

CLAIMS

1. A polynucleotide molecule comprising, in a 5' to 3' orientation, (i) a first universal target sequence, (ii) a nucleotide sequence encoding an antigenic peptide, (iii) a second universal target sequence that is distinct from the first universal target sequence, (iv) a Beta 2 Microglobulin (β 2M) sequence, and (v) a Multiple Histocompatibility Complex (MHC) allele sequence.
2. The polynucleotide of claim 1, comprising, in a 5' to 3' orientation, (i) a promoter sequence, (ii) the first universal target sequence comprising the sequence shown in SEQ ID NO: 3, (iii) the nucleotide sequence encoding an antigenic peptide, wherein the antigenic peptide is a tumor neoantigen, (iv) the second universal target sequence comprising the sequence shown in SEQ ID NO: 4, (v) the β 2M sequence comprising the sequence shown in SEQ ID NO: 106, (vi) the MHC allele sequence comprising a sequence selected from the group consisting of the sequences shown in SEQ ID NOs: 109-174, (vii) a first affinity tag sequence comprising the sequence shown in SEQ ID NO: 29, (viii) a protease cleavage site sequence comprising the sequence shown in SEQ ID NO: 31, and (ix) a second affinity tag sequence comprising the sequence shown in SEQ ID NO: 35.
3. The polynucleotide of claim 1, wherein the antigenic peptide is selected from the group consisting of a tumor antigen, a neoantigen, a tumor neoantigen, a viral antigen, bacterial antigen, phosphoantigen, and a microbial antigen.
4. The polynucleotide of any of the above claims, wherein the antigenic peptide is a neoantigen.
5. The polynucleotide of claim 4, wherein the neoantigen is selected by analyzing tumor sequencing data from a subject to identify one or more somatic mutations.
6. The polynucleotide of claim 5, wherein the analyzing is performed using an in silico predictive algorithm.
7. The polynucleotide of claim 6, wherein the predictive algorithm further comprises an MHC binding algorithm to predict binding between the neoantigen and an MHC allele.
8. The polynucleotide of any of the above claims, wherein the MHC allele is a mammalian MHC allele.
9. The polynucleotide of any of the above claims, wherein the MHC allele is a human MHC allele.

10. The polynucleotide of claim 8, wherein the MHC allele is a class I HLA allele.
11. The polynucleotide of claim 10, wherein the HLA allele comprises an HLA-A, HLA-B, or HLA-C allele.
12. The polynucleotide of claim 10, wherein the HLA allele is an HLA allele of the subject.
13. The polynucleotide of claim 10, wherein the HLA allele is selected from the group consisting of: HLA-A*01:01, HLA-A*02:01, HLA-A*03:01, HLA-A*24:02, HLA-A*30:02, HLA-A*31:01, HLA-A*32:01, HLA-A*33:01, HLA-A*68:01, HLA-A*11:01, HLA-A*23:01, HLA-A*30:01, HLA-A*33:03, HLA-A*25:01, HLA-A*26:01, HLA-A*29:02, HLA-A*68:02, HLA-B*07:02, HLA-B*14:02, HLA-B*18:01, HLA-B*27:02, HLA-B*39:01, HLA-B*40:01, HLA-B*44:02, HLA-B*46:01, HLA-B*50:01, HLA-B*57:01, HLA-B*58:01, HLA-B*08:01, HLA-B*15:01, HLA-B*15:03, HLA-B*35:01, HLA-B*40:02, HLA-B*42:01, HLA-B*44:03, HLA-B*51:01, HLA-B*53:01, HLA-B*13:02, HLA-B*15:07, HLA-B*27:05, HLA-B*35:03, HLA-B*37:01, HLA-B*38:01, HLA-B*41:02, HLA-B*44:05, HLA-B*49:01, HLA-B*52:01, HLA-B*55:01, HLA-C*02:02, HLA-C*03:04, HLA-C*05:01, HLA-C*07:01, HLA-C*01:02, HLA-C*04:01, HLA-C*06:02, HLA-C*07:02, HLA-C*16:01, HLA-C*03:03, HLA-C*07:04, HLA-C*08:01, HLA-C*08:02, HLA-C*12:02, HLA-C*12:03, HLA-C*14:02, HLA-C*15:02, and HLA-C*17:01.
14. The polynucleotide of claim 10, wherein the HLA allele comprises a sequence selected from the group consisting of SEQ ID NOs: 109-174.
15. The polynucleotide of any of the above claims, wherein the β 2M allele is a mammalian β 2M allele.
16. The polynucleotide of claim 15, wherein the β 2M allele is a human β 2M allele.
17. The polynucleotide of claim 16, wherein the β 2M allele comprises the sequence shown in SEQ ID NO: 106.
18. The polynucleotide of any of the above claims, wherein the nucleotide sequence encoding an antigenic peptide is between 20-70, between 20-30, between 25-35, between 20-45, between 30-45, between 40-70, or between 45-70 nucleotides in length.
19. The polynucleotide of any of the above claims, wherein the nucleotide sequence encoding an antigenic peptide is between 20-30 nucleotides in length.

