

BINDING POLYPEPTIDES AND METHODS OF MAKING THE SAME

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 62/438,712, filed
5 December 23, 2016. The contents of the aforesaid application are hereby incorporated by reference in
their entirety.

BACKGROUND

Monoclonal antibody therapies are a class of immunotherapies that involve monoclonal
10 antibodies (mAbs) that are capable of specifically interacting with disease-relevant biological
molecules. In recent years, the disease areas that therapeutic antibodies can target have significantly
expanded, and a number of monoclonal antibodies and antibody-derivative products have been
approved for therapeutic use in the United States and many other countries. Monoclonal antibody
therapies are currently used or investigated for treating various diseases or conditions, including, for
15 example, infectious diseases, cancer, immune diseases, organ transplantation, cardiovascular diseases,
and metabolic diseases.

Given the ability of monoclonal antibodies and antibody-derivative products in modulating
various biological functions, the need exists for developing new approaches for generation of
antibodies suitable for treating, preventing, and diagnosing disorders.

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SUMMARY

This disclosure provides, at least in part, binding polypeptides (*e.g.*, antibody molecules or T-
cell receptor (TCR) molecules) that comprise one or more of the structural or functional properties
disclosed herein. In an example, libraries of the binding polypeptides, methods for making the
25 polypeptides or libraries, nucleic acid molecules encoding the binding polypeptides, expression
vectors, host cells, compositions (*e.g.*, pharmaceutical compositions), kits, and containers, are also
provided. The polypeptides (*e.g.*, antibody molecules or TCR molecules) disclosed herein can be
used (alone or in combination with other agents or therapeutic modalities) to treat, prevent and/or
diagnose disorders, such as disorders and conditions disclosed herein.

30 In an aspect, there is provided 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:

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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,

wherein the HC strand and the LC strand are both sense strands, or wherein the HC strand and the LC strand are both antisense strands; 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.

In another aspect, there is provided a method of making a library comprising a plurality of unique members, the method comprising:

making the plurality of members by the method as defined above,

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.

In another aspect, there is provided a library made by the method as defined above, optionally, wherein the library is a display library.

In another aspect, there is provided a method of making a binding polypeptide, the method comprising:

a) acquiring a library as defined above; 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.

In another aspect, there is provided 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

5 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, wherein the HC strand and the LC strand are

10 both sense strands, or wherein the HC strand and the LC strand are both antisense strands, 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,

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

In another aspect, there is provided 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;

20 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,

25 wherein the HCVR and LCVR are matched, wherein the HC strand and the LC strand are both sense strands, or wherein the HC strand and the LC strand are both antisense strands, 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.

30 In an example, 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, the method comprising:

a) acquiring an isolated production reaction site, *e.g.*, a production micro-chamber,

35 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 a 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), and

b) covalent linking, *e.g.*, ligation, of an HC strand to an LC strand,

wherein 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 cell other than the cell (*e.g.*, a different cell, *e.g.*, a different B cell),

thereby 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 example, the HC element comprises, or consists of, an HCVRS, or a functional fragment thereof (*e.g.*, an antigen binding fragment thereof). In an example, the LC element comprises, or consists of, an LCVRS, or a functional fragment thereof (*e.g.*, an antigen binding fragment thereof).

In an example, the HC ds cDNA comprises a segment that encodes an HCVRS. In an example, the LC ds cDNA comprises a segment that encodes an LCVRS. In an example, the HC ds cDNA comprises a segment that encodes an HCVRS, and the LC ds cDNA comprises a segment that encodes an LCVRS.

In an example, the cell is an immune cell, *e.g.*, a B cell, *e.g.*, a human B cell. In an example, the cell is a mammalian cell or an avian cell.

In an example, the nucleic acid sequence is configured such that, when expressed, the HC element and the LC element (*e.g.*, the HCVRS and the LCVRS) form a functional antigen binding molecule, *e.g.*, an scFv, an Fab, or an scFab. In an example, the antigen binding molecule, *e.g.*, an scFv, is functional *in vitro*, *ex vivo*, or *in vivo*, *e.g.*, as determined by a method or assay described herein.

