

HCVRs; and a plurality of LC ds cDNAs comprising a segment that encodes an LC element of the LCVR from the cell, *e.g.*, an LCVRS.

In an embodiment, the HC ds cDNA is identical, or substantially identical, to the first ds cDNA. For example, the sense strand of the HC ds cDNA is at least 80%, 85%, 90%, 95%, 98%, 99%, or 100% identical to, or differs by no more than 1, 2, 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 nucleotides from, the sense strand of the first ds cDNA, and/or the antisense strand of the HC ds cDNA is at least 80%, 85%, 90%, 95%, 98%, 99%, or 100% identical to, or differs by no more than 1, 2, 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 nucleotides from, the antisense strand of the first ds cDNA.

In an embodiment, the LC ds cDNA is identical, or substantially identical, to the second ds cDNA. For example, the sense strand of the LC ds cDNA is at least 80%, 85%, 90%, 95%, 98%, 99%, or 100% identical to, or differs by no more than 1, 2, 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 nucleotides from, the sense strand of the second ds cDNA, and/or the antisense strand of the LC ds cDNA is at least 80%, 85%, 90%, 95%, 98%, 99%, or 100% identical to, or differs by no more than 1, 2, 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 nucleotides from, the antisense strand of the second ds cDNA.

In an embodiment, the HC strand is a sense strand. In an embodiment, the LC strand is a sense strand. In an embodiment, the HC strand is an antisense strand. In an embodiment, the LC strand is an antisense strand. In an embodiment, both the HC strand and the LC strand are sense strands. In an embodiment, both the HC strand and the LC strand are antisense strands.

In an embodiment, the capture substrate comprises a bead, *e.g.*, a magnetic bead. In an embodiment, the capture substrate comprises a moiety (*e.g.*, an oligonucleotide) which binds to cDNA, *e.g.*, (i) a moiety which binds to the HC strand; (ii) a moiety which binds to the LC strand; or (iii) both (i) and (ii). In an embodiment, the moiety which binds to the HC strand is different from the moiety which binds to the LC strand, *e.g.*, to facilitate creating conditions favorable to capturing similar levels of each DNA molecule type. In an embodiment, the moiety which binds to the HC strand is identical to the moiety which binds to the LC strand.

In an embodiment, the first mRNA and the second mRNA are disposed on an mRNA loaded capture substrate.

In another aspect, the disclosure features an isolated production reaction site, comprising:

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; 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 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,

5 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, more basepairs.

In an embodiment, the isolated production reaction site, *e.g.*, the production micro-chamber, comprises: a reagent mixture suitable for producing, from the first and second mRNAs (*e.g.*, after the first and second mRNAs are released from the mRNA loaded capture substrate into a solution), a first ds cDNA comprising a segment that encodes an HC element of the HCVR of the cell, *e.g.*, an

10 HCVRs, and a second ds cDNA comprising a segment that encodes an LC element of the LCVR of the cell, *e.g.*, an LCVRs.

In an embodiment, the isolated production reaction site, *e.g.*, production micro-chamber, comprises primers that mediate the production of the first ds cDNA. In an embodiment, the isolated

In an embodiment, the disclosure features 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, comprising:

- 5 a) acquiring an isolated cell reaction site (*e.g.*, an isolated cell reaction site described herein), *e.g.*, a cell isolation micro-chamber, comprising: i) a cell (*e.g.*, a cell described herein); and ii) a capture substrate (*e.g.*, a capture substrate described herein) capable of binding a first mRNA encoding an HCVR from the cell and a second mRNA encoding an LCVR from the cell;
- 10 b) maintaining the isolated cell reaction site, *e.g.*, the cell isolation micro-chamber, under conditions that allow lysis of the cell and binding of the capture substrate with the first mRNA and the second mRNA to form an mRNA loaded capture substrate,
- wherein the isolated cell reaction site, *e.g.*, cell isolation micro-chamber, does not include a nucleic acid encoding an HCVR or an LCVR from a cell other than the cell (*e.g.*, a different cell);
- 15 c) contacting the mRNA loaded capture substrate with a reaction mixture, *e.g.*, a reaction mixture comprising reverse transcriptase, that uses the loaded mRNA as a template to make cDNA (this can occur, *e.g.*, in the isolated cell reaction site, in an isolated production reaction site, or in neither, *e.g.*, not in an isolated reaction site);
- 20 d) acquiring an isolated production reaction site (*e.g.*, an isolated production reaction site described herein), *e.g.*, a production micro-chamber, 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 an HC element of the HCVR from the cell, *e.g.*, a heavy chain variable region sequence (HCVRS); 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 an LC element of the LCVR from the cell, *e.g.*, a light chain variable region sequence (LCVRS),
- 25 wherein the isolated production reaction site, *e.g.*, a production micro-chamber, does not include a nucleic acid encoding an LCVR or an HCVR from a cell other than the cell (*e.g.*, a different cell); and
- e) covalent linking, *e.g.*, ligation, of the HC strand to the LC strand.

