

SUMMARY

- [0006]** The present disclosure provides synthetic modular polypeptide libraries and nucleic acids encoding such synthetic modular polypeptide libraries. Also provided are methods of making synthetic modular polypeptide libraries and nucleic acids encoding synthetic modular polypeptide libraries. Methods of screening a synthetic modular polypeptide library to identify a selected phenotype associated with a member of a synthetic modular polypeptide library are also provided where such methods find use in both in vitro and in vivo assays.
- [0007]** In one aspect, there is provided a method of identifying a selected phenotype associated with a synthetic modular polypeptide in a cell, the method comprising: a) introducing a barcoded library of nucleic acids into host cells, thereby generating a heterogeneous population of genetically modified host cells, wherein the barcoded library of nucleic acids comprises a plurality of members, each of which plurality of members comprises a nucleotide sequence encoding a different synthetic modular polypeptide; and b) identifying a genetically modified host cell within the heterogeneous population that displays the selected phenotype in response to a stimulus.
- [0008]** Also provided is a method, wherein the synthetic modular polypeptide is a chimeric antigen receptor (CAR) polypeptide and wherein the stimulus is an antigen-presenting cell that displays on its surface an antigen that is bound by the CAR.
- [0009]** Also provided is a method of identifying a selected phenotype associated with a synthetic modular polypeptide in a cell, wherein the stimulus is in contact with a co-stimulatory molecule.
- [0010]** Also provided is a method of identifying a selected phenotype associated with a synthetic modular polypeptide in a cell, wherein the synthetic modular polypeptide is a modular receptor polypeptide.
- [0011]** Also provided is a method of identifying a selected phenotype associated with a synthetic modular polypeptide in a cell, wherein the modular receptor polypeptide is a chimeric Notch receptor polypeptide.
- [0012]** Also provided is a method of identifying a selected phenotype associated with a synthetic modular polypeptide in a cell, wherein the stimulus is a ligand for the chimeric Notch receptor polypeptide.
- [0013]** Also provided is a method of identifying a selected phenotype associated with a synthetic modular polypeptide in a cell, wherein the synthetic modular polypeptide is a modular scaffold protein.
- [0014]** Also provided is a method of identifying a selected phenotype associated with a synthetic modular polypeptide in a cell, wherein the synthetic modular polypeptide is a modular protein kinase or phosphatase protein.

comprises 100 or more unique members and the identifying comprises screening 100 or more unique members of the library for the selected phenotype.

[0026] Also provided is a method of identifying a selected phenotype associated with a synthetic modular polypeptide in a cell, wherein the method includes a barcoded library of nucleic acids comprising a plurality of members, wherein the plurality of members comprises a nucleotide sequence encoding a synthetic modular polypeptide comprising a modular domain selected from the group consisting of: an antigen-binding domain, a specific binding domain or a specific binding partner protein, a co-stimulatory domain, a co-inhibitory domain, an intracellular signaling domain, a transmembrane domain, a scaffold protein domain, a protein kinase protein domain, a protein phosphatase protein domain, a receptor tyrosine kinase protein domain, a lipid kinase protein domain, a lipid phosphatase protein domain, an ubiquitinylase protein domain, a deubiquitinylase protein domain, a SUMOylase protein domain, an acetylase protein domain, a deacetylase protein domain, a methylase protein domain, a demethylase protein domain, a nuclease protein domain, a recombinase protein domain, a transcription factor protein domain and combinations thereof.

[0027] In one aspect, there is provided a barcoded library of nucleic acids, the library comprising: a plurality of unique polynucleotides each comprising a nucleotide sequence encoding a unique synthetic modular polypeptide wherein each unique polynucleotide comprises: i) a coding region encoding the unique synthetic modular polypeptide comprising a first coding sequence encoding a first module linked in-frame to a second coding sequence encoding a second module, ii) a barcode region comprising a first barcode specific to the first coding sequence linked to a second barcode specific to the second coding sequence, wherein the first and second barcodes are in the reverse 5' to 3' order as compared to the first and second coding sequences; and wherein sequencing of each barcode region allows for the identification each unique synthetic modular polypeptide.