In an example, acquiring an isolated production reaction site, *e.g.*, a production micro-chamber, 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 an HCVR from a cell; and (ii) a second ds cDNA comprising a strand complementary to a second mRNA encoding an LCVR from the cell (the cDNA loaded capture substrate), and

b) 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 HC ds cDNAs comprising a segment that encodes an HC element of the HCVR from the cell, *e.g.*, an

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

In an example, the capture substrate comprises a bead, *e.g.*, a magnetic bead. In an example, 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 example, 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 example, the moiety which binds to the HC strand is identical to the moiety which binds to the LC strand.

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

In another example, 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,

5 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.

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

production reaction site, *e.g.*, production micro-chamber, comprises primers that mediate the production of the second ds cDNA.

In an example, a cDNA strand that is complementary to a first mRNA that encodes an HCVR from a cell is made by reverse transcription of the first mRNA. In an example, a cDNA strand that is complementary to a second mRNA that encodes an LCVR from a cell is made by reverse transcription of the second mRNA.

In an example, the reverse transcription takes place in the isolated production reaction site, *e.g.*, a production-micro chamber. In an example, the reverse transcription takes place in an isolated cell reaction site, *e.g.*, a cell isolation micro-chamber. In an example, the reverse transcription takes place outside the isolated production reaction site, *e.g.*, a production micro-chamber, or outside an isolated cell reaction site, *e.g.*, a cell isolation micro-chamber. In an example, the reverse transcription takes place outside the isolated production reaction site, *e.g.*, a production-micro chamber, and outside an isolated cell reaction site, *e.g.*, a cell isolation micro-chamber. In an example, the reverse transcription takes place outside an isolated reaction site, *e.g.*, outside a micro-chamber.

In an example, the amplification comprises 30 or fewer cycles, *e.g.*, 20 or fewer cycles, *e.g.*, 15 or fewer, 14 or fewer, 13 or fewer, 12 or fewer, 11 or fewer, 10 or fewer, 9 or fewer, 8 or fewer, 7 or fewer, 6 or fewer, or 5 or fewer cycles.

In an example, the reverse transcription and/or amplification uses one or more primers, *e.g.*, comprising a sequence specific for an HCVRS and/or an LCVRS.

In an example, the reverse transcription and/or amplification comprises using two or more primers that mediate the production of the HC ds cDNA, wherein at least one primer comprises a nucleotide modification, and wherein at least one primer does not comprise a nucleotide modification. In an example, the amplification comprises using two or more primers that mediate the production of the LC ds cDNA, wherein at least one primer comprises a nucleotide modification, and wherein at least one primer does not comprise a nucleotide modification.

In an example, at least one primer comprises a nucleotide modification, *e.g.*, which reduces, *e.g.*, inhibits, DNA synthesis, *e.g.*, by a DNA polymerase. In an example, at least one primer does not comprise a nucleotide modification, *e.g.*, which reduces, *e.g.*, inhibits, DNA synthesis, *e.g.*, by a DNA polymerase.

In an example, the nucleotide modification inhibits a DNA polymerase from extending the DNA. Without wishing to be bound by theory, it is believed that in an example, any chemical entity that reduces (*e.g.*, blocks) DNA polymerase extension can be used in accordance with the methods described herein.

In an example, the nucleotide modification is an insertion of a spacer to the primer, *e.g.*, between two adjacent nucleotides in the primer. In an example, the spacer is a flexible spacer. In an example, the spacer is a carbon spacer (*e.g.*, $-(CH_2)_n-$, wherein $n=3, 4, 5, 6, 7, 8, 9, 10$, or

more), two or more (*e.g.*, three, four, five, six, seven, eight, nine, ten, or more) abasic nucleotides, or a polyethylene glycol (PEG) spacer. In an example, the spacer is a PEG spacer. In an example, the nucleotide modification is 2'-O-methyl, 2'-OH, 2'-NH₂, or uracil, *e.g.*, to a ribose.

In an example, the nucleotide modification is located internally or at the 3' end of the primer. In an example, at least one primer comprises (i) a first member; (ii) a second member; and optionally (iii) a third member, *e.g.*, comprising a nucleotide modification described herein, *e.g.*, located between (i) and (ii).

In an example, the first member is capable of annealing with the second member. In an example, the first member is capable of annealing with the second member in the same primer, *e.g.*, through intra-molecular hybridization, *e.g.*, to form 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 another example, the first member is capable of annealing hybridizing with the second member in a different primer, *e.g.*, through inter-molecular hybridization, *e.g.*, to form 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. Without wishing to be bound by theory, it is believed that in an example, there are at least two secondary structures that the modified primers can form and facilitate reduction (*e.g.*, prevention) of competition to substrate (*e.g.*, bead) capture. For example, the secondary structure can be a hairpin-like structure formed by intra-molecular hybridization (within the same primer), or the secondary structure can be a duplex structure formed by inter-molecular hybridization (between two different primers).