30 In an embodiment, one or more (*e.g.*, two, three, four, or all) of the steps a)-e) are performed in accordance with a method described herein. In an embodiment, each of the steps a)-e) is performed in accordance with a method described herein.

35 In an embodiment, the disclosure features 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, comprising:

chain element (LC element) of a light chain variable region (LCVR), and wherein the HCVR and LCVR are matched, made by a method described herein,

wherein each unique nucleic acid sequence of the plurality comprises an HC element and an LC element from a different unique cell (*e.g.*, a cell described herein),

5 thereby making a library comprising a plurality of unique members.

In an embodiment, the plurality of unique members comprises at least 10^4 , 10^5 , 10^6 , 10^7 , 10^8 , or 10^9 unique members. In an embodiment, 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. In an embodiment, at
10 least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%, of the members in the library are unique members (which encode matched HC element and LC element sequences). In an embodiment, less than 20%, 15%, 10%, 5%, 4%, 3%, 2%, or 1%, of the members in the library are unique members (which encode matched HC element and LC element sequences).

15 In an embodiment, the disclosure features a library comprising a plurality of unique members, wherein,

i) each unique member of the plurality comprises a segment that encodes an HC element, *e.g.*, an HCVR, and a segment that encodes an LC element, *e.g.*, an LCVR, wherein the HC element and the LC element in each unique member is matched;

20 ii) each unique member of the plurality comprises a segment that encodes an HC element, *e.g.*, an HCVR, and a segment that encodes an LC element, *e.g.*, an LCVR, from a different unique cell; and

iii) the library comprises one or more (*e.g.*, two, three, four, or all) of the following properties:

25 a) the library is made by a method described herein;

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

c) 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 ,
30 10^5 to 10^6 , 10^6 to 10^7 , 10^7 to 10^8 , or 10^8 to 10^9 unique members;

d) at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%, of the members in the library are unique members (which encode matched HC element and LC element sequences); or

e) less than 20%, 15%, 10%, 5%, 4%, 3%, 2%, or 1%, of the members in the library
35 are unique members (which encode matched HC element and LC element sequences).

In an embodiment, each unique member of the plurality is configured such that, when expressed, the HC element, *e.g.*, the HCVRs, and the LC element, *e.g.*, the LCVRs, form a functional antigen binding molecule, *e.g.*, an scFv, an Fab, or an scFab.

In another aspect, the disclosure features a library made by the method as disclosed herein, optionally, wherein the library is a display library. In an embodiment, the library is a display library. In an embodiment, each of the members of the plurality further encodes a polypeptide that results in display of the member on the surface of a display entity. In an embodiment, the library is a phage display library. In an embodiment, the library is a yeast display library. In an embodiment, the library is a mammalian display library.

In an aspect, the disclosure features a method of making a binding polypeptide (*e.g.*, a polypeptide comprising an HC element and an LC element), the method comprising: a) acquiring a library described herein, *e.g.*, by a method described herein; and b) expressing a polypeptide encoded by a unique nucleic acid of the library.

In an embodiment, the method further comprises contacting the polypeptide with an antigen. In an embodiment, the method further comprises retrieving (*e.g.*, isolating or purifying) the nucleic acid that encodes a polypeptide that binds the antigen.

In an embodiment, the disclosure features an isolated production reaction site, *e.g.*, a production micro-chamber, which is an isolated production reaction site described herein (*e.g.*, comprising a nucleic acid encoding an HCVR and a nucleic acid encoding a LCVR, wherein the HCVR and the LCVR are matched).

In an embodiment, the isolated production reaction site, *e.g.*, a production micro-chamber, does not include a nucleic acid encoding an HCVR or an LCVR from a different cell.

In an embodiment, the isolated production reaction site, *e.g.*, a production micro-chamber, comprises one, two, or all of: (i) one or more primers specific to V gene sequences of the HC and LC; (ii) one or more primers specific to overhangs introduced onto the HC and LC cDNAs; or (iii) one or more primers comprising a first member, a second member, and a third member comprising a nucleotide modification (*e.g.*, a spacer) located between the first and second members, wherein the first member is capable of annealing with the second member of the same primer or a different primer, *e.g.*, forming a 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.