20. The polynucleotide of any of the above claims, wherein the first universal target sequence is between 4-50, between 4-15, between 15-40, between 15-35, between 15-30, between 20-40, between 25-40, or between 30-40 nucleotides in length.
21. The polynucleotide of any of the above claims, wherein the first universal target sequence is between 25-35 nucleotides in length.
22. The polynucleotide of claim 1-20, wherein the first universal target sequence is at least about 15 nucleotides in length.
23. The polynucleotide of any of the above claims, wherein the sequence of the first universal target comprises the sequence shown in SEQ ID NO: 3.
24. The polynucleotide of any of the above claims, wherein the second universal target sequence is between 4-50, between 4-15, between 15-40, between 15-35, between 15-30, between 20-40, between 25-40, or between 30-40 nucleotides in length.
25. The polynucleotide of any of the above claims, wherein the second universal target sequence is between 25-35 nucleotides in length.
26. The polynucleotide of claims 1-24, wherein the second universal target sequence is at least about 15 nucleotides in length.
27. The polynucleotide of any of the above claims, wherein the sequence of the second universal target comprises the sequence shown in SEQ ID NO: 4.
28. The polynucleotide of any of the above claims, wherein the first and second universal target sequences comprise polymerase chain reaction (PCR) primer target sequences.
29. The polynucleotide of any of the above claims, wherein the first and second universal target sequences comprise restriction enzyme cleavage sites.
30. The polynucleotide of claims 1-29, wherein the first universal target sequence further comprises a signal sequence.
31. The polynucleotide of claim 30, wherein the signal sequence encodes a signal sequence comprising a Human Growth Hormone signal sequence, a hIG1 Kappa light chain signal sequence, a Beta 2 microglobulin, signal sequence, or an IL2 signal sequence.
32. The polynucleotide of claim 31, wherein the signal sequence comprises a sequence comprising the sequence shown in SEQ ID NOs: 1, 24, 26, or 28.

33. The polynucleotide of claim 30, wherein the signal sequence encodes the Human Growth Hormone (HGH) signal sequence.
34. The polynucleotide of claim 33, wherein the signal sequence comprises the sequence shown in SEQ ID NO: 1.
35. The polynucleotide of claim 30, wherein the signal sequence is between 40-90, 40-60, 45-70, 50-80, 60-90, 55-70, 60-80, or 70-90 nucleotides in length.
36. The polynucleotide of any of the above claims, wherein the 3' end of the polynucleotide sequence further comprises, in a 5' to 3' orientation, a purification cluster sequence comprising (i) a first affinity tag sequence, (ii) a protease cleavage site sequence, and (iii) a second affinity tag sequence.
37. The polynucleotide of claim 36, wherein the first and second affinity tags are selected from the group consisting of: AviTag, streptavidin-tag, polyhistidine (His6)-tag, FLAG-tag, HA-tag, and Myc-tag.
38. The polynucleotide of claim 36, wherein the first affinity tags comprises a sequence comprising the sequence shown in SEQ ID NO: 29, and the second affinity tag comprises a sequence comprising the sequence shown in SEQ ID NO: 33.
39. The polynucleotide of claim 36, wherein the protease cleavage site sequence is selected from the group consisting of: a TEV cleavage site sequence, a thrombin cleavage site sequence, a Factor Xa cleavage site sequence, an enteropeptidase cleavage site sequence, and a rhinovirus 3C protease cleavage site sequence.
40. The polynucleotide of claim 39, wherein the protease cleavage site sequence comprises a TEV cleavage site the sequence shown in SEQ ID NO: 31.
41. The polynucleotide of claim 39, wherein the first affinity tag sequence comprises the sequence shown in SEQ ID NO: 29, the protease cleavage site comprises the sequence shown in SEQ ID NO: 31, and the second affinity tag comprises the sequence shown in SEQ ID NO: 33.
42. The polynucleotide of any of the above claims, wherein the second universal target sequence further comprises a first linker sequence.
43. The polynucleotide of claim 42, wherein the first linker sequence comprises the sequence shown in SEQ ID NO: 10, 14, 16, or 18.