[0028] Also provided is a barcoded library of nucleic acids, wherein the first and second coding sequences are directly linked without any intervening non-coding nucleotides.

[0029] Also provided is a barcoded library of nucleic acids, wherein the plurality of unique polynucleotides comprises at least 1000 unique polynucleotides.

[0030] Also provided is a barcoded library of nucleic acids, wherein the barcode region is 5' of the coding region.

[0031] Also provided is a barcoded library of nucleic acids, wherein the coding region is 5' of the barcode region.

[0032] Also provided is a barcoded library of nucleic acids, that includes a plurality of unique polynucleotides each comprising a nucleotide sequence encoding a unique synthetic modular polypeptide wherein the unique synthetic modular polypeptide comprises a co-stimulatory domain.

wherein the unique synthetic modular polypeptides encoded by each unique polynucleotide of the plurality are modular protein kinases or phosphatase proteins.

[0041] Also provided is a barcoded library of nucleic acids, which includes a plurality of unique polynucleotides each comprising a nucleotide sequence encoding a unique synthetic modular polypeptide wherein the unique synthetic modular polypeptides encoded by each unique polynucleotide of the plurality are modular transcriptional regulator proteins.

[0042] Also provided is a barcoded library of nucleic acids, which includes a plurality of unique polynucleotides each comprising a nucleotide sequence encoding a unique synthetic modular polypeptide wherein the unique synthetic modular polypeptides encoded by each unique polynucleotide of the plurality are modular epigenetic regulator proteins.

[0043] Also provided is a barcoded library of nucleic acids, which includes a plurality of unique polynucleotides each comprising a nucleotide sequence encoding a unique synthetic modular polypeptide wherein the unique synthetic modular polypeptides encoded by each unique polynucleotide of the plurality are modular recombinase or nuclease proteins.

[0044] Also provided is a barcoded library of nucleic acids, which includes a plurality of unique polynucleotides each comprising a nucleotide sequence encoding a unique synthetic modular polypeptide wherein the unique synthetic modular polypeptides encoded by each unique polynucleotide of the plurality comprise a modular domain selected from the group consisting of: an antigen-binding domain, a specific binding domain or a specific binding partner protein, a co-stimulatory domain, a co-inhibitory domain, an intracellular signaling domain, a transmembrane domain, a scaffold protein domain, a protein kinase protein domain, a protein phosphatase protein domain, a receptor tyrosine kinase protein domain, a lipid kinase protein domain, a lipid phosphatase protein domain, an ubiquitinylase protein domain, a deubiquitinylase protein domain, a SUMOylase protein domain, an acetylase protein domain, a deacetylase protein domain, a methylase protein domain, a demethylase protein domain, a nuclease protein domain, a recombinase protein domain, a transcription factor protein domain and combinations thereof.

[0045] In one aspect, there is provided a cellular library, the library comprising: a plurality of cells each comprising a unique polynucleotide comprising a nucleotide sequence encoding a unique synthetic modular polypeptide wherein each unique polynucleotide comprises: i) a coding region encoding the unique synthetic modular polypeptide comprising a first coding sequence encoding a first module linked in-frame to a second coding sequence encoding a second module, ii) a barcode region comprising a first barcode specific to the first coding sequence linked to a second barcode specific to the second coding sequence, wherein the first and second barcodes are in the reverse 5' to 3' order as compared to the first

inhibitory domain, an intracellular signaling domain, a transmembrane domain, a scaffold protein domain, a protein kinase protein domain, a protein phosphatase protein domain, a receptor tyrosine kinase protein domain, a lipid kinase protein domain, a lipid phosphatase protein domain, an ubiquitinylase protein domain, a deubiquitinylase protein domain, a SUMOylase protein domain, an acetylase protein domain, a deacetylase protein domain, a methylase protein domain, a demethylase protein domain, a nuclease protein domain, a recombinase protein domain, a transcription factor protein domain and combinations thereof.