In an example, the first member comprises a sequence that is complementary to the sequence of an oligonucleotide attached to the capture substrate. In an example, 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 example, 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 example, the universal priming sequence is different from the sequence that is complementary to at least a portion of the first member. In an example, the second member comprises a sequence for homologous recombination (*e.g.*, in a yeast or mammalian cell).

In an example, at least one primer comprises a sequence encoding at least a portion of a linker sequence, or a complementary sequence thereof. In an example, the primer that comprises a sequence encoding at least a portion of a linker sequence, or a complementary sequence thereof, is phosphorylated, *e.g.*, 5' phosphorylated. Without wishing to be bound by theory, it is believed that in an example, any sequence with the general properties of flexibility (*e.g.*, facilitated by glycine) and hydrophilicity can work effectively in accordance with the methods described herein. Exemplary linkers can generally have overrepresentation of one or more of Gly, Ser, Thr, or Ala and

underrepresentation of hydrophobic residues, *e.g.*, one or more of Trp, Tyr, Phe, Cys, Met, Leu, or Ile. The length of the primer may vary, *e.g.*, 3-50 amino acid residues (*e.g.*, 5-45, 10-40, 15-35, 20-30, 10-20, 10-30, 20-40, or 30-40 amino acid residues). In an example, the linker sequence comprises, or consists of, ((Gly)^m-Ser)ⁿ, where m=3, 4, 5, or more and n=1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more. In an example, the linker sequence
5 comprises, or consists of, (Gly-Gly-Gly-Gly-Ser)ⁿ, where n=1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more.

In an example, the primer is a primer described herein, *e.g.*, in Examples.

In an example, the reverse transcription, the amplification, or both, occurs in a solution in the isolated production reaction site, *e.g.*, production micro-chamber. In an example, the reverse transcription, the amplification, or both, does not occur on the substrate (*e.g.*, bead). For example, the reverse transcription, the
10 amplification, or both, can occur on in a solution within a droplet.

In an example, the HC ds cDNA comprises a 5' overhang, *e.g.*, a 5' overhang that is capable of hybridizing to an oligonucleotide attached to a capture substrate. In an example, the HC ds cDNA comprises a blunt end, *e.g.*, a blunt end comprising a 5' phosphate. In an example, the LC ds cDNA comprises a 5' overhang, *e.g.*, a 5' overhang that is capable of hybridizing to an oligonucleotide attached to a capture substrate.
15 In an example, the LC ds cDNA comprises a blunt end, *e.g.*, a blunt end comprising a 5' phosphate. In an example, the HC ds cDNA and the LC ds cDNA comprise sticky ends, *e.g.*, both have 5' overhangs.

In an example, the HC strand and the LC strand are covalently linked, *e.g.*, ligated, to produce a single stranded nucleic acid sequence, wherein the HC and LC strands are both sense strands or both antisense strands. In an example, a denatured HC strand of the HC ds cDNA to a denatured LC strand of the LC ds cDNA are
20 covalently linked, *e.g.*, ligated, wherein the HC and LC strands are both sense strands or both antisense strands. In an example, the HC strand is present in the HC ds cDNA and the LC strand is present in the LC ds cDNA, and wherein the HC ds cDNA and the LC ds cDNA are covalently linked, *e.g.*, ligated, *e.g.*, to produce a double stranded nucleic acid sequence.

In an example, the covalent linking, *e.g.*, ligation, occurs in the isolated production reaction site. In an
25 example, the isolated production reaction site, *e.g.*, a production micro-chamber, or the isolated linkage reaction site, *e.g.*, a linkage micro-chamber, comprises a reagent that is capable of covalently linking, *e.g.*, ligating, the HC and LC strands or the HC and LC ds cDNAs. In an example, the isolated production reaction site, *e.g.*, a production micro-chamber comprises an enzyme that covalently couples the HC and LC strands or the HC and LC ds cDNAs. In an example, the enzyme is a ligase, *e.g.*, a thermal stable ligase. In an example, the covalent
30 linking comprises ligase thermocycling.