In an embodiment, the isolated production reaction site, *e.g.*, a production micro-chamber, does not comprise a reagent that can covalently link nucleic acids, *e.g.*, a ligase, *e.g.*, a thermostable ligase. In another embodiment, the isolated production reaction site, *e.g.*, a production micro-chamber, comprises a reagent that can covalently link nucleic acids, *e.g.*, a ligase, *e.g.*, a thermostable ligase.

In another aspect, the disclosure features 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 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, more basepairs, optionally, wherein the spacer is a flexible spacer or a PEG spacer.

In an embodiment, the disclosure features a self-annealing oligonucleotide comprising a first member, a second member, and third member comprising a nucleotide modification (*e.g.*, a spacer) located between the first and second members, wherein the first member is capable of annealing with the second member of the same oligonucleotide (*e.g.*, for a method of making a nucleic acid sequence comprising a sequence that encodes an HC element of an HCVR and a LC element of an LCVR, wherein the HCVR and LCVR are matched).

In an embodiment, the first and second members are capable of forming a hairpin 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. In an embodiment, the first member is 5-40 nucleotides, *e.g.*, 5-10, 5-20, 5-30, 30-40, 20-40, 10-30, 10-30, or 15-25 nucleotides, in length. In an embodiment, the second member is 5-40 nucleotides, *e.g.*, 5-10, 5-20, 5-30, 30-40, 20-40, 10-30, 10-30, or 15-25 nucleotides, in length.

In an embodiment, the spacer is a spacer described herein, *e.g.*, a flexible spacer or a PEG spacer.

In an embodiment, the first member comprises a sequence that is complementary to the sequence of an oligonucleotide attached to a capture substrate.

In an embodiment, the second member comprises (*e.g.*, from 5' to 3') one, two, or all of: (i) a sequence that is complementary to at least a portion of the first member; (ii) a universal priming sequence (*e.g.*, for PCR amplification or next-generation sequencing); and (iii) a sequence complementary to a target sequence, *e.g.*, an HCVRS and/or an LCVRS. In an embodiment, the universal priming sequence is identical, or substantially identical, to the sequence that is complementary to at least a portion of the first member. In another embodiment, the universal priming sequence is different from the sequence that is complementary to at least a portion of the first member. In an embodiment, the second member comprises a sequence for homologous recombination (*e.g.*, in a yeast or mammalian cell).

In another aspect, the disclosure features an isolated linkage reaction site, comprising:

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; 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

5 encoding an HCVR or an LCVR from a cell other than the cell,

optionally, further comprising a ligase.

In an embodiment, the disclosure features an isolated linkage reaction site, *e.g.*, a linkage micro-chamber, which is an isolated linkage reaction site described herein (*e.g.*, comprising a nucleic acid encoding an HCVR and a nucleic acid encoding a LCVR, wherein the HCVR and the LCVR are

10 matched).

In an embodiment, the isolated linkage reaction site, *e.g.*, a linkage micro-chamber, does not include a nucleic acid encoding an HCVR or an LCVR from a different cell.

In an embodiment, the isolated linkage reaction site, *e.g.*, a linkage micro-chamber, comprises a splint oligonucleotide (*e.g.*, a splint oligonucleotide described herein) that is capable of hybridizing

15 to a sequence comprising the junction of the HC strand and the LC strand, or a sequence

complementary thereof, to form a duplexed region at the site of ligation.

In an embodiment, the isolated linkage reaction site, *e.g.*, a linkage micro-chamber, comprises a reagent that can covalently link nucleic acids, *e.g.*, a ligase, *e.g.*, a thermostable ligase.

In an embodiment, the disclosure features a method of making a nucleic acid sequence comprising a sequence that encodes a TCR α chain element (AC element) of a TCR α chain variable region (ACVR) and a TCR β chain element (BC element) of a TCR β chain variable region (BCVR), and wherein the ACVR and BCVR are matched, comprising:

5 a) acquiring an isolated cell reaction site (*e.g.*, an isolated cell reaction site described herein), *e.g.*, a cell isolation micro-chamber, comprising: i) a cell (*e.g.*, a cell described herein); and ii) a capture substrate (*e.g.*, a capture substrate described herein) capable of binding a first mRNA encoding an ACVR from the cell and a second mRNA encoding a BCVR from the cell;

10 b) maintaining the isolated cell reaction site, *e.g.*, the cell isolation micro-chamber, 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, *e.g.*, cell isolation micro-chamber, does not include a nucleic acid encoding an ACVR or a BCVR from a cell other than the cell (*e.g.*, a different cell);

