variant genes. Further provided herein are oligonucleic acid libraries, wherein the 50 to 500 non-identical oligonucleic acids are attached to a surface of a structure and located within a discrete cluster. Further provided herein are oligonucleic acid libraries, wherein the 50 to 500 non-identical oligonucleic acids collectively encode at least 50 variant genes.

[0013a] Provided herein are methods for modulating protein activity, the method comprising:

a) providing predetermined sequences encoding for a plurality of oligonucleotides, wherein the predetermined sequences comprise predetermined ratios of codon sequence variance for multiple codon positions compared to a single reference sequence;

b) synthesizing the plurality of oligonucleotides;

c) mixing the plurality of oligonucleotides with a DNA polymerase and the single reference sequence to form a library of variant nucleic acids, wherein the library of variant nucleic acids comprises nucleic acids encoding for at least 90% of the predetermined sequences;

d) transferring the library of variant nucleic acids to cells and expressing a plurality of variant proteins; and

e) identifying an activity associated with a variant protein of the plurality of variant proteins, wherein the activity is modulated relative to that associated with a protein encoded by the single reference sequence.

[0013b] Provided herein are methods for method for modulating cellular activity, the method comprising:

a) providing predetermined sequences encoding for at least about 30,000 oligonucleotides, wherein each of the at least about 30,000 oligonucleotides encodes for a predetermined variant sequence compared to a single reference sequence;

b) providing a structure having a surface;

c) synthesizing the at least about 30,000 oligonucleotides, wherein each of the at least about 30,000 oligonucleotides extends from the surface;

d) mixing the least about 30,000 oligonucleotides with a DNA polymerase and the single reference sequence to form a library of variant nucleic acids;

e) transferring the library of variant nucleic acids to a first set of cells; and

f) contacting a second set of cells with at least one expression product isolated from the first set of cells, and measuring a change in a cellular activity in the second set of cells.

INCORPORATION BY REFERENCE

[0014] 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

[0015] **Figures 1A-1D** depict a process workflow for the synthesis of variant biological molecules incorporating a PCR mutagenesis step.

[0016] **Figures 2A-2D** depict a process workflow for the generation of an oligonucleic acid comprising a nucleic acid sequence which differs from a reference oligonucleic acid sequence at a single predetermined codon site.

[0017] **Figures 3A-3F** depict an alternative workflow for the generation of a set of oligonucleic acid variants from a template oligonucleic acid, with each variant comprising a different nucleic acid sequence at a single codon position. Each variant oligonucleic acid encodes for a different amino acid at their single codon position, the different codons represented by X, Y, and Z.

[0018] **Figures 4A-4E** depict a reference amino acid sequence (**FIG. 4A**) having a number of amino acids, each residue indicated by a single circle, and variant amino acid sequences (**FIGS. 4B, 4C, 4D, & 4E**) generated using methods described herein. The reference amino acid sequence and variant sequences are encoded by nucleic acids and variants thereof generated by processes described herein.

[0019] **Figures 5A-5B** depict a reference amino acid sequence (**FIG. 5A,** SEQ ID NO: 24) and a library of variant amino acid sequences (**FIG. 5B,** SEQ ID NOS 25-31, respectively, in order of appearance), each variant comprising a single residue variant (indicated by an "X"). The reference amino acid sequence and variant sequences are encoded by nucleic acids and variants thereof generated by processes described herein.