In an example, the covalent linking, *e.g.*, ligation, occurs in a site different from the isolated production reaction site, *e.g.*, occurs in an isolated linkage reaction site, *e.g.*, a linkage micro-chamber. In an example, the HC strand and the LC strand are transferred from the isolated

production site to the isolated linkage reaction site, *e.g.*, a linkage micro-chamber, and the covalent linking occurs in the isolated linkage reaction site, *e.g.*, a linkage micro-chamber. In an example, the isolated linkage reaction site, *e.g.*, a linkage micro-chamber, comprises a reagent that is capable of covalently linking, *e.g.*, ligating, the HC and LC strands or the HC and LC ds cDNAs. In an example, the isolated linkage reaction site, *e.g.*, a linkage micro-chamber, comprises an enzyme that covalently couples the HC and LC strands or the HC and LC ds cDNAs. In an example, the enzyme is a ligase, *e.g.*, a thermal stable ligase. In an example, the covalent linking comprises ligase thermocycling.

In an example, the covalent linking, *e.g.*, ligation, comprises: (a) heating the isolated linkage reaction site, *e.g.*, the linkage micro-chamber, under conditions (*e.g.*, at 95°C) that allow denaturation of the HC strand and the LC strand; (b) cooling the isolated linkage reaction site, *e.g.*, the linkage micro-chamber, under conditions (*e.g.*, at 50-65°C) that allow hybridization of the splint oligonucleotide to the HC strand and the LC strand; (c) maintaining the isolated linkage reaction site, *e.g.*, the linkage micro-chamber, under conditions (*e.g.*, at 45-65°C) that allow ligation of the HC strand and the LC strand (*e.g.*, formation of phosphodiester bond between the HC strand and the LC strand); and (d) repeating steps (a), (b), and (c) sequentially for 2, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, or more cycles.

In an example, the HC strand and the LC strand are covalently linked, *e.g.*, ligated, in the presence of a splint oligonucleotide. In an example, the splint oligonucleotide is hybridized to a sequence comprising the junction of the HC strand and the LC strand, or a sequence complementary thereof, and forms a duplexed region at the site of ligation. In an example, the splint oligonucleotide comprises a modification (*e.g.*, an NH₂ group) that inhibits DNA synthesis, *e.g.*, by a DNA polymerase. In an example, the modification is at the 3' end of the splint oligonucleotide.

In an example, a strand complimentary to the covalently linked, *e.g.*, ligated, HC and LC strands is produced by amplification.

In an example, the method, *e.g.*, the step of covalent linkage, does not include a step of overlap extension polymerase chain reaction (OE-PCR), also known as splicing by overlap extension or splicing by overhang extension (SOE) PCR.

In an example, the method further comprises, prior to acquiring the isolated production reaction site, *e.g.*, a production micro-chamber, acquiring an mRNA loaded capture substrate.

In an example, acquiring the mRNA loaded capture substrate comprising: a) acquiring an isolated cell reaction site, *e.g.*, a cell isolation micro-chamber, 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, *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 HCVR or an LCVR from a cell other than the cell (*e.g.*, a different cell).

In an example, the isolated cell reaction site, *e.g.*, cell isolation micro-chamber, comprises a lysing reagent, *e.g.*, a detergent. In an example, the cell is lysed by heat or an enzyme. In an example, the capture substrate comprises a moiety (*e.g.*, an oligonucleotide) which binds mRNA, *e.g.*, an oligo(dT).

In an example, the method further comprises releasing the mRNA loaded capture substrate from the isolated cell reaction site, *e.g.*, the cell isolation micro-chamber. In an example, the releasing step is performed in the presence of a poly(dA) or poly(dT) oligonucleotide, *e.g.*, to reduce cross-binding of non-captured mRNA.

In an example, the mRNA loaded capture substrate is transferred from the isolated cell reaction site, *e.g.*, the cell isolation micro-chamber, to the isolated production reaction site, *e.g.*, the production micro-chamber.

In an example, the method further comprises releasing the nucleic acid sequence from the isolated production reaction site, *e.g.*, the production micro-chamber. In an example, the method further comprises amplifying the nucleic acid sequence. In an example, amplification of the nucleic acid sequence occurs outside the isolated production reaction site, *e.g.*, the production micro-chamber, *e.g.*, after the nucleic acid is released from the isolated production reaction site, *e.g.*, the production micro-chamber. In an example, amplification of the nucleic acid sequence occurs at the isolated production reaction site, *e.g.*, the production micro-chamber.