44. The polynucleotide of any of the above claims, further comprising a second linker sequence between the β 2M sequence and the MHC allele sequence.
45. The polynucleotide of claim 44, wherein the second linker sequence comprises the sequence shown in SEQ ID NO: 10 or 20.
46. The polynucleotide of any of the above claims, further comprising a third linker sequence between the MHC allele sequence and the first affinity tag.
47. The polynucleotide of claim 46, wherein the third linker sequence comprises the sequence shown in SEQ ID NO: 12 or 22.
48. The polynucleotide of any of the above claims, wherein the 5' end of the polynucleotide sequence further comprises a promoter sequence linked to the 5' end of the first universal target.
49. The polynucleotide of claim 48, wherein the promoter sequence is selected from the group consisting of CMV, EF1 α , and SV40 promoters.
50. The polynucleotide of claim 48, wherein the promoter sequence is a CMV promoter sequence.
51. The polynucleotide of claim 50, wherein the promoter sequence is a CMV promoter sequence.
52. The polynucleotide of any of the above claims, wherein the 3' end of the polynucleotide sequence further comprises a polyA sequence.
53. The polynucleotide of claim 52, wherein the polyA sequence is selected from a bGH polyA sequence, an SV40 polyA sequence, an hGH polyA sequence, and an rbGlob polyA sequence.
54. The polynucleotide of claim 52, wherein the polyA sequence comprises a bGH polyA sequence as shown in SEQ ID NO: 179.
55. An expression construct comprising the polynucleotide molecule of any of the above claims.
56. The construct of claim 55, wherein the expression construct comprises a plasmid or a viral vector.
57. A host cell comprising the polynucleotide molecule or the expression construct of any of claims 1-56.

58. The cell of claim 57, wherein the polynucleotide is integrated into the cell genome.
59. The host cell of claim 57, wherein the polynucleotide is extrachromosomal.
60. The cell of any of claim 57-59, wherein the host cell is a mammalian cell.
61. The cell of claim 60, wherein the cell is a human cell.
62. The cell of any of claims 57-60, wherein the cell is selected from the group consisting of: a stem cell, a tumor cell, an immortalized cell, and a fetal cell.
63. The cell of any of claim 57-59, wherein the host cell is a prokaryotic cell.
64. The cell of claim 63, wherein the cell is an *Escherichia coli* cell.
65. The cell of any one of claims 57-64, wherein the cell expresses a BirA protein or fragment thereof.
66. A library comprising the composition of any of the above claims, wherein the library comprises greater than or equal to two distinct polynucleotide molecules, wherein each distinct polynucleotide molecule comprises (i) the first universal sequence, (ii) the nucleotide sequence encoding a antigenic peptide, wherein the nucleotide sequence is not the same for each of the greater than or equal to two polynucleotide molecules (iii) the second universal target sequence, (iv) the β 2M sequence, and (v) the MHC allele sequence.
67. The library of claim 66, wherein the MHC allele sequence is not the same for each of the greater than or equal to two polynucleotide molecules.
68. The library of claim 66 or 67, wherein the library comprises 20 to 500 distinct polynucleotide molecules.
69. The library of claim 66 or 67, wherein the library comprises at least 66 distinct polynucleotide molecules.
70. The library of claim 69, wherein the library comprises at least the HLA-A*01:01, HLA-A*02:01, HLA-A*03:01, HLA-A*24:02, HLA-A*30:02, HLA-A*31:01, HLA-A*32:01, HLA-A*33:01, HLA-A*68:01, HLA-A*11:01, HLA-A*23:01, HLA-A*30:01, HLA-A*33:03, HLA-A*25:01, HLA-A*26:01, HLA-A*29:02, HLA-A*68:02, HLA-B*07:02, HLA-B*14:02, HLA-B*18:01, HLA-B*27:02, HLA-B*39:01, HLA-B*40:01, HLA-B*44:02, HLA-B*46:01, HLA-B*50:01, HLA-B*57:01, HLA-B*58:01, HLA-B*08:01, HLA-B*15:01, HLA-B*15:03, HLA-B*35:01, HLA-B*40:02, HLA-B*42:01, HLA-B*44:03, HLA-B*51:01, HLA-B*53:01, HLA-B*13:02, HLA-B*15:07, HLA-

B*27:05, HLA-B*35:03, HLA-B*37:01, HLA-B*38:01, HLA-B*41:02, HLA-B*44:05, HLA-B*49:01, HLA-B*52:01, HLA-B*55:01, HLA-C*02:02, HLA-C*03:04, HLA-C*05:01, HLA-C*07:01, HLA-C*01:02, HLA-C*04:01, HLA-C*06:02, HLA-C*07:02, HLA-C*16:01, HLA-C*03:03, HLA-C*07:04, HLA-C*08:01, HLA-C*08:02, HLA-C*12:02, HLA-C*12:03, HLA-C*14:02, HLA-C*15:02, and HLA-C*17:01 alleles.