15 c) acquiring an isolated production reaction site (*e.g.*, an isolated production reaction site described herein), *e.g.*, a production micro-chamber, comprises: contacting the mRNA loaded capture substrate with a reaction mixture, *e.g.*, a reaction mixture comprising reverse transcriptase, that uses the loaded mRNA as a template, to produce: a first double-stranded cDNA (ds cDNA) comprising a strand that is complementary to a first mRNA that encodes an ACVR from a cell; and a second ds cDNA comprising a strand complementary to a second mRNA encoding a BCVR from the cell (the
20 cDNA loaded capture substrate);

wherein the isolated production reaction site, *e.g.*, a production micro-chamber, does not include a nucleic acid encoding an ACVR or a BCVR from a cell other than the cell (*e.g.*, a different cell).

25 d) maintaining the isolated production reaction site, *e.g.*, the production micro-chamber, under conditions that allow amplification of the first and second ds cDNAs, to produce: a plurality of AC ds cDNAs comprising a segment that encodes an AC element of the ACVR from the cell, *e.g.*, an ACVRs; and a plurality of BC ds cDNAs comprising a segment that encodes a BC element of the BCVR from the cell, *e.g.*, a BCVRs;

30 e) acquiring an isolated linkage reaction site (*e.g.*, an isolated linkage reaction site described herein), *e.g.*, a linkage micro-chamber, comprising: covalent linking, *e.g.*, ligation, of a strand of the AC ds cDNA (AC strand) to a strand of the BC ds cDNA (BC strand), wherein the AC and BC strands are both sense strands or antisense strands; and

f) amplifying the covalently linked, *e.g.*, ligated, AC and BC strands.

35 In an embodiment, one or more (*e.g.*, two, three, four, five, or all) of the steps a)-f) are performed in accordance with a method described herein. In an embodiment, each of the steps a)-f) is performed in accordance with a method described herein.

In an aspect, the disclosure features a method of making a library comprising a plurality of unique members, the method comprising:

making the plurality of members, wherein each of the members comprises a sequence that encodes a TCR α chain element (AC element) of a TCR α chain variable region (ACVR) and a TCR β chain element (BC element) of a TCR β chain variable region (BCVR), and wherein the ACVR and BCVR are matched, made by a method described herein,

wherein each unique nucleic acid sequence of the plurality comprises an AC element and a BC element from a different unique cell (*e.g.*, a cell described herein),

thereby making a library comprising a plurality of unique members.

10 In an embodiment, the plurality of unique members comprises at least 10^4 , 10^5 , 10^6 , 10^7 , 10^8 , or 10^9 unique members. In an embodiment, 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. In an embodiment, at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%, of the members in the library are unique members (which encode matched AC element and BC element sequences). In an embodiment, less than 20%, 15%, 10%, 5%, 4%, 3%, 2%, or 1%, of the members in the library are unique members (which encode matched AC element and BC element sequences).

20 In an embodiment, the disclosure features a library comprising a plurality of unique members, wherein,

i) each unique member of the plurality comprises a segment that encodes an AC element, *e.g.*, an ACVRS, and a segment that encodes a BC element, *e.g.*, a BCVRS, wherein the AC element and the BC element in each unique member is matched;

25 ii) each unique member of the plurality comprises a segment that encodes an AC element, *e.g.*, an ACVRS, and a segment that encodes a BC element, *e.g.*, a BCVRS, from a different unique cell; and

iii) the library comprises one or more (*e.g.*, two, three, four, or all) of the following properties:

a) the library is made by a method described herein;

30 b) the plurality of unique members comprises at least 10^4 , 10^5 , 10^6 , 10^7 , 10^8 , or 10^9 unique nucleic acid sequences;

c) 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;

35 d) at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%, of the members in the library are unique members (which encode matched AC element and BC element sequences); or

e) less than 20%, 15%, 10%, 5%, 4%, 3%, 2%, or 1%, of the members in the library are unique members (which encode matched AC element and BC element sequences).

5 In an embodiment, each unique member of the plurality is configured such that, when expressed, the AC element, *e.g.*, the ACVRS, and the BC element, *e.g.*, the BCVRS, form a functional antigen binding molecule, *e.g.*, a single chain or a complex of a TCR α chain and a β chain.

In an embodiment, the library is a display library. In an embodiment, each of the members of the plurality further encodes a polypeptide that results in display of the member on the surface of a display entity. In an embodiment, the library is a phage display library. In an embodiment, the library is a yeast display library. In an embodiment, the library is a mammalian display library.

