

What is claimed is:

1. A method of making a nucleic acid sequence comprising a sequence that encodes a heavy chain element (HC element) of an antibody heavy chain variable region (HCVR) and a light chain element (LC element) of an antibody light chain variable region (LCVR), and wherein the HCVR and LCVR are matched, the method comprising:
 - a) acquiring an isolated production reaction site, comprising:
 - i) a heavy chain (HC) strand, wherein the HC strand is a strand of a heavy chain double-stranded cDNA (HC ds cDNA) comprising a segment that encodes the HC element of the HCVR from a cell; and
 - ii) a light chain (LC) strand, wherein the LC strand is a strand of a light chain double-stranded cDNA (LC ds cDNA) comprising a segment that encodes the LC element of the LCVR from the cell, and
 - b) covalent linking of the HC strand to the LC strand, wherein the HC strand and the LC strand are covalently linked to produce a single-stranded nucleic acid sequence, wherein the isolated production reaction site does not comprise a nucleic acid encoding an HCVR or an LCVR from a cell other than the cell, thereby making the nucleic acid sequence.
2. The method of claim 1, wherein:
 - (a) the HC element comprises, or consists of, a heavy chain variable region sequence (HCVRS), or an antigen binding fragment thereof, and/or the LC element comprises, or consists of, a light chain variable region sequence (LCVRS), or an antigen binding fragment thereof; and/or
 - (b) the nucleic acid sequence is configured such that, when expressed, the HC element and the LC element form a functional antigen binding molecule *in vitro*, *ex vivo*, or *in vivo*.
3. The method of claim 1 or 2, wherein:
 - (a) acquiring the isolated production reaction site comprises:
 - a) acquiring a capture substrate bound to:
 - i) a first double-stranded cDNA (ds cDNA) comprising a strand that is complementary to a first mRNA that encodes the HCVR from the cell; and
 - ii) a second ds cDNA comprising a strand complementary to a second mRNA encoding the LCVR from the cell, to produce a loaded capture substrate, and
 - b) maintaining the isolated production reaction site under conditions that allow amplification of the first and second ds cDNAs, to produce:
 - a plurality of HC ds cDNAs comprising the segment that encodes the HC element of the HCVR from the cell; and

a plurality of LC ds cDNAs comprising the segment that encodes an LC element of the LCVR from the cell;

(b) the capture substrate comprises a bead and a moiety which binds to cDNA; and/or

(c) the isolated production reaction site comprises a reagent mixture suitable for producing, from the first and second mRNAs, a first ds cDNA comprising the segment that encodes the HC element of the HCVR of the cell, and a second ds cDNA comprising a segment that encodes the LC element of the LCVR of the cell,

optionally, wherein:

(a) the first and second ds cDNAs are amplified in the presence of primers, wherein at least one of the primers comprises a first member, a second member, and a nucleotide modification between the first and second members, wherein the nucleotide modification reduces DNA synthesis, optionally wherein the nucleotide modification comprises an insertion of a spacer between two adjacent nucleotides or a modification to a ribose;

(b) the first member is capable of annealing with the second member in the same primer or a different primer, forming a double-stranded structure comprising a duplex region of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, more basepairs; and/or

(c) at least one of the primers is phosphorylated and comprises a sequence encoding at least a portion of a linker sequence, or a complementary sequence thereof.

4. The method of any of claims 1-3, wherein:

(a) the HC ds cDNA comprises a 5' overhang and a blunt end and the LC ds cDNA comprises a 5' overhang and a blunt end;

(b) the HC and LC strands are both sense strands or both antisense strands;

(c) the covalent linking occurs in an isolated linkage reaction site comprising a ligase; and/or

(d) the HC strand and the LC strand are covalently linked in the presence of a splint oligonucleotide, wherein the splint oligonucleotide is hybridized to a sequence comprising the junction of the HC strand and the LC strand to form a duplexed region at the site of linkage, optionally wherein the splint oligonucleotide comprises a modification that inhibits DNA synthesis.