71. The library of claim 69, wherein the library comprises at least the sequences shown in SEQ ID NOs: 109-174.

72. A polypeptide comprising, in an amino to carboxyl terminus orientation, (i) a first universal target peptide, (ii) an antigenic peptide, (iii) a second universal target peptide that is distinct from the first universal target peptide, (iv) a β 2M peptide, and (v) an MHC peptide.

73. The polypeptide of claim 72, comprising, in a 5' to 3' orientation, (i) a promoter peptide comprising the sequence shown in SEQ ID NO: 2, (ii) the first universal target peptide, (iii) the nucleotide sequence encoding an antigenic peptide, wherein the antigenic peptide is a tumor neoantigen, (iv) the second universal target peptide comprising the sequence shown in SEQ ID NO: 15 or 17, (v) the β 2M peptide comprising the sequence shown in SEQ ID NO: 105, (vi) the MHC peptide comprising a sequence shown in SEQ ID NOs: 38-103, (vii) a first affinity peptide comprising the sequence shown in SEQ ID NO: 30, (viii) a protease cleavage site peptide comprising the sequence shown in SEQ ID NO: 32, and (ix) a second affinity peptide comprising the sequence shown in SEQ ID NO: 36.

74. The polypeptide of claim 72, wherein the antigenic peptide is selected from the group consisting of a tumor antigen, a neoantigen, a tumor neoantigen, a viral antigen, bacterial antigen, phosphoantigen, and a microbial antigen.

75. The polypeptide of claims 72 or 74, wherein the antigenic peptide is a neoantigen.

76. The polypeptide of claims 72-75, wherein the neoantigen is selected by analyzing tumor sequencing data from a subject to identify one or more somatic mutations.

77. The polypeptide of claims 72-76, wherein the analyzing is performed using an in silico predictive algorithm.

78. The polypeptide of claims 72-77, wherein the predictive algorithm further comprises an MHC binding algorithm to predict binding between the neoantigen and an MHC peptide.

79. The polypeptide of claim 72-78, wherein the MHC peptide is a mammalian MHC peptide.

80. The polypeptide of claim 72-78, wherein the MHC peptide is a human MHC peptide.
81. The polypeptide of claims 72-79, wherein the MHC peptide is a class I HLA peptide.
82. The polypeptide of claims 72-81, wherein the HLA peptide comprises an HLA-A, HLA-B, or HLA-C peptide.
83. The polypeptide of claim 82, wherein the HLA peptide is an HLA peptide of the subject.
84. The polypeptide of claim 82, wherein the HLA peptide comprises HLA-A*01:01, HLA-A*02:01, HLA-A*03:01, HLA-A*24:02, HLA-A*30:02, HLA-A*31:01, HLA-A*32:01, HLA-A*33:01, HLA-A*68:01, HLA-A*11:01, HLA-A*23:01, HLA-A*30:01, HLA-A*33:03, HLA-A*25:01, HLA-A*26:01, HLA-A*29:02, HLA-A*68:02, HLA-B*07:02, HLA-B*14:02, HLA-B*18:01, HLA-B*27:02, HLA-B*39:01, HLA-B*40:01, HLA-B*44:02, HLA-B*46:01, HLA-B*50:01, HLA-B*57:01, HLA-B*58:01, HLA-B*08:01, HLA-B*15:01, HLA-B*15:03, HLA-B*35:01, HLA-B*40:02, HLA-B*42:01, HLA-B*44:03, HLA-B*51:01, HLA-B*53:01, HLA-B*13:02, HLA-B*15:07, HLA-B*27:05, HLA-B*35:03, HLA-B*37:01, HLA-B*38:01, HLA-B*41:02, HLA-B*44:05, HLA-B*49:01, HLA-B*52:01, HLA-B*55:01, HLA-C*02:02, HLA-C*03:04, HLA-C*05:01, HLA-C*07:01, HLA-C*01:02, HLA-C*04:01, HLA-C*06:02, HLA-C*07:02, HLA-C*16:01, HLA-C*03:03, HLA-C*07:04, HLA-C*08:01, HLA-C*08:02, HLA-C*12:02, HLA-C*12:03, HLA-C*14:02, HLA-C*15:02, or HLA-C*17:01.
85. The polypeptide of claim 82, wherein the HLA peptide comprises a sequence selected from the group consisting of SEQ ID NOs: 38-103.
86. The polypeptide of claims 72-85, wherein the HLA peptide comprises a Y84A or a Y84C mutation.
87. The polypeptide of claims 72-85, wherein the β 2M peptide is a mammalian β 2M peptide.
88. The polypeptide of claim 87, wherein the β 2M peptide is a human β 2M peptide.
89. The polypeptide of claim 88, wherein the β 2M peptide comprises the sequence shown in SEQ ID NO: 105.
90. The polypeptide of claim 88, wherein the β 2M peptide comprises the sequence shown in SEQ ID NO: 107, comprising an S88C mutation at amino acid 88.