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In an embodiment, the disclosure features a method of making a binding polypeptide (*e.g.*, a polypeptide comprising an AC element and a BC element), the method comprising: a) acquiring a library described herein, *e.g.*, by a method described herein; and b) expressing a polypeptide encoded by a unique nucleic acid of the library.

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In an embodiment, the method further comprises contacting the polypeptide with an antigen. In an embodiment, the method further comprises retrieving (*e.g.*, isolating or purifying) the nucleic acid that encodes a polypeptide that binds the antigen.

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In an embodiment, the disclosure features an isolated production reaction site, *e.g.*, a production micro-chamber, which is an isolated production reaction site described herein (*e.g.*, comprising a nucleic acid encoding an ACVR and a nucleic acid encoding a BCVR, wherein the ACVR and the BCVR are matched).

In an embodiment, the isolated production reaction site, *e.g.*, a production micro-chamber, does not include a nucleic acid encoding an ACVR or a BCVR from a different cell.

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In an embodiment, the isolated production reaction site, *e.g.*, a production micro-chamber, comprises one, two, or all of: (i) one or more primers specific to V gene sequences of the AC and BC; (ii) one or more primers specific to overhangs introduced onto the AC and BC cDNAs; or (iii) one or more primers comprising a first member, a second member, and a third member comprising a nucleotide modification (*e.g.*, a spacer) located between the first and second members, wherein the first member is capable of annealing with the second member of the same primer or a different primer, *e.g.*, forming a 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.

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In an embodiment, the isolated production reaction site, *e.g.*, a production micro-chamber, does not comprise a reagent that can covalently link nucleic acids, *e.g.*, a ligase, *e.g.*, a thermostable ligase. In another embodiment, the isolated production reaction site, *e.g.*, a production micro-chamber, comprises a reagent that can covalently link nucleic acids, *e.g.*, a ligase, *e.g.*, a thermostable ligase.

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In an embodiment, the disclosure features a self-annealing oligonucleotide comprising a first member, a second member, and third member comprising a nucleotide modification (*e.g.*, a spacer) located between the first and second members, wherein the first member is capable of annealing with the second member of the same oligonucleotide (*e.g.*, for a method of making a nucleic acid sequence comprising a sequence that encodes an AC element of an ACVR and a BC element of a BCVR, wherein the ACVR and BCVR are matched).

In an embodiment, the first and second members are capable of forming a hairpin 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. In an embodiment, the first member is 5-40 nucleotides, *e.g.*, 5-10, 5-20, 5-30, 30-40, 20-40, 10-30, 10-30, or 15-25 nucleotides, in length. In an embodiment, the second member is 5-40 nucleotides, *e.g.*, 5-10, 5-20, 5-30, 30-40, 20-40, 10-30, 10-30, or 15-25 nucleotides, in length.

In an embodiment, the spacer is a spacer described herein, *e.g.*, a flexible spacer or a PEG spacer.

In an embodiment, the first member comprises a sequence that is complementary to the sequence of an oligonucleotide attached to a capture substrate.

In an embodiment, the second member comprises (*e.g.*, from 5' to 3') one, two, or all of: (i) a sequence that is complementary to at least a portion of the first member; (ii) a universal priming sequence (*e.g.*, for PCR amplification or next-generation sequencing); and (iii) a sequence complementary to a target sequence, *e.g.*, an ACVRS and/or a BCVRS. In an embodiment, the universal priming sequence is identical, or substantially identical, to the sequence that is complementary to at least a portion of the first member. In another embodiment, the universal priming sequence is different from the sequence that is complementary to at least a portion of the first member. In an embodiment, the second member comprises a sequence for homologous recombination (*e.g.*, in a yeast or mammalian cell).

In an embodiment, the disclosure features an isolated linkage reaction site, *e.g.*, a linkage micro-chamber, which is an isolated linkage reaction site described herein (*e.g.*, comprising a nucleic acid encoding an ACVR and a nucleic acid encoding a BCVR, wherein the ACVR and the BCVR are matched).

In an embodiment, the isolated linkage reaction site, *e.g.*, a linkage micro-chamber, does not include a nucleic acid encoding an ACVR or a BCVR from a different cell.

In an embodiment, the isolated linkage reaction site, *e.g.*, a linkage micro-chamber, comprises a splint oligonucleotide (*e.g.*, a splint oligonucleotide described herein) that is capable of hybridizing to a sequence comprising the junction of the AC strand and the BC strand, or a sequence complementary thereof, to form a duplexed region at the site of ligation.

