

45. An engineered expression (EEC) construct having 1, 2, 3, 4, or 5 copies of the sequence as set forth in CAUACUCA.

46. The EEC of claim 44, wherein the enhancer sequence is operably linked to a promoter.

47. The EEC of claim 46, wherein the promoter is a minimal promoter.

48. An engineered expression construct comprising an in vitro-synthesized RNA comprising a coding sequence within an open reading frame that encodes a protein for translation in a mammalian cell, wherein said in vitro-synthesized RNA further comprises one of a 5' untranslated region comprising CAUACUCA and a 3' untranslated region comprising one of SEQ ID NOs: 4, 5, 6, or 7.

49. An engineered expression construct comprising an in vitro-synthesized RNA comprising a coding sequence within an open reading frame that encodes a protein for translation in a mammalian cell, wherein said in vitro-synthesized RNA further comprises one of a 5' untranslated region comprising SEQ ID NO: 2, or SEQ ID NO: 3 and a 3' untranslated region comprising SEQ ID NOs: 4, 5, 6, or 7.

50. An engineered expression construct comprising an in vitro-synthesized RNA comprising an

open reading frame that encodes a protein for translation in a mammalian cell, wherein said in vitro-synthesized RNA further comprises a 5' untranslated region comprising a T7 polymerase promoter, the sequence as set forth in CAUACUCA, and a Kozak sequence.

51. The engineered expression construct of claim 2, wherein the T7 promoter is selected from a T7 Class III promoter.

52. An engineered expression construct comprising an in vitro-synthesized RNA comprising an open reading frame that encodes a protein for translation in a mammalian cell, wherein said in vitro-synthesized RNA comprises a 3' untranslated region comprising SEQ ID NOs: 4, 5, 6, or 7 and a stop codon.

53. The engineered expression construct of claim 52, wherein the stop codon is UAA, UAG, or UGA.

54. An engineered expression construct comprising an in vitro-synthesized RNA comprising an open reading frame that encodes a protein for translation in a mammalian cell, wherein said in vitro-synthesized RNA comprises a 3' untranslated region comprising either a) CCUC and GAGG or b) GAGG and CCUC, wherein either set of the 3' untranslated region sequences is separated by no fewer than seven nucleotides.

55. An engineered expression construct (EEC) comprising an in vitro-synthesized RNA comprising an open reading frame that encodes a protein for translation in a mammalian cell, wherein said in vitro-synthesized RNA comprises a 3' untranslated region comprising either a) AAACCUC and GAGG or b) AAAGAGG and CCUC, wherein either set of the 3' untranslated region sequences is separated by no fewer than seven nucleotides.

56. A 5' untranslated region (UTR) comprising a sequence as set forth in CAUACUCA that is in between a minimal promoter and a Kozak sequence.

57. The 5'UTR of claim 56, wherein the minimal promoter is a T7 promoter.

58. The 5'UTR of claim 57, wherein the T7 promoter has the sequence as set forth in GGGAGA.

59. The 5'UTR of claim 56, wherein the Kozak sequence has the sequence as set forth in GCCRCCAUG, wherein R is adenosine or guanine.

60. The 5'UTR of claim 56, comprising the sequence as set forth in SEQ ID NO: 2 operably linked to a start codon.

61. The 5'UTR of claim 56, comprising the sequence as set forth in SEQ ID NO: 3 operably linked to a start codon.

62. The 5'UTR of claim 60, wherein the sequence as set forth in SEQ ID NO: 2 operably linked to a start codon has the sequence as set forth in SEQ ID NO: 38.

63. The 5'UTR of claim 61, wherein the sequence as set forth in SEQ ID NO: 3 operably linked to

a start codon has the sequence as set forth in SEQ ID NO: 39.

64. The 5'UTR of claim 56, wherein the 5' UTR is less than 30 nucleotides.

65. A 3'UTR comprising a spacer and a stem loop structure operably linked to a stop codon, wherein the stop codon has the sequence UAA, UGA, or UAG and the spacer has the sequence [N₁₋₃]AUA or [N₁₋₃]AAA.

66. The 3'UTR of claim 65, wherein the spacer has the sequence UGCAUA or UGCAAA.

67. The 3'UTR of claim 65, wherein the stem loop structure comprises hybridizing sequences as set forth in CCUC and GAGG.

68. The 3'UTR of claim 65, wherein the stem loop structure comprises hybridizing sequences as set forth in AAACCUC and GAGG or as set forth in AAAGAGG and CCUC.

69. The 3'UTR of claim 65, wherein the stem loop structure has a loop segment having at least 7 nucleotides.

70. The 3'UTR of claim 65, wherein the stem loop structure has a loop segment having 7 – 15 nucleotides.

71. The 3'UTR of claim 65, wherein the stem loop structure has a loop segment having the sequence as set forth in UAACGGUCUU (SEQ ID NO: 34).

72. The 3'UTR of claim 65, wherein the 3' UTR is less than 30 nucleotides.

73. The 3'UTR of claim 65, wherein the 3' UTR further comprises a polyadenine (polyA) tail.

74. The 3'UTR of claim 73, wherein the polyA tail has 60 residues or less.

75. The 3'UTR of claim 73, wherein the polyA tail has 40 residues.

76. The 3'UTR of claim 65, wherein the 3' UTR has the sequence as set forth in SEQ ID NO: 4, 5, 6, 7, 8, or 9.

77. The 3'UTR of claim 65, wherein the 3' UTR has the sequence as set forth in SEQ ID NO: 10, 11, or 12.

78. The 3'UTR of claim 65, wherein the 3' UTR has the sequence as set forth in SEQ ID NO: 13, 14, 15, 16, 17, 18, 19, 20, or 21.