91. The polypeptide of claims 72-90, wherein the antigenic peptide is 7-15 amino acids, 7-10, 8-9, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acids in length.
92. The polypeptide of claims 72-91, wherein the first universal target peptide further comprises a signal peptide.
93. The polypeptide of claim 92, wherein the signal peptide comprising a Human Growth Hormone signal peptide, a hIG1 Kappa light chain signal peptide, a Beta 2 microglobulin signal peptide, or an IL2 signal peptide.
94. The polypeptide of claim 93, wherein the signal peptide comprises a sequence comprising the sequence shown in SEQ ID NOs: 2, 23, 25, or 27.
95. The polypeptide of claims 93, wherein the signal peptide comprises the Human Growth Hormone (HGH) signal peptide sequence shown in SEQ ID NO: 2.
96. The polypeptide of claims 72-96, wherein the signal sequence is between 15-45, 15-30, 20-45, 20-30, or 30-45 amino acids in length.
97. The polypeptide of claims 72-96, wherein the carboxyl terminus of the polypeptide further comprises, in an amino to carboxylic terminus orientation, a purification cluster comprising (i) a first affinity peptide, (ii) a protease cleavage site, and (iii) a second affinity peptide.
98. The polypeptide of claim 97, wherein the first and second affinity peptides are selected from the group consisting of: AviTag, streptavidin-tag, polyhistidine (His6)-tag, FLAG-tag, HA-tag, and Myc-tag.
99. The polypeptide of claim 98, wherein the first affinity peptide comprises a sequence comprising SEQ ID NO: 30 and the second affinity peptide comprises a sequence comprising SEQ ID NO: 34.
100. The polypeptide of claims 97, wherein the protease cleavage site is selected from the group consisting of: a TEV cleavage site, a thrombin cleavage site, a Factor Xa, an enteropeptidase cleavage site, and a rhinovirus 3C protease cleavage site.
101. The polypeptide of claim 100, wherein the protease cleavage site comprises the TEV cleavage site sequence shown in SEQ ID NO: 32.

102. The polypeptide of claim 97, wherein the purification cluster comprises the AviTag peptide sequence shown in SEQ ID NO: 30, the TEV cleavage site sequence shown in SEQ ID NO: 32, and the His6 peptide sequence shown in SEQ ID NOs: 34 or 36.
103. The polypeptide of claim 102, wherein the purification cluster comprises two or more copies of the His6 peptide sequence shown in SEQ ID NO: 34.
104. The polypeptide of claims 97, wherein the purification cluster comprises the sequence shown in SEQ ID NO: 37.
105. The polypeptide of any one of claims 72-104, wherein the second universal target peptide further comprises a linker comprising the sequence shown in SEQ ID NO: 9, 11, 15, or 17.
106. The polypeptide of any one of claims 72-105, further comprising a second linker sequence between the β 2M sequence and the MHC allele sequence.
107. The polypeptide of claim 106, wherein the second linker sequence comprises the sequence shown in SEQ ID NO: 9 or 19.
108. The polypeptide of any one of claims 72-107, further comprising a third linker sequence between the MHC allele sequence and the first affinity tag.
109. The polypeptide of claim 108, wherein the third linker sequence comprises the sequence shown in SEQ ID NO: 13 or 21.
110. The polypeptide of claims 72-109 wherein the polypeptide is biotinylated.
111. A library comprising the polypeptide of claims 72-110, wherein the library comprises greater than or equal to two distinct polypeptide molecules, wherein the distinct polypeptide molecule comprises (i) the first universal target peptide, (ii) the antigenic peptide, wherein the antigenic peptide is not the same for each of the greater than or equal to two polypeptide molecules, (iii) the second universal target peptide that is distinct from the first universal target peptide, (iv) the β 2M peptide, and (v) the MHC peptide.
112. The library of claim 111, wherein the MHC peptide is not the same for each of the greater than or equal to two polypeptide molecules.
113. The library of claims 111 or 112, wherein the library comprises 20 to 500 distinct polypeptides.