In an embodiment, the disclosure features a method of making a nucleic acid sequence comprising a sequence that encodes a TCR γ chain element (GC element) of a TCR γ chain variable region (GCVR) and a TCR δ chain element (DC element) of a TCR δ chain variable region (DCVR), and wherein the GCVR and DCVR are matched, comprising:

a) acquiring an isolated cell reaction site (*e.g.*, an isolated cell reaction site described herein), *e.g.*, a cell isolation micro-chamber, comprising: i) a cell (*e.g.*, a cell described herein); and ii) a capture substrate (*e.g.*, a capture substrate described herein) capable of binding a first mRNA encoding a GCVR from the cell and a second mRNA encoding a DCVR from the cell;

b) maintaining the isolated cell reaction site, *e.g.*, the cell isolation micro-chamber, 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, *e.g.*, cell isolation micro-chamber, does not include a nucleic acid encoding a GCVR or a DCVR from a cell other than the cell (*e.g.*, a different cell);

c) acquiring an isolated production reaction site (*e.g.*, an isolated production reaction site described herein), *e.g.*, a production micro-chamber, comprises: contacting the mRNA loaded capture substrate with a reaction mixture, *e.g.*, a reaction mixture comprising reverse transcriptase, that uses the loaded mRNA as a template, to produce: a first double-stranded cDNA (ds cDNA) comprising a strand that is complementary to a first mRNA that encodes a GCVR from a cell; and a second ds cDNA comprising a strand complementary to a second mRNA encoding a DCVR from the cell (the cDNA loaded capture substrate);

wherein the isolated production reaction site, *e.g.*, a production micro-chamber, does not include a nucleic acid encoding a GCVR or a DCVR from a cell other than the cell (*e.g.*, a different cell).

d) maintaining the isolated production reaction site, *e.g.*, the production micro-chamber, under conditions that allow amplification of the first and second ds cDNAs, to produce: a plurality of GC ds cDNAs comprising a segment that encodes a GC element of the GCVR from the cell, *e.g.*, a GCVRS; and a plurality of DC ds cDNAs comprising a segment that encodes a DC element of the DCVR from the cell, *e.g.*, a DCVRS;

e) acquiring an isolated linkage reaction site (*e.g.*, an isolated linkage reaction site described herein), *e.g.*, a linkage micro-chamber, comprising: covalent linking, *e.g.*, ligation, of a strand of the GC ds cDNA (GC strand) to a strand of the DC ds cDNA (DC strand), wherein the GC and DC strands are both sense strands or antisense strands; and

f) amplifying the covalently linked, *e.g.*, ligated, GC and DC strands.

In an embodiment, one or more (*e.g.*, two, three, four, five, or all) of the steps a)-f) are performed in accordance with a method described herein. In an embodiment, each of the steps a)-f) is performed in accordance with a method described herein.

In an aspect, the disclosure features a method of making a library comprising a plurality of unique members, the method comprising:

5 making the plurality of members, wherein each of the members comprises a sequence that encodes a TCR γ chain element (GC element) of a TCR γ chain variable region (GCVR) and a TCR δ chain element (DC element) of a TCR δ chain variable region (DCVR), and wherein the GCVR and DCVR are matched, made by a method described herein,

wherein each unique nucleic acid sequence of the plurality comprises a GC element and a DC element from a different unique cell (*e.g.*, a cell described herein),

10 thereby making a library comprising a plurality of unique members.

In an embodiment, the plurality of unique members comprises at least 10^4 , 10^5 , 10^6 , 10^7 , 10^8 , or 10^9 unique members. In an embodiment, 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. In an embodiment, at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%, of the members in the library are unique members (which encode matched GC element and DC element sequences). In an embodiment, less than 20%, 15%, 10%, 5%, 4%, 3%, 2%, or 1%, of the members in the library are unique members (which encode matched GC element and DC element sequences).

20 In an embodiment, the disclosure features a library comprising a plurality of unique members, wherein,

i) each unique member of the plurality comprises a segment that encodes a GC element, *e.g.*, a GCVR, and a segment that encodes a DC element, *e.g.*, a DCVR, wherein the GC element and the DC element in each unique member is matched;

25 ii) each unique member of the plurality comprises a segment that encodes a GC element, *e.g.*, a GCVR, and a segment that encodes a DC element, *e.g.*, a DCVR, from a different unique cell; and

iii) the library comprises one or more of the following properties:

a) the library is made by a method described herein;

30 b) the plurality of unique members comprises at least 10^4 , 10^5 , 10^6 , 10^7 , 10^8 , or 10^9 unique nucleic acid sequences;

c) 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;

35 d) at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%, of the members in the library are unique members (which encode matched GC element and DC element sequences); or

e) less than 20%, 15%, 10%, 5%, 4%, 3%, 2%, or 1%, of the members in the library are unique members (which encode matched GC element and DC element sequences).