[0020] **Figures 6A-6B** depict a reference amino acid sequence (**FIG. 6A**) and a library of variant amino acid sequences (**FIG. 6B**), each variant comprising two sites of single position variants. Each variant is indicated by differently patterned circles. The reference amino acid

CLAIMS

WHAT IS CLAIMED IS:

1. A method for modulating protein activity, the method comprising:

a) providing predetermined sequences encoding for a plurality of oligonucleotides, wherein the predetermined sequences comprise predetermined ratios of codon sequence variance for multiple codon positions compared to a single reference sequence;

b) synthesizing the plurality of oligonucleotides;

c) mixing the plurality of oligonucleotides with a DNA polymerase and the single reference sequence to form a library of variant nucleic acids, wherein the library of variant nucleic acids comprises nucleic acids encoding for at least 90% of the predetermined sequences;

d) transferring the library of variant nucleic acids to cells and expressing a plurality of variant proteins; and

e) identifying an activity associated with a variant protein of the plurality of variant proteins, wherein the activity is modulated relative to that associated with a protein encoded by the single reference sequence.

2. The method of claim 1, wherein the activity comprises cellular reproduction, growth, adhesion, death, migration, energy production, oxygen utilization, metabolic activity, cell signaling, aging, response to free radical damage, or any combination thereof.

3. The method of claim 1, wherein the cells are eukaryotic cells or prokaryotic cells.

4. The method of claim 1, wherein the library of variant nucleic acids encodes sequences for variant genes or fragments thereof.

5. The method of claim 1, wherein the variant protein of the plurality of variant proteins is an antibody, enzyme or peptide.

6. The method of claim 1, wherein the variant protein of the plurality of variant proteins has enhanced or reduced binding affinity for another molecule or enhanced or reduced enzymatic activity.

7. The method of claim 1, wherein the plurality of oligonucleotides comprises at least about 30,000 oligonucleotides.

8. A method for modulating cellular activity, the method comprising:

a) providing predetermined sequences encoding for at least about 30,000 oligonucleotides, wherein each of the at least about 30,000 oligonucleotides encodes for a predetermined variant sequence compared to a single reference sequence;

b) providing a structure having a surface;

c) synthesizing the at least about 30,000 oligonucleotides, wherein each of the at least about 30,000 oligonucleotides extends from the surface;

d) mixing the least about 30,000 oligonucleotides with a DNA polymerase and the single reference sequence to form a library of variant nucleic acids;

e) transferring the library of variant nucleic acids to a first set of cells; and

f) contacting a second set of cells with at least one expression product isolated from the first set of cells, and measuring a change in a cellular activity in the second set of cells.

9. The method of claim 8, wherein the cellular activity comprises reproduction, growth, adhesion, death, migration, energy production, oxygen utilization, metabolic activity, cell signaling, response to free radical damage, or any combination thereof.

10. The method of claim 8, wherein the first set of cells or the second set of cells are eukaryotic cells or prokaryotic cells.

11. The method of claim 8, wherein the library of variant nucleic acids encodes sequences for variant genes or fragments thereof, a transcription regulatory sequence, or a sequence that, when transcribed, encodes for mRNA, miRNA, or shRNA.

12. The method of claim 11, further comprising translation of each of the variant genes to form a protein.

13. The method of claim 12, wherein the protein is an antibody, enzyme, or peptide.

14. The method of claim 13, wherein the library of variant nucleic acids encodes for at least a portion of a variable region or constant region of the antibody or at least one CDR region of the antibody.

15. The method of claim 12, wherein the protein has enhanced or reduced binding affinity for another molecule, or wherein the protein has enhanced or reduced enzymatic activity.

16. The method of claim 15, wherein the transcription regulatory sequence is a promoter, UTR, or a terminator sequence.

17. The method of claim 1, wherein the predetermined sequences comprise different predetermined ratios of codon sequence variance for each of the multiple codon positions.

18. The method of claim 1, wherein the predetermined sequences comprise different predetermined ratios of codon sequence variance for at least 5 codon positions.

19. The method of claim 1, wherein the library of variant nucleic acids comprises nucleic acids encoding for at least 95% of the predetermined sequences.

20. The method of claim 1, wherein the library of variant nucleic acids comprises nucleic acids encoding for a variant codon sequence in at least two adjacent codon positions or in at least two non-adjacent codon positions compared to the single reference sequence.