114. The library of claims 111 or 112, wherein the library comprises at least 66 distinct polypeptides.

115. The library of claim 114, wherein the library comprises HLA-A*01:01, HLA-A*02:01, HLA-A*03:01, HLA-A*24:02, HLA-A*30:02, HLA-A*31:01, HLA-A*32:01, HLA-A*33:01, HLA-A*68:01, HLA-A*11:01, HLA-A*23:01, HLA-A*30:01, HLA-A*33:03, HLA-A*25:01, HLA-A*26:01, HLA-A*29:02, HLA-A*68:02, HLA-B*07:02, HLA-B*14:02, HLA-B*18:01, HLA-B*27:02, HLA-B*39:01, HLA-B*40:01, HLA-B*44:02, HLA-B*46:01, HLA-B*50:01, HLA-B*57:01, HLA-B*58:01, HLA-B*08:01, HLA-B*15:01, HLA-B*15:03, HLA-B*35:01, HLA-B*40:02, HLA-B*42:01, HLA-B*44:03, HLA-B*51:01, HLA-B*53:01, HLA-B*13:02, HLA-B*15:07, HLA-B*27:05, HLA-B*35:03, HLA-B*37:01, HLA-B*38:01, HLA-B*41:02, HLA-B*44:05, HLA-B*49:01, HLA-B*52:01, HLA-B*55:01, HLA-C*02:02, HLA-C*03:04, HLA-C*05:01, HLA-C*07:01, HLA-C*01:02, HLA-C*04:01, HLA-C*06:02, HLA-C*07:02, HLA-C*16:01, HLA-C*03:03, HLA-C*07:04, HLA-C*08:01, HLA-C*08:02, HLA-C*12:02, HLA-C*12:03, HLA-C*14:02, HLA-C*15:02, and HLA-C*17:01 polypeptides.

116. The polypeptide of claims 72-115, wherein the polypeptide is attached to a particle, wherein the particle is selected from the group consisting of: a surface, a nanoparticle, a bead, and a polymer.

117. The polypeptide of claim 116, wherein the polypeptide is attached via a linker to the particle.

118. The polypeptide of claims 116 or 117, wherein the nanoparticle is a magnetic nanoparticle or a polystyrene nanoparticle.

119. The polypeptide of claim 118, wherein the magnetic nanoparticle comprises magnetic iron oxide.

120. The polypeptide of claim 116 wherein the bead is an agarose bead or a sepharose bead.

121. The polypeptide of claims 116-120, further comprising a fluorophore.

122. The polypeptide of claims 121, wherein the fluorophore is attached, with or without a linker, to the particle.

123. A library comprising the polypeptide of claims 116-122, wherein the library comprises greater than or equal to two distinct polypeptide molecules, wherein the antigenic

peptide is not the same for each of the greater than or equal to two polypeptide molecules, and wherein each distinct polypeptide is attached to a particle.

124. The library of claim 123, wherein the MHC peptide is not the same for each of the greater than or equal to two polypeptide molecules.

125. The library of claim 123 or 124, wherein the library further comprises a unique defined barcode sequence operably associated with the identity of each distinct polypeptide.

126. The library of claim 125, wherein the library comprises 20 to 500 distinct polypeptides.

127. The library of claim 125, wherein the library comprises at least 66 distinct polypeptides.

128. A kit comprising the composition of any of the above claims and instructions for use.

129. A method of manufacturing a polynucleotide molecule comprising the steps of:

- a. obtaining a first polynucleotide sequence comprising, in a 5' to 3' orientation, (i) a first universal target sequence comprising a restriction site, (ii) a second universal target sequence comprising a restriction site that is not the same as the first universal target sequence, (iii) a β 2M sequence, and (iv) an MHC allele sequence;
- b. obtaining a second polynucleotide comprising, in a 5' to 3' orientation (i) a portion of the first universal target sequence, (i) a sequence encoding an antigenic peptide, and (iii) a portion of the second universal target sequence;
- c. obtaining a third polynucleotide comprising, in a 5' to 3' orientation, (i) the reverse complement of a portion of the second universal target sequence, (ii) the reverse complement of the sequence encoding an antigenic peptide, and (iii) the reverse complement of a portion of the first universal target sequence;
- d. mixing the second and third polynucleotides together such that the complementary sequences anneal;
- e. performing a restriction digest on the first polynucleotide sequence with at least one restriction enzyme; and

- f. ligating the first and second polynucleotides together by mixing the digested first polynucleotide and annealed second and third polynucleotides together with DNA ligation reaction reagents.

130. The method of claim 129, further comprising phosphorylating the 5' nucleotide of the second and third polynucleotides after the complementary sequences anneal, wherein the phosphorylating comprises incubating the first or second and third polynucleotides with an enzyme.

