

CLAIMS

WHAT IS CLAIMED IS:

1. A nucleic acid library, wherein the nucleic acid library comprises at least 3000 variant nucleic acids, wherein each variant nucleic acid encodes for a variant gene or gene fragment for a T cell receptor (TCR) protein, and wherein a region selected for variation spans up to 1000 bases in length.
2. The nucleic acid library of claim 1, wherein the variant gene or gene fragment is from a variable domain.
3. The nucleic acid library of claim 2, wherein the variable domain is a variable domain of TCR alpha, TCR beta, TCR gamma, or TCR delta.
4. The nucleic acid library of claim 2, wherein the variable domain is specific to a cancer antigen.
5. The nucleic acid library of claim 4, wherein the cancer antigen is MAGE A3, MAGE A12, MAGE A2, MAGE A6, NY-ESO-1, or CEA.
6. The nucleic acid library of claim 1, wherein the variant gene or gene fragment is from a constant domain.
7. The nucleic acid library of claim 1, wherein the variant gene or gene fragment allows for generation of a TCR protein having increased specificity, avidity, affinity, stability, or expression.
8. A polynucleotide library, wherein the polynucleotide library comprises at least 3000 variant nucleic acids, wherein each polynucleotide is at least 15 bases in length, wherein each polynucleotide encodes for a variant within a variable domain of a T cell receptor (TCR) protein or fragment thereof, wherein the variable domain comprises up to 1000 bases, and wherein each variant nucleic acid comprises at least one variation at a preselected codon for an amino acid residue in an antigen contacting interface.
9. The polynucleotide library of claim 8, wherein the variable domain is a variable domain of TCR alpha, TCR beta, TCR gamma, or TCR delta.
10. The polynucleotide library of claim 8, wherein the antigen is a cancer antigen.
11. The polynucleotide library of claim 10, wherein the cancer antigen is MAGE A3, MAGE A12, MAGE A2, MAGE A6, NY-ESO-1, or CEA.
12. The polynucleotide library of claim 8, wherein each variant nucleic acid comprises a plurality of variations at preselected codons for amino acid residues in the antigen contacting interface.

13. The polynucleotide library of claim 12, wherein the plurality of variations at preselected codons for amino acid residues comprises up to 100 residues.

14. The polynucleotide library of claim 12, wherein the plurality of variations at preselected codons for amino acid residues comprises up to 30 residues.

15. The polynucleotide library of claim 12, wherein the plurality of variations at preselected codons for amino acid residues comprises up to 5 residues.

16. The polynucleotide library of claim 8, wherein the variant allows for generation of a TCR protein having increased specificity, avidity, affinity, stability, or expression.

17. The polynucleotide library of claim 8, wherein each variant nucleic acid further comprises at least one variation at a preselected codon for an amino acid residue within a constant domain of the T cell receptor (TCR) protein or fragment thereof.

18. A polynucleotide library, wherein the polynucleotide library comprises at least 3000 variant polynucleotides, wherein each polynucleotide is at least 15 bases in length, wherein each polynucleotide encodes for a variant within a constant domain of a T cell receptor (TCR) protein or fragment thereof, wherein the constant domain comprises up to 1000 bases, and wherein the variant comprises at least one variation at a preselected codon for an amino acid residue in an antigen contacting interface.

19. The polynucleotide library of claim 18, wherein the constant domain is a constant domain of TCR alpha, TCR beta, TCR gamma, or TCR delta.

20. The polynucleotide library of claim 18, wherein the antigen is a cancer antigen.

21. The polynucleotide library of claim 20, wherein the cancer antigen is MAGE A3, MAGE A12, MAGE A2, MAGE A6, NY-ESO-1, or CEA.

22. The polynucleotide library of claim 18, wherein each polynucleotide comprises a plurality of variations at preselected codons for amino acid residues.

23. The polynucleotide library of claim 22, wherein the plurality of variations at preselected codons for amino acid residues comprises up to 100 residues.