79. The 3'UTR of claim 65, wherein the 3'UTR is operably linked to a coding sequence.

80. The 3'UTR of claim 79, wherein the coding sequence encodes a therapeutic protein, a vaccine antigen, a cytokine, or a fluorescent protein.

81. The 3'UTR of claim 79, wherein the coding sequence encodes an immune evading factor.

82. The 3'UTR of claim 81, wherein the immune evading factor comprises B18R, E3, K3, NS1, or ORF8.

83. The 3'UTR of claim 79, wherein the coding sequence does not comprise an immune evading factor.

84. The 3'UTR of claim 79, wherein the coding sequence encodes a cell-penetrating protein.
85. The 3'UTR of claim 84, wherein the cell-penetrating protein comprises penetratin, the minimal domain of TAT, VP22, ZEBRA, melittin, mastoporan, maurocalcin, crotamine, buforin, poly-arginine, or transportan.

CLAIMS

1. An in vitro-synthesized RNA wherein the in-vitro synthesized RNA comprises
 - a 5' untranslated region (UTR) that is less than 30 nucleotides and has the sequence as set forth in SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO:38, or SEQ ID NO: 39;
 - a 3' UTR that has the sequence as set forth in SEQ ID NO: 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21; and
 - an open reading frame that encodes a protein between the 5' UTR and the 3' UTR and wherein the in-vitro-synthesized RNA does not comprise modified nucleosides and does not comprise microRNA binding sites.
2. The in vitro-synthesized RNA of claim 1, wherein
 - the 5' UTR has the sequence as set forth in SEQ ID NO: 2 and the 3' UTR has the sequence as set forth in SEQ ID NO: 10,
 - the 5' UTR has the sequence as set forth in SEQ ID NO: 3 and the 3' UTR has the sequence as set forth in SEQ ID NO: 10,
 - the 5' UTR has the sequence as set forth in SEQ ID NO: 38 and the 3' UTR has the sequence as set forth in SEQ ID NO: 10,
 - the 5' UTR has the sequence as set forth in SEQ ID NO: 39 and the 3' UTR has the sequence as set forth in SEQ ID NO: 10,
 - the 5' UTR has the sequence as set forth in SEQ ID NO: 2 and the 3' UTR has the sequence as set forth in SEQ ID NO: 13,
 - the 5' UTR has the sequence as set forth in SEQ ID NO: 3 and the 3' UTR has the sequence as set forth in SEQ ID NO: 13,
 - the 5' UTR has the sequence as set forth in SEQ ID NO: 38 and the 3' UTR has the sequence as set forth in SEQ ID NO: 13, or
 - the 5' UTR has the sequence as set forth in SEQ ID NO: 39 and the 3' UTR has the sequence as set forth in SEQ ID NO: 13.
3. The in vitro-synthesized RNA of claim 1, formulated for administration to a subject.
4. The in-vitro synthesized RNA of claim 1, wherein the protein comprises a vaccine antigen, Green Fluorescent Protein (GFP), Human Interleukin-2 (IL2) or Human POU5F1 (or OCT3/4).
5. An engineered expression construct (EEC) comprising an enhancer consisting of the sequence as set forth in CAUACUCA.
6. The EEC of claim 5, further comprising a promoter.
7. The EEC of claim 6, wherein the promoter is a minimal promoter.

8. The EEC of claim 7, wherein the promoter has the sequence GGGAGA.
9. The EEC of claim 7, wherein the enhancer and minimal promoter are operably linked to an open reading frame that encodes a protein.
10. The EEC of claim 5, wherein the EEC does not comprise modified nucleosides.
11. The EEC of claim 5, wherein the EEC does not comprise microRNA binding sites.
12. The EEC of claim 5, wherein the EEC does not comprise modified nucleosides and does not comprise microRNA binding sites.
13. The EEC of claim 5, comprising a 5' UTR having the sequence as set forth in SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 38, or SEQ ID NO: 39.
14. The EEC of claim 5, comprising a 3' UTR having the sequence as set forth in SEQ ID NO: 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21.
15. The EEC of claim 5, comprising
 - a 5' UTR having the sequence as set forth in SEQ ID NO: 2 and a 3' UTR having the sequence as set forth in SEQ ID NO: 10,
 - a 5' UTR having the sequence as set forth in SEQ ID NO: 3 and a 3' UTR having the sequence as set forth in SEQ ID NO: 10,
 - a 5' UTR having the sequence as set forth in SEQ ID NO: 38 and a 3' UTR having the sequence as set forth in SEQ ID NO: 10,
 - a 5' UTR having the sequence as set forth in SEQ ID NO: 39 and a 3' UTR having the sequence as set forth in SEQ ID NO: 10,
 - a 5' UTR having the sequence as set forth in SEQ ID NO: 2 and a 3' UTR having the sequence as set forth in SEQ ID NO: 13,
 - a 5' UTR having the sequence as set forth in SEQ ID NO: 3 and a 3' UTR having the sequence as set forth in SEQ ID NO: 13,
 - a 5' UTR having the sequence as set forth in SEQ ID NO: 38 and a 3' UTR having the sequence as set forth in SEQ ID NO: 13, or
 - a 5' UTR having the sequence as set forth in SEQ ID NO: 39 and a 3' UTR having the sequence as set forth in SEQ ID NO: 13.
16. The EEC of claim 5, further comprising a Kozak sequence.
17. The EEC of claim 5, further comprising a promoter and a Kozak sequence wherein the enhancer is between the promoter and the Kozak sequence.