

synthesizing a nucleic acid library, wherein the variable domain is a variable domain of TCR alpha, TCR beta, TCR gamma, or TCR delta. Further provided herein is a method of synthesizing a nucleic acid library, wherein the at least one variation is in a constant domain coding region of the TCR gene or gene fragment. Further provided herein is a method of synthesizing a nucleic acid library, wherein the constant domain is a constant domain of TCR alpha, TCR beta, TCR gamma, or TCR delta. Further provided herein is a method of synthesizing a nucleic acid library, wherein the antigen is a cancer antigen. Further provided herein is a method of synthesizing a nucleic acid library, wherein the cancer antigen is MAGE A3, MAGE A12, MAGE A2, MAGE A6, NY-ESO-1, or CEA. Further provided herein is a method of synthesizing a nucleic acid library, wherein each variant sequence comprises up to 100 variations at preselected codons for amino acid residues in the antigen contacting interface. Further provided herein is a method of synthesizing a nucleic acid library, wherein each variant sequence comprises up to 30 variations at preselected codons for amino acid residues in the antigen contacting interface. Further provided herein is a method of synthesizing a nucleic acid library, 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. Further provided herein is a method of synthesizing a nucleic acid library, wherein the second activity is cancer cell killing, protein expression, or protein stability.

[0012a] Provided herein is 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; c. screening binding activity for proteins encoded by the first set of polynucleotide sequences; d. 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; e. synthesizing the second set of preselected polynucleotide sequences; and f. screening a second activity for proteins encoded by the second set of polynucleotide sequences.

INCORPORATION BY REFERENCE

[0013] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] **Figures 1A-1D** depict a process workflow for the synthesis of variant biological molecules incorporating a PCR mutagenesis step.

[0015] **Figures 2A-2D** depict a process workflow for the generation of a nucleic acid comprising a nucleic acid sequence which differs from a reference nucleic acid sequence at a single predetermined codon site.

CLAIMS

WHAT IS CLAIMED IS:

1. 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;
 - c. screening binding activity for proteins encoded by the first set of polynucleotide sequences;
 - d. 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;
 - e. synthesizing the second set of preselected polynucleotide sequences; and
 - f. screening a second activity for proteins encoded by the second set of polynucleotide sequences.
2. The method of claim 1, wherein the at least one variation is in a variable domain coding region of the TCR gene or gene fragment.
3. The method of claim 2, wherein the variable domain is a variable domain of TCR alpha, TCR beta, TCR gamma, or TCR delta.
4. The method of claim 1, wherein the at least one variation is in a constant domain coding region of the TCR gene or gene fragment.
5. The method of claim 4, wherein the constant domain is a constant domain of TCR alpha, TCR beta, TCR gamma, or TCR delta.
6. The method of claim 1, wherein the antigen is a cancer antigen.
7. The method of claim 6, wherein the cancer antigen is MAGE A3, MAGE A12, MAGE A2, MAGE A6, NY-ESO-1, or CEA.
8. The method of claim 4, wherein each variant sequence comprises up to 100 variations at preselected codons for amino acid residues in the antigen contacting interface.
9. The method of claim 4, wherein each variant sequence comprises up to 30 variations at preselected codons for amino acid residues in the antigen contacting interface.

10. The method of claim 1, wherein the second activity is cancer cell killing, protein expression, or protein stability.

11. The method of claim 4, wherein each variant sequence comprises up to 5 variations at preselected codons for amino acid residues in the antigen contacting interface.

12. The method of claim 1, wherein the variant allows for generation of a TCR protein having increased specificity, avidity, affinity, stability, or expression.

13. The method of claim 1, 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.

14. The method of claim 1, wherein a region selected for variation spans up to 1000 bases in length.