24. The polynucleotide library of claim 22, wherein the plurality of variations at preselected codons for amino acid residues comprises up to 30 residues.

25. The polynucleotide library of claim 22, wherein the plurality of variations at preselected codons for amino acid residues comprises up to 5 residues.

26. A nucleic acid library, the nucleic acid library comprising at least 10,000 variant nucleic acids, wherein each variant nucleic acid is about 500 to about 1000 bases in length, wherein each variant nucleic acid encodes for a variant of a reference sequence that encodes an exon sequence

for a T cell receptor protein or fragment thereof, and wherein the reference sequence is from a subject that has a cancer.

27. A nucleic acid library, the library comprising at least about 1,000,000 variant nucleic acids, wherein each variant nucleic acid is about 500 to about 1000 bases in length, wherein each variant nucleic acid encodes for a variant of a reference sequence that encodes an exon sequence for a T cell receptor protein or fragment thereof, wherein the reference sequence of the T cell receptor protein or fragment thereof is selected based on comparing T cell receptor gene sequence from a plurality of subjects for a nucleic acid sequence that is common amongst the subjects, and wherein at least a portion of the subjects are diagnosed with a cancer.

28. The nucleic acid library of any one of claims 26 or 27, wherein the cancer is a solid cancer or a hematopoietic cancer.

29. The nucleic acid library of any one of claims 26 or 27, wherein the library comprises about 10,000,000 variant nucleic acids.

30. The nucleic acid library of any one of claims 26 or 27, wherein each variant nucleic acid is about 600 to about 900 bases in length.

31. The nucleic acid library of any one of claims 26 or 27, wherein each variant nucleic acid is in a vector sequence.

32. The nucleic acid library of claim 31, wherein the vector sequence is a viral vector sequence.

33. A protein library comprising proteins encoded by the nucleic acid library of any one of claims 1 to 7 or 26 to 32.

34. A cell library comprising the nucleic acid library of any one of claims 1 to 7 or 26 to 32.

35. A method of synthesizing a nucleic acid library, comprising:

a. providing a first set of preselected polynucleotide sequences encoding for at least 3000 variant sequences of a TCR gene or gene fragment, wherein each variant sequence comprises at least one variation at a preselected codon for an amino acid residue in an antigen contacting interface;

b. synthesizing the first set of preselected polynucleotide sequences; and

c. screening binding activity for proteins encoded by the first set of polynucleotide sequences.

36. The method of claim 35, wherein the at least one variation is in a variable domain coding region of the TCR gene or gene fragment.

37. The method of claim 36, wherein the variable domain is a variable domain of TCR alpha, TCR beta, TCR gamma, or TCR delta.

38. The method of claim 35, wherein the at least one variation is in a constant domain coding region of the TCR gene or gene fragment.
39. The method of claim 38, wherein the constant domain is a constant domain of TCR alpha, TCR beta, TCR gamma, or TCR delta.
40. The method of claim 35, wherein the antigen is a cancer antigen.
41. The method of claim 40, wherein the cancer antigen is MAGE A3, MAGE A12, MAGE A2, MAGE A6, NY-ESO-1, or CEA.
42. The method of claim 38, wherein each variant sequence comprises up to 100 variations at preselected codons for amino acid residues in the antigen contacting interface.
43. The method of claim 38, wherein each variant sequence comprises up to 30 variations at preselected codons for amino acid residues in the antigen contacting interface.
44. The method of claim 38, further comprising
- a. providing a second set of preselected polynucleotide sequences encoding for at least one variant sequence of a TCR gene or gene fragment, where each variant sequence comprises at least one variation at a preselected codon for an amino acid residue in the TCR gene or gene fragment in a region encoding a constant domain;
 - b. synthesizing the second set of preselected polynucleotide sequences; and
 - c. screening a second activity for proteins encoded by the second set of polynucleotide sequences.
45. The method of claim 44, wherein the second activity is cancer cell killing, protein expression, or protein stability.