In an example, the method further comprises sequencing all or a portion of the nucleic acid sequence.

In an example, the method further comprises inserting all or a portion of nucleic acid sequence into a vector. In an example, the vector supplies an additional HC element or LC element not included in the nucleic acid sequence. In an example, the vector supplies an HC CDR1, an HC CDR2, or both. In an example, the method further comprises expressing the vector.

In an example, the method further comprises expressing the nucleic acid sequence to produce a polypeptide comprising a segment that encodes an HC element of the HCVR, *e.g.*, an HCVRs, and a segment that encodes an LC element of the LCVR, *e.g.*, an LCVRs. In an example, the LC element is N-terminal to the HC element in the polypeptide. In an example, the HC element is C-terminal to the LC element in the polypeptide.

In an example, the method further comprises contacting the polypeptide with an antigen. In an example, the method further comprises determining if the polypeptide binds the antigen, *in vitro*, *ex vivo*, or *in vivo*, *e.g.*, by a method or assay described herein.

In an example, 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;
- b) maintaining the isolated cell reaction site, *e.g.*, the cell isolation micro-chamber, under
10 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);
- c) contacting the mRNA loaded capture substrate with a reaction mixture, *e.g.*, a reaction
15 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);
- 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,
20 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.

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

In an example, 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
35 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:

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;

5 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 HCVR or an LCVR from a cell other than the cell (*e.g.*, a different cell);

10 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 HCVR from a cell; and a second ds

15 cDNA comprising a strand complementary to a second mRNA encoding an LCVR 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 an LCVR or an HCVR from a cell other than the cell (*e.g.*, a different cell).

20 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 HC ds cDNAs comprising a segment that encodes an HC element of the HCVR from the cell, *e.g.*, an 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;

25 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 HC ds cDNA (HC strand) to a strand of the LC ds cDNA (LC strand), wherein the HC and LC strands are both sense strands or antisense strands; and

 f) amplifying the covalently linked, *e.g.*, ligated, HC and LC strands.

30 In an example, 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 example, each of the steps a)-f) is performed in accordance with a method described herein.

 In an example, the disclosure features a method of making a library comprising a plurality of

35 unique members, the method comprising:

 making the plurality of members, 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), 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 example, 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 example, 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 example, 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 example, 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 example, 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 example, 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 example, the disclosure features a library made by the method as disclosed herein, optionally, wherein the library is a display library. In an example, the library is a display library. In an example, 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 example, the library is a phage display library. In an example, the library is a yeast display library. In an example, the library is a mammalian display library.

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

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

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

In an example, 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 HCVR and/or an LCVR. In an example, 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 example, the universal priming sequence is different from the sequence that is complementary to at least a portion of the first member. In an example, the second member comprises a sequence for homologous recombination (*e.g.*, in a yeast or mammalian cell).

In another example, 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 example, 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 example, 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 example, 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 HC strand and the LC strand, or a sequence complementary thereof, to form a duplexed region at the site of ligation.

15

In an example, 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 example, the disclosure features a method of making a nucleic acid sequence comprising a sequence that encodes an α chain element (AC element) of a 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 the BCVR are matched, the method comprising:

- 5 a) acquiring an isolated production reaction site, *e.g.*, a production micro-chamber, 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, *e.g.*, an α chain variable region sequence (ACVRS); and
 - 10 ii) a β chain (BC) strand, wherein the BC strand is a strand of a β chain ds cDNA (BC ds cDNA) comprising a segment that encodes a BC element of the BCVR from the cell, *e.g.*, a β chain variable region sequence (BCVRS), and
- b) covalent linking, *e.g.*, ligation, of the first strand to the second strand, wherein the isolated production reaction site, *e.g.*, a production micro-chamber, does not
- 15 include a nucleic acid encoding an ACVR or a BCVR from a cell other than the cell (*e.g.*, a different cell, *e.g.*, a different T cell),

thereby 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 the BCVR are matched.

In an example, the AC element comprises, or consists of, an ACVRS, or a functional

20 fragment thereof (*e.g.*, an antigen binding fragment thereof). In an example, the BC element comprises, or consists of, a BCVRS, or a functional fragment thereof (*e.g.*, an antigen binding fragment thereof).