131. The method of claim 130, wherein the enzyme is T4 kinase.

132. The method of claims 129-131, wherein the first polynucleotide further comprises a promoter sequence.

133. The method of claim 132, wherein the promoter sequence is selected from the group consisting of CMV, EF1 α , and SV40 promoters.

134. The method of claim 133, wherein the promoter sequence is CMV.

135. The method of claims 129-134, wherein the 3' end of the second polynucleotide sequence further comprises, in a 5' to 3' orientation, a purification cluster sequence comprising (i) a first affinity tag sequence, (ii) a protease cleavage site sequence, and (iii) a second affinity tag sequence.

136. The method of claims 129-135, wherein the 3' end of the second polynucleotide sequence further comprises a polyA sequence.

137. The method of claims 129-136, wherein the first polynucleotide is an expression construct.

138. The method of claims 129-136, further comprising inserting the manufactured polynucleotide into an expression construct.

139. The method of claims 129-138, wherein the obtaining the third and fourth polynucleotide sequence steps further comprises obtaining a predictive data set of a tumor sequencing data from a subject.

140. The method of claims 139, wherein the obtaining the third and fourth polynucleotide sequence steps further comprises predicting the sequence encoding the antigenic peptide from the tumor sequencing data.

141. The method of claims 139 or 140, wherein the obtaining the third and fourth polynucleotide sequence steps further comprises synthesizing the polynucleotide encoding the antigenic peptide based on the predicted sequence.

142. The method of any of claim 129-141, wherein the method further comprises manufacturing a library comprising greater than or equal to two distinct polynucleotide molecules, wherein the distinct polynucleotide molecule comprises (i) the first universal target sequence, (ii) the nucleotide sequence encoding an antigenic peptide, wherein the antigenic peptide is not the same for each of the greater than or equal to two polypeptide molecules, (iii) the second universal target sequence that is distinct from the first universal target sequence, (iv) the β 2M peptide, and (v) the MHC allele sequence.

143. The method of claim 142, wherein the MHC allele is not the same for each of the greater than or equal to two polynucleotide molecules.

144. The method of claim 143, wherein the library comprises 20 to 500 distinct polynucleotide molecules.

145. The method of claim 143, wherein the library comprises at least 66 distinct polynucleotide molecules.

146. The method of claims 129-144, wherein the first polynucleotide is an expression construct.

147. The method of claims 129-144, further comprising inserting the manufactured polynucleotide into an expression construct.

148. The method of claim 146, wherein the method further comprises manufacturing a library comprising greater than or equal to two expression constructs comprising distinct polynucleotide molecules, wherein the distinct polynucleotide molecule comprises (i) the first universal target sequence, (ii) the nucleotide sequence encoding an antigenic peptide, wherein the nucleotide sequence is not the same for each of the greater than or equal to two polynucleotide molecules, (iii) the second universal target sequence that is distinct from the first universal target sequence, (iv) the β 2M sequence, and (v) the MHC allele sequence.

149. The method of claim 148, wherein the MHC allele is not the same for each of the greater than or equal to two polynucleotide molecules.

150. The method of claim 149, wherein the MHC allele is selected from the sequences shown in the group consisting of SEQ ID NOs: 109-174.

151. The method of any of claims 129-150, further comprising expressing a polypeptide molecule from the polynucleotide molecule.
152. The method of claim 151, wherein the polynucleotide is transfected or transduced into a cell.
153. The method of claim 152, wherein the polynucleotide is integrated into the cell genome.
154. The method of claim 152, wherein the polynucleotide remains extrachromosomal in the cell.
155. The method of claims 152-154 wherein the cell is a mammalian cell, optionally wherein the cell is a tumor cell line.
156. The method of claims 152-154 wherein the cell is a prokaryotic cell.
157. The method of claim 156, wherein the cell is an *Escherichia coli* cell.
158. The method of any of claims 151-155, further comprising quantifying the expression of the polypeptide.
159. The method of claim 158, wherein a non-expressed polypeptide sequence is used to refine the antigenic peptide prediction analysis step of claim 140.
160. The method of any of claim 146-158, further comprising biotinylating the polypeptide.
161. The method of claim 160, wherein the polypeptide is biotinylated with a BirA protein.
162. The method of any of claims 146-160, further comprising purifying the polypeptide.
163. The method of claim 162, wherein the purifying step comprises affinity chromatography.
164. The method of claim 163, wherein the affinity chromatography comprises immobilized metal affinity chromatography, comprising a support, a chelator and a divalent metal.
165. The method of claim 164, wherein the chelator for the affinity chromatography is nitrilotriacetic acid (NTA) or iminodiacetic acid (IDA).
166. The method of claim 164, wherein the divalent metal is selected from the group consisting of: Nickel (Ni), Cobalt (Co), Copper (Cu), and Iron (Fe).