[0062] In one aspect, there is provided a method of making a barcoded library of nucleic acids each encoding a unique synthetic modular polypeptide, the method comprising: contacting a first polynucleotide comprising a first module coding sequence linked to a first barcode sequence with a second polynucleotide comprising a second module coding sequence linked to a second barcode sequence under conditions sufficient for insertion of the first polynucleotide into the second polynucleotide at the junction between the second coding sequence and the second barcode sequence thereby generating a barcoded bi-modular polynucleotide, wherein the barcoded bi-modular polynucleotide comprises the second modular coding sequence linked in-frame with the first modular coding sequence linked to the first barcode sequence linked to the second barcode sequence.

[0063] Also provided is a method of making a barcoded library of nucleic acids each encoding a unique synthetic modular polypeptide wherein the method further comprises contacting the first and second polynucleotides with a third polynucleotide comprising a third module coding sequence linked to a third barcode sequence wherein the third polynucleotide inserts into the first polynucleotide at the junction between the first coding sequence and the first barcode sequence thereby generating a barcoded tri-modular polynucleotide, wherein the barcoded tri-modular polynucleotide comprises the second modular coding sequence linked in-frame with the first modular coding sequence linked in-frame with the third modular coding sequence linked to the third barcode sequence linked to the first barcode sequence linked to the second barcode sequence.

[0064] Also provided is a method of making a barcoded library of nucleic acids each encoding a unique synthetic modular polypeptide wherein the first and second modular coding sequences are linked in-frame without any intervening non-coding nucleotides.

[0065] Also provided is a method of making a barcoded library of nucleic acids each encoding a unique synthetic modular polypeptide wherein the barcoded library comprises 1000 or more unique nucleic acids each encoding a unique synthetic modular polypeptide.

[0066] Also provided is a method of making a barcoded library of nucleic acids each encoding a unique synthetic modular polypeptide wherein the barcode sequences are 5' to the module coding sequences or 3' to the module coding sequences.

- [0067] Also provided is a method of making a barcoded library of nucleic acids each encoding a unique synthetic modular polypeptide wherein the insertion of the first polynucleotide into the second polynucleotide at the junction between the second coding sequence and the second barcode sequence is mediated by the activity of a restriction enzyme which recognizes a restriction enzyme recognition site on the second polynucleotide and cleaves the second polynucleotide between the second coding sequence and the second barcode sequence, including wherein the restriction enzyme used is a Type II restriction enzyme, including wherein the restriction enzyme used is a Type IIS restriction enzyme.
- [0068] Also provided is a method of making a barcoded library of nucleic acids each encoding a unique synthetic modular polypeptide wherein the first polynucleotide also comprises the restriction enzyme recognition site that is present on the second polynucleotide.
- [0069] Also provided is a method of making a barcoded library of nucleic acids each encoding a unique synthetic modular polypeptide wherein the method further comprises contacting barcoded bi-modular polynucleotides with a reporter encoding polynucleotide under conditions sufficient for insertion of the reporter encoding polynucleotide into the barcoded bi-modular polynucleotide at the junction between the first modular coding sequence and the first barcode sequence thereby generating a reporter-linked barcoded bi-modular polynucleotide, wherein the reporter-linked barcoded bi-modular polynucleotide comprises the second modular coding sequence linked in-frame with the first modular coding sequence linked in-frame with the reporter coding sequence linked to the first barcode sequence linked to the second barcode sequence. Also provided is a method of making a barcoded library wherein the reporter coding sequence encodes an epitope tag or a fluorescent reporter.
- [0070] In one aspect, there is provided a chimeric antigen receptor (CAR) identified by screening a library of synthetic modular CAR polypeptide encoding nucleic acids, wherein the CAR comprises at least one co-modulatory domain listed in **Table 3** or **Table 4**.
- [0071] Also provided is a CAR identified by screening a library of synthetic modular CAR polypeptide encoding nucleic acids wherein the CAR comprises a anti-CD19 antigen-binding domain.
- [0072] Also provided is a CAR identified by screening a library of synthetic modular CAR polypeptide encoding nucleic acids wherein the CAR comprises a CD3 zeta primary signaling domain.
- [0073] Also provided is a CAR identified by screening a library of synthetic modular CAR polypeptide encoding nucleic acids wherein the CAR stimulates T cell activity and comprises at least one co-stimulatory domain listed in **Table 3**.
- [0074] Also provided is a CAR identified by screening a library of synthetic modular CAR polypeptide encoding nucleic acids wherein the CAR inhibits T cell activity and comprises at least one co-inhibitory domain listed in **Table 4**.