In an embodiment, each unique member of the plurality is configured such that, when expressed, the GC element, *e.g.*, the GCVRS, and the DC element, *e.g.*, the DCVRS, form a functional antigen binding molecule, *e.g.*, a single chain or a complex of a TCR γ chain and a δ chain.

In another aspect, the disclosure features a library made by the method disclosed herein. In an embodiment, the library is a display library. In an embodiment, each of the members of the plurality further encodes a polypeptide that results in display of the member on the surface of a display entity. In an embodiment, the library is a phage display library. In an embodiment, the library is a yeast display library. In an embodiment, the library is a mammalian display library.

In an aspect, the disclosure features a method of making a binding polypeptide (*e.g.*, a polypeptide comprising a GC element and a DC element), the method comprising: a) acquiring a library described herein, *e.g.*, by a method described herein; and b) expressing a polypeptide encoded by a unique nucleic acid of the library.

In an embodiment, the method further comprises contacting the polypeptide with an antigen. In an embodiment, the method further comprises retrieving (*e.g.*, isolating or purifying) the nucleic acid that encodes a polypeptide that binds the antigen.

In an embodiment, the disclosure features an isolated production reaction site, *e.g.*, a production micro-chamber, which is an isolated production reaction site described herein (*e.g.*, comprising a nucleic acid encoding a GCVR and a nucleic acid encoding a DCVR, wherein the GCVR and the DCVR are matched).

In an embodiment, the isolated production reaction site, *e.g.*, a production micro-chamber, does not include a nucleic acid encoding a GCVR or a DCVR from a different cell.

In an embodiment, the isolated production reaction site, *e.g.*, a production micro-chamber, comprises one, two, or all of: (i) one or more primers specific to V gene sequences of the GC and DC; (ii) one or more primers specific to overhangs introduced onto the GC and DC cDNAs; or (iii) one or more primers comprising a first member, a second member, and a third member comprising a nucleotide modification (*e.g.*, a spacer) located between the first and second members, wherein the first member is capable of annealing with the second member of the same primer or a different primer, *e.g.*, forming a 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.

In an embodiment, the isolated production reaction site, *e.g.*, a production micro-chamber, does not comprise a reagent that can covalently link nucleic acids, *e.g.*, a ligase, *e.g.*, a thermostable ligase. In another embodiment, the isolated production reaction site, *e.g.*, a production micro-chamber, comprises a reagent that can covalently link nucleic acids, *e.g.*, a ligase, *e.g.*, a thermostable ligase.

In an embodiment, the disclosure features a self-annealing oligonucleotide comprising a first member, a second member, and third member comprising a nucleotide modification (*e.g.*, a spacer) located between the first and second members, wherein the first member is capable of annealing with the second member of the same oligonucleotide (*e.g.*, for a method of making a nucleic acid sequence comprising a sequence that encodes a GC element of a GCVR and a DC element of a DCVR, wherein the GCVR and DCVR are matched).

In an embodiment, the first and second members are capable of forming a hairpin 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. In an embodiment, the first member is 5-40 nucleotides, *e.g.*, 5-10, 5-20, 5-30, 30-40, 20-40, 10-30, 10-30, or 15-25 nucleotides, in length. In an embodiment, the second member is 5-40 nucleotides, *e.g.*, 5-10, 5-20, 5-30, 30-40, 20-40, 10-30, 10-30, or 15-25 nucleotides, in length.

In an embodiment, the spacer is a spacer described herein, *e.g.*, a flexible spacer or a PEG spacer.

In an embodiment, the first member comprises a sequence that is complementary to the sequence of an oligonucleotide attached to a capture substrate.

In an embodiment, the second member comprises (*e.g.*, from 5' to 3') one, two, or all of: (i) a sequence that is complementary to at least a portion of the first member; (ii) a universal priming sequence (*e.g.*, for PCR amplification or next-generation sequencing); and (iii) a sequence complementary to a target sequence, *e.g.*, a GCVRS and/or a DCVRS. In an embodiment, the universal priming sequence is identical, or substantially identical, to the sequence that is complementary to at least a portion of the first member. In another embodiment, the universal priming sequence is different from the sequence that is complementary to at least a portion of the first member. In an embodiment, the second member comprises a sequence for homologous recombination (*e.g.*, in a yeast or mammalian cell).