In an example, the AC ds cDNA comprises a segment that encodes an ACVRS. In an example, the BC ds cDNA comprises a segment that encodes a BCVRS. In an example, the AC ds

25 cDNA comprises a segment that encodes an ACVRS, and the BC ds cDNA comprises a segment that encodes a BCVRS.

In an example, the cell is an immune cell, *e.g.*, a T cell, *e.g.*, a human T cell. In an example, the cell is a mammalian cell or an avian cell.

In an example, the nucleic acid sequence is configured such that, when expressed, the AC

30 element and the BC element (*e.g.*, the ACVRS and 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 example, the antigen binding molecule, *e.g.*, a TCR α chain and/or a β chain, is functional *in vitro*, *ex vivo*, or *in vivo*, *e.g.*, as determined by a method or assay described herein.

In an example, acquiring an isolated production reaction site, *e.g.*, a production micro-

35 chamber, 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 an ACVR from a cell; and (ii) a second ds cDNA comprising a strand complementary to a second mRNA encoding a BCVR from the cell (the cDNA loaded capture substrate), and

5 b) 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.

10 In an example, the AC ds cDNA is identical, or substantially identical, to the first ds cDNA. For example, the sense strand of the AC 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 AC 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
15 nucleotides from, the antisense strand of the first ds cDNA.

 In an example, the BC ds cDNA is identical, or substantially identical, to the second ds cDNA. For example, the sense strand of the BC 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 BC ds cDNA is at least 80%, 85%,
20 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 example, the AC strand is a sense strand. In an example, the BC strand is a sense strand. In an example, the AC strand is an antisense strand. In an example, the BC strand is an antisense strand. In an example, both the AC strand and the BC strand are sense strands. In an example, both the AC strand and the
25 BC strand are antisense strands.

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

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

 In an example, the isolated production reaction site, *e.g.*, the production micro-chamber, comprises:
35 a reagent mixture suitable for producing, from the first and second mRNAs (*e.g.*, after the

first and second mRNAs are released from the loaded mRNA capture substrate into a solution), a first cDNA comprising a segment that encodes an AC element of the ACVR of the cell, *e.g.*, an ACVRS, and a second cDNA comprising a segment that encodes a BC element of the BCVR of the cell, *e.g.*, a BCVRS.

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

10 In an example, a cDNA strand that is complementary to a first mRNA that encodes an ACVR from a cell is made by reverse transcription of the first mRNA. In an example, a cDNA strand that is complementary to a second mRNA that encodes a BCVR from a cell is made by reverse transcription of the second mRNA.

15 In an example, the reverse transcription takes place in the isolated production reaction site, *e.g.*, a production-micro chamber. In an example, the reverse transcription takes place in an isolated cell reaction site, *e.g.*, a cell isolation micro-chamber. In an example, the reverse transcription takes place outside the isolated production reaction site, *e.g.*, a production micro-chamber, or outside an isolated cell reaction site, *e.g.*, a cell isolation micro-chamber. In an example, the reverse transcription takes place outside the isolated production reaction site, *e.g.*, a production-micro chamber, and outside an isolated cell reaction site, *e.g.*, a cell isolation micro-chamber. In an example, the reverse transcription takes place outside an isolated reaction site, *e.g.*, outside a micro-chamber.

20 In an example, the amplification comprises 20 or fewer cycles, *e.g.*, 15 or fewer, 14 or fewer, 13 or fewer, 12 or fewer, 11 or fewer, 10 or fewer, 9 or fewer, 8 or fewer, 7 or fewer, 6 or fewer, or 5 or fewer cycles.

25 In an example, the reverse transcription and/or amplification uses one or more primers, *e.g.*, comprising a sequence specific for an ACVRS and/or a BCVRs.

 In an example, the reverse transcription and/or amplification comprises using two or more primers that mediate the production of the AC ds cDNA, wherein at least one primer comprises a nucleotide modification, and wherein at least one primer does not comprise a nucleotide modification.

30 In an example, the amplification comprises using two or more primers that mediate the production of the BC ds cDNA, wherein at least one primer comprises a nucleotide modification, and wherein at least one primer does not comprise a nucleotide modification.

35 In an example, at least one primer comprises a nucleotide modification, *e.g.*, which reduces, *e.g.*, inhibits, DNA synthesis, *e.g.*, by a DNA polymerase. In an example, at least one primer does not comprise a nucleotide modification, *e.g.*, which reduces, *e.g.*, inhibits, DNA synthesis, *e.g.*, by a DNA polymerase.