[0075] In one aspect, there is provided a nucleic acid encoding a CAR identified by screening a library of synthetic modular CAR polypeptide encoding nucleic acids, including e.g., any of those CARs described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0076] FIG. 1 depicts a barcoded module sequence subcloned for library assembly according to one embodiment of the disclosure.

[0077] FIG. 2 depicts the barcoded module sequence of FIG. 1 following Type IIS restriction enzyme digestion in preparation for library assembly.

[0078] FIG. 3 depicts an example sequence fragment according to FIG. 1 (before Type IIS restriction enzyme (RE) digestion; SEQ ID NO:16) and FIG. 2 (after Type IIS RE digestion; SEQ ID NO:17) containing sequence encoding for a CD28 co-stimulatory domain (translation of Type IIS RE digested fragment; SEQ ID NO:18).

[0079] FIG. 4 provides Table 1. Sequence identifiers for the sequences in Table 1 are as follows: index 1 – SEQ ID NO:19; index 2 – SEQ ID NO:20; index 3 – SEQ ID NO:21; index 4 – SEQ ID NO:22; index 5 – SEQ ID NO:23; index 6 – SEQ ID NO:24; index 7 – SEQ ID NO:25; index 8 – SEQ ID NO:26; index 9 – SEQ ID NO:27; index 10 – SEQ ID NO:28; index 11 – SEQ ID NO:29; index 12 – SEQ ID NO:30; index 13 – SEQ ID NO:31; index 14 – SEQ ID NO:32; index 15 – SEQ ID NO:33; index 16 – SEQ ID NO:34; index 17 – SEQ ID NO:35; index 18 – SEQ ID NO:36; index 19 – SEQ ID NO:37; index 20 – SEQ ID NO:38; index 21 – SEQ ID NO:39; index 22 – SEQ ID NO:40; index 23 – SEQ ID NO:41; index 24 – SEQ ID NO:42; index 25 – SEQ ID NO:43; index 26 – SEQ ID NO:44; index 27 – SEQ ID NO:45; index 28 – SEQ ID NO:46; index 29 – SEQ ID NO:47; index 30 – SEQ ID NO:48; index 31 – SEQ ID NO:49; index 32 – SEQ ID NO:50; index 33 – SEQ ID NO:51; index 34 – SEQ ID NO:52; index 35 – SEQ ID NO:53; index 36 – SEQ ID NO:54; index 37 – SEQ ID NO:55; index 38 – SEQ ID NO:56; index 39 – SEQ ID NO:57; index 40 – SEQ ID NO:58; index 41 – SEQ ID NO:59; index 42 – SEQ ID NO:60; index 43 – SEQ ID NO:61; index 44 – SEQ ID NO:62; index 45 – SEQ ID NO:63; index 46 – SEQ ID NO:64; index 47 – SEQ ID NO:65; index 48 – SEQ ID NO:66; index 49 – SEQ ID NO:67; index 50 – SEQ ID NO:68; index 51 – SEQ ID NO:69; index 52 – SEQ ID NO:70; index 53 – SEQ ID NO:71; index 54 – SEQ ID NO:72; index 55 – SEQ ID NO:73; index 56 – SEQ ID NO:74; index 57 – SEQ ID NO:75; index 58 – SEQ ID NO:76; index 59 – SEQ ID NO:77; index 60 – SEQ ID NO:26; index 61 – SEQ ID NO:26; index 62 – SEQ ID NO:26.

[0080] FIG. 5 depicts the general scheme of step-wise assembly of a barcoded two-dimensional co-modulatory module library according to one embodiment of the instant disclosure.

[0081] FIG. 6 depicts the general scheme of generation of a 62-by-62 two-dimensional co-modulatory module library according to one embodiment of the instant disclosure.