167. The method of claim 164, wherein the chelator is NTA and the divalent metal is Ni.
168. The method of any of claims 164-167, wherein the support is selected from the group consisting of: agarose beads, sepharose beads, and magnetic beads.
169. The method of any of claims 160-168, further comprising quantifying the biotinylation level of the purified polypeptide.
170. The method of any of claims 162-169, further comprising attaching the polypeptide to a least one particle, wherein the particle is selected from the group consisting of: a surface, a nanoparticle, a bead, and a polymer.
171. The method of claim 170, wherein the nanoparticle is a magnetic nanoparticle or a polystyrene nanoparticle.
172. The method of claim 171, wherein the magnetic nanoparticle comprises magnetic iron oxide.
173. The method of claim 170, wherein the bead is an agarose bead or a sepharose bead.
174. The method of any of claims 170-173, wherein the particle further comprises a fluorophore.
175. The method of claim 174, wherein the fluorophore is attached, with or without a linker, to the at least one particle.
176. The method of any of claims 151-175, further comprising producing a library comprising greater than or equal to two distinct polypeptides attached to at least one particle, wherein the distinct polypeptide comprises (i) the first universal target peptide, (ii) the antigenic peptide, wherein the antigenic peptide is not the same for each of the greater than or equal to two polypeptide molecules, (iii) the second universal target peptide that is distinct from the first universal target peptide, (iv) the β 2M peptide, and (v) the MHC peptide.
177. The method of claim 176, wherein the MHC peptide is not the same for each of the greater than or equal to two polypeptide molecules.
178. The method of claim 177, wherein the MHC peptide is selected from the sequences shown in the group consisting of SEQ ID NO: 39-104.
179. The method of claim 176 or 177, wherein the library comprises 20 to 500 distinct single polypeptides.

180. The method of claim 176 or 177, wherein the library comprises at least 66 distinct single polypeptides.

181. A method of manufacturing a polynucleotide molecule comprising the steps of:

- a. obtaining a first polynucleotide sequence comprising, in a 5' to 3' orientation, (i) a first universal target sequence comprising a restriction site, (ii) a second universal target sequence comprising a restriction site that is not the same as the first universal target sequence, (iii) a β 2M sequence, and (iv) an MHC allele sequence;
- b. obtaining a second polynucleotide comprising, in a 5' to 3' orientation, (i) the first universal target sequence, (ii) a sequence encoding an antigenic peptide, and (iii) the second universal target sequence;
- c. performing a restriction digest on the first polynucleotide sequence with at least one restriction enzyme;
- d. performing a restriction digest on the second polynucleotide sequence with at least one restriction enzyme; and
- e. ligating the first and second polynucleotides together by mixing the digested first and second polynucleotides together with DNA ligation reaction reagents.

182. A method of manufacturing a polynucleotide molecule comprising the steps of:

- a. obtaining a first polynucleotide sequence comprising a first universal target sequence;
- b. obtaining a second polynucleotide sequence comprising, in a 5' to 3' orientation, (i) a second universal target sequence, (ii) a β 2M sequence, and (iii) an MHC allele sequence;
- c. obtaining a third polynucleotide sequence comprising, in a 5' to 3' orientation, a sequence encoding an antigenic peptide and the second universal target sequence;
- d. obtaining a fourth polynucleotide sequence comprising, in a 5' to 3' orientation, the reverse complement of the antigenic peptide and the reverse complement of the first universal target sequence;
- e. combining the first, second, third, and fourth polynucleotides in a solution;

- f. adding polymerase chain reaction (PCR) reagents; and
 - g. performing a PCR reaction wherein a complementary region of the second universal target sequence in the second and third polynucleotides anneal to each other and a complementary region of the first universal target sequence in the first and fourth polynucleotides anneal to each other; and wherein the annealed sequences provide priming sequences for a PCR extension and amplification reaction.
183. A method for isolating an antigen specific T cell, the method comprising the steps of:
- a. providing a polypeptide comprising, in an amino terminus to carboxyl terminus orientation, (i) a first universal target peptide, (ii) an antigenic peptide, (iii) a second universal target peptide that is distinct from the first universal target peptide, (iv) a β 2M peptide, and (v) an MHC peptide, wherein the polypeptide is linked to one particle;
 - b. providing a sample known or suspected to comprise one or more T cells;
 - c. contacting the polypeptide with the sample, wherein the contacting comprises providing conditions sufficient for a single T cell to bind the polypeptide attached to the particle, and
 - d. isolating the single T cell associated with the particle.