In an example, the nucleotide modification inhibits a DNA polymerase from extending the DNA. Without wishing to be bound by theory, it is believed that in an example, any chemical entity that reduces (*e.g.*, blocks) DNA polymerase extension can be used in accordance with the methods described herein.

In an example, the nucleotide modification is an insertion of a spacer to the primer, *e.g.*, between two adjacent nucleotides in the primer. In an example, the spacer is a flexible spacer. In an example, the spacer is a carbon spacer (*e.g.*, $-(CH_2)_n-$, wherein $n=3, 4, 5, 6, 7, 8, 9, 10$, or more), two or more (*e.g.*, three, four, five, six, seven, eight, nine, ten, or more) abasic nucleotides, or a polyethylene glycol (PEG) spacer. In an example, the spacer is a PEG spacer. In an example, the nucleotide modification is 2'-O-methyl, 2'-OH, 2'-NH₂, or uracil, *e.g.*, to a ribose.

In an example, the nucleotide modification is located internally or at the 3' end of the primer. In an example, at least one primer comprises (i) a first member; (ii) a second member; and optionally (iii) a third member, *e.g.*, comprising a nucleotide modification described herein, *e.g.*, located between (i) and (ii).

In an example, the first member is capable of annealing with the second member. In an example, the first member is capable of annealing with the second member in the same primer, *e.g.*, through intra-molecular hybridization, *e.g.*, to form 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 another example, the first member is capable of annealing hybridizing with the second member in a different primer, *e.g.*, through inter-molecular hybridization, *e.g.*, to form 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. Without wishing to be bound by theory, it is believed that in an example, there are at least two secondary structures that the modified primers can form and facilitate reduction (*e.g.*, prevention) of competition to substrate (*e.g.*, bead) capture. For example, the secondary structure can be a hairpin-like structure formed by intra-molecular hybridization (within the same primer), or the secondary structure can be a duplex structure formed by inter-molecular hybridization (between two different primers).

In an example, the first member comprises a sequence that is complementary to the sequence of an oligonucleotide attached to the capture substrate. In an example, 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 example, 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 example, the universal priming sequence is different from the sequence that is complementary to at least a portion of the first member. In an example, the second member comprises a sequence for homologous recombination (*e.g.*, in a yeast or mammalian cell).

In an example, at least one primer comprises a sequence encoding at least a portion of a linker sequence, or a complementary sequence thereof. In an example, the primer that comprises a sequence encoding at least a portion of a linker sequence, or a complementary sequence thereof, is phosphorylated, *e.g.*, 5' phosphorylated. Without wishing to be bound by theory, it is believed that in an example, any sequence with the general properties of flexibility (*e.g.*, facilitated by glycine) and hydrophilicity can work effectively in accordance with the methods described herein. Exemplary linkers can generally have overrepresentation of one or more of Gly, Ser, Thr, or Ala and underrepresentation of hydrophobic residues, *e.g.*, one or more of Trp, Tyr, Phe, Cys, Met, Leu, or Ile. The length of the primer may vary, *e.g.*, 3-50 amino acid residues (*e.g.*, 5-45, 10-40, 15-35, 20-30, 10-20, 10-30, 20-40, or 30-40 amino acid residues). In an example, the linker sequence comprises, or consists of, ((Gly)*m*-Ser)*n*, where *m*=3, 4, 5, or more and *n*=1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more. In an example, the linker sequence comprises, or consists of, (Gly-Gly-Gly-Ser)*n*, where *n*=1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more.

In an example, the primer is a primer described herein, *e.g.*, in Examples.

In an example, the reverse transcription, the amplification, or both, occurs in a solution in the isolated production reaction site, *e.g.*, production micro-chamber. In an example, the reverse transcription, the amplification, or both, does not occur on the substrate (*e.g.*, bead). For example, the reverse transcription, the amplification, or both, can occur on in a solution within a droplet.

In an example, the AC ds cDNA comprises a 5' overhang, *e.g.*, a 5' overhang that is capable of hybridizing to an oligonucleotide attached to a capture substrate. In an example, the AC ds cDNA comprises a blunt end, *e.g.*, a blunt end comprising a 5' phosphate. In an example, the BC ds cDNA comprises a 5' overhang, *e.g.*, a 5' overhang that is capable of hybridizing to an oligonucleotide attached to a capture substrate. In an example, the BC ds cDNA comprises a blunt end, *e.g.*, a blunt end comprising a 5' phosphate. In an example, the AC ds cDNA and the BC ds cDNA comprise sticky ends, *e.g.*, both have 5' overhangs.

In an example, the AC strand and the BC strand are covalently linked, *e.g.*, ligated, to produce a single stranded nucleic acid sequence, wherein the AC and BC strands are both sense strands or both antisense strands. In an example, a denatured AC strand of the AC ds cDNA to a denatured BC strand of the BC ds cDNA are covalently linked, *e.g.*, ligated, wherein the AC and BC strands are both sense strands or both antisense strands. In an example, the AC strand is present in the AC ds cDNA and the BC strand is present in the BC ds cDNA, and wherein the AC ds cDNA and the BC ds cDNA are covalently linked, *e.g.*, ligated, *e.g.*, to produce a double stranded nucleic acid sequence.

In an example, the covalent linking, *e.g.*, ligation, occurs in the isolated production reaction site. In an example, the isolated production reaction site, *e.g.*, a production micro-chamber, or the isolated linkage reaction site, *e.g.*, a linkage micro-chamber, comprises a reagent that is capable of covalently linking, *e.g.*, ligating, the AC and BC strands or the AC and BC ds cDNAs.

In an example, the isolated production reaction site, *e.g.*, a production micro-chamber comprises an enzyme that covalently couples the AC and BC strands or the AC and BC ds cDNAs. In an example, the enzyme is a ligase, *e.g.*, a thermal stable ligase. In an example, the covalent linking comprises ligase thermocycling.

5 In an example, the covalent linking, *e.g.*, ligation, occurs in a site different from the isolated production reaction site, *e.g.*, occurs in an isolated linkage reaction site, *e.g.*, a linkage micro-chamber. In an example, the AC strand and the BC strand are transferred from the isolated production site to the isolated linkage reaction site, *e.g.*, a linkage micro-chamber, and the covalent linking occurs in the isolated linkage reaction site, *e.g.*, a linkage micro-chamber. In an example, the isolated linkage reaction site, *e.g.*, a linkage micro-chamber,
10 comprises a reagent that is capable of covalently linking, *e.g.*, ligating, the AC and BC strands or the AC and BC ds cDNAs. In an example, the isolated linkage reaction site, *e.g.*, a linkage micro-chamber, comprises an enzyme that covalently couples the AC and BC strands or the AC and BC ds cDNAs. In an example, the enzyme is a ligase, *e.g.*, a thermal stable ligase. In an example, the covalent linking comprises ligase thermocycling.

15 In an example, the covalent linking, *e.g.*, ligation, comprises: (a) heating the isolated linkage reaction site, *e.g.*, the linkage micro-chamber, under conditions (*e.g.*, at 95°C) that allow denaturation of the AC strand and the BC strand; (b) cooling the isolated linkage reaction site, *e.g.*, the linkage micro-chamber, under conditions (*e.g.*, at 50-65°C) that allow hybridization of the splint oligonucleotide to the AC strand and the BC strand; (c) maintaining the isolated linkage reaction site, *e.g.*, the linkage micro-chamber, under conditions (*e.g.*,
20 at 45-65°C) that allow ligation of the AC strand and the BC strand (*e.g.*, formation of phosphodiester bond between the AC strand and the BC strand); and (d) repeating steps (a), (b), and (c) sequentially for 2, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, or more cycles.

In an example, the AC strand and the BC strand are covalently linked, *e.g.*, ligated, in the presence of a splint oligonucleotide. In an example, the splint oligonucleotide is hybridized to a sequence comprising the
25 junction of the AC strand and the BC strand, or a sequence complementary thereof, and forms a duplexed region at the site of ligation. In an example, the splint oligonucleotide comprises a modification (*e.g.*, an NH₂ group) that inhibits DNA synthesis, *e.g.*, by a DNA polymerase. In an example, the modification is at the 3' end of the splint oligonucleotide.

30 In an example, a strand complimentary to the covalently linked, *e.g.*, ligated, AC and BC strands is produced by amplification.

In an example, the method, *e.g.*, the step of covalent linkage, does not include a step of overlap extension polymerase chain reaction (OE-PCR), also known as splicing by overlap extension or splicing by overhang extension (SOE) PCR.

In an example, the method further comprises, prior to acquiring the isolated production reaction site,
35 *