5. The method of any of claims 1-4, further comprising:

(a) prior to acquiring the isolated production reaction site, acquiring an mRNA loaded capture substrate comprising:

a) acquiring an isolated cell reaction site, comprising:

i) a cell; and

ii) a capture substrate capable of binding a first mRNA encoding an HCVR from the cell and a second mRNA encoding an LCVR from the cell; and

b) maintaining the isolated cell reaction site under conditions that allow lysis of the cell and binding of the capture substrate with the first mRNA and the second mRNA to form the mRNA loaded capture substrate,

wherein the isolated cell reaction site does not include a nucleic acid encoding an HCVR or an LCVR from a cell other than the cell,

optionally further comprising releasing the mRNA loaded capture substrate from the isolated cell reaction site in the presence of a poly(dA) or poly(dT) oligonucleotide;

(b) amplifying the nucleic acid sequence;

(c) sequencing all or a portion of the nucleic acid sequence;

(d) inserting all or a portion of nucleic acid sequence into a vector; and/or

(e) expressing the nucleic acid sequence to produce a polypeptide comprising the segment that encodes the HC element of the HCVR, and the segment that encodes the LC element of the LCVR, optionally further comprising contacting the polypeptide with an antigen and determining if the polypeptide binds the antigen.

6. A method of making a library comprising a plurality of unique members, the method comprising:

making the plurality of members by the method of any of claims 1-5,

wherein each of the members comprises a sequence that encodes a heavy chain element (HC element) of a heavy chain variable region (HCVR) and a light chain element (LC element) of a light chain variable region (LCVR), wherein the HCVR and the LCVR are matched, and wherein each unique nucleic acid sequence of the plurality comprises an HC element and an LC element from a different unique cell,

thereby making the library,

optionally, wherein the library comprises one, two, three, or all of the following properties:

a) the plurality of unique members comprises at least 10^4 , 10^5 , 10^6 , 10^7 , 10^8 , or 10^9 unique members;

b) the plurality of unique members comprises 10^4 to 10^9 , 10^4 to 10^8 , 10^4 to 10^7 , 10^4 to 10^6 , 10^4 to 10^5 , 10^8 to 10^9 , 10^7 to 10^9 , 10^6 to 10^9 , 10^5 to 10^9 , 10^5 to 10^8 , 10^6 to 10^7 , 10^4 to 10^5 , 10^5 to 10^6 , 10^6 to 10^7 , 10^7 to 10^8 , or 10^8 to 10^9 unique members;

c) at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%, of the members in the library are unique members; or

d) less than 20%, 15%, 10%, 5%, 4%, 3%, 2%, or 1%, of the members in the library are unique members.

7. A library made by the method of claim 6, optionally, wherein the library is a display library.

8. A method of making a binding polypeptide, the method comprising:
a) acquiring a library of claim 7; and
b) expressing a polypeptide encoded by a unique nucleic acid of the library,
5 optionally, further comprising contacting the polypeptide with an antigen and obtaining a nucleic acid that encodes a polypeptide that binds the antigen.

9. An isolated production reaction site, comprising:
a) a heavy chain (HC) strand, wherein the HC strand is a strand of a heavy chain double-
10 stranded cDNA (HC ds cDNA) comprising a segment that encodes an HC element of the HCVR from a cell; and
b) a light chain (LC) strand, wherein the LC strand is a strand of a light chain double-stranded cDNA (LC ds cDNA) comprising a segment that encodes an LC element of the LCVR from the cell,
c) a primer comprising a first member, a second member, and a nucleotide modification
15 between the first and second members, wherein the nucleotide modification reduces DNA synthesis, wherein the HCVR and LCVR are matched, and wherein the isolated production reaction site does not comprise a nucleic acid encoding an LCVR or an HCVR from a cell other than the cell,
optionally, wherein the first member is capable of annealing with the second member in the same primer or a different primer, forming double-stranded structure comprising a duplex region of 4,
20 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more basepairs.

10. A self-annealing oligonucleotide comprising a first member, a second member, and a spacer located between the first and second members, wherein the first member is capable of annealing with the second member in the same oligonucleotide or a different oligonucleotide, forming
25 a hairpin structure or a double-stranded structure comprising a duplex region of 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more basepairs,
optionally, wherein the spacer is a flexible spacer or a PEG spacer.

11. An isolated linkage reaction site, comprising:
30 a) a heavy chain (HC) strand, wherein the HC strand is a strand of a heavy chain double-stranded cDNA (HC ds cDNA) comprising a segment that encodes an HC element of the HCVR from a cell;
b) a light chain (LC) strand, wherein the LC strand is a strand of a light chain double-stranded cDNA (LC ds cDNA) comprising a segment that encodes an LC element of the LCVR from the cell;
35 and
c) a splint oligonucleotide that is capable of hybridizing to a sequence comprising the junction of the HC strand and the LC strand, to form a duplexed region at the site of linkage,

wherein the HCVR and LCVR are matched, wherein the HC strand and the LC strand are covalently linked, and wherein the isolated linkage reaction site does not comprise a nucleic acid encoding an HCVR or an LCVR from a cell other than the cell,
optionally, further comprising a ligase.

5