In an embodiment, the disclosure features an isolated linkage reaction site, *e.g.*, a linkage micro-chamber, which is an isolated linkage reaction site described herein (*e.g.*, comprising a nucleic acid encoding a GCVR and a nucleic acid encoding a DCVR, wherein the GCVR and the DCVR are matched).

In an embodiment, the isolated linkage reaction site, *e.g.*, a linkage micro-chamber, does not include a nucleic acid encoding a GCVR or a DCVR from a different cell.

In an embodiment, the isolated linkage reaction site, *e.g.*, a linkage micro-chamber, comprises a splint oligonucleotide (*e.g.*, a splint oligonucleotide described herein) that is capable of hybridizing to a sequence comprising the junction of the GC strand and the DC strand, or a sequence complementary thereof, to form a duplexed region at the site of ligation.

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 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 strand and the LC strand are covalently linked to produce a single stranded nucleic acid sequence, wherein 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,

optionally, further comprising contacting the polypeptide with an antigen and obtaining a nucleic acid that encodes a polypeptide that binds the antigen.

9. A method of making a nucleic acid sequence comprising a sequence that encodes an α chain element (AC element) of a T-cell receptor (TCR) α chain variable region (ACVR) and a β chain element (BC element) of a TCR β chain variable region (BCVR), and wherein the ACVR and BCVR are matched, the method comprising:

a) acquiring an isolated production reaction site comprising:

i) an α chain (AC) strand, wherein the AC strand is a strand of an α chain double-stranded cDNA (AC ds cDNA) comprising a segment that encodes an AC element of the ACVR from a cell; and

ii) a β chain (BC) strand, wherein the BC strand is a strand of a β chain double-stranded cDNA (BC ds cDNA) comprising a segment that encodes a BC element of the BCVR from the cell, and

b) covalent linking of an AC strand to a BC strand,

wherein the isolated production reaction site does not comprise a nucleic acid encoding a BCVR or an ACVR from a cell other than the cell,

thereby making the nucleic acid sequence,

optionally, wherein the nucleic acid sequence is configured such that, when expressed, the AC element and the BC element form a functional antigen binding molecule, optionally wherein the covalent linking occurs in an isolated linkage reaction site comprising a ligase.

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

making the plurality of members by the method of claim 9,

wherein each of the members comprises a sequence that encodes a α chain element (AC element) of a α chain variable region (ACVR) and a β chain element (BC element) of a β chain variable region (BCVR), wherein the ACVR and BCVR are matched, and wherein each unique nucleic acid sequence of the plurality comprises an AC element and a BC element from a different unique cell,

thereby making the library.

11. A method of making a nucleic acid sequence comprising a sequence that encodes an γ chain element (GC element) of a TCR γ chain variable region (GCVR) and a δ chain element (DC element) of a TCR δ chain variable region (DCVR), and wherein the GCVR and DCVR are matched, the method comprising:

a) acquiring an isolated production reaction site, comprising:

i) a γ chain (GC) strand, wherein the GC strand is a strand of a γ chain double-stranded cDNA (GC ds cDNA) comprising a segment that encodes a GC element of the GCVR from a cell; and

ii) a δ chain (DC) strand, wherein the DC strand is a strand of a δ chain double-stranded cDNA (DC ds cDNA) comprising a segment that encodes a DC element of the DCVR from the cell, and

b) covalent linking of a GC strand to a DC strand,

wherein the isolated production reaction site does not comprise a nucleic acid encoding a DCVR or a GCVR from a cell other than the cell,

thereby making the nucleic acid sequence,

optionally, wherein the nucleic acid sequence is configured such that, when expressed, the GC element and the DC element form a functional antigen binding molecule, optionally wherein the covalent linking occurs in an isolated linkage reaction site comprising a ligase.

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

making the plurality of members by the method of claim 11,

wherein each of the members comprises a sequence that encodes a γ chain element (GC element) of a γ chain variable region (GCVR) and a δ chain element (DC element) of a δ chain variable region (DCVR), wherein the GCVR and DCVR are matched, and wherein each unique nucleic acid sequence of the plurality comprises a GC element and a DC element from a different unique cell,

thereby making the library.

13. A library made by the method of claim 10 or 12.

14. A method of making a binding polypeptide, the method comprising:

a) acquiring the library of claim 13; and

b) expressing a polypeptide encoded by a unique nucleic acid of the library.

15. An isolated production reaction site, comprising:

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; 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 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, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, more basepairs.

16. 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 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, more basepairs,

optionally, wherein the spacer is a flexible spacer or a PEG spacer.

17. An isolated linkage reaction site, comprising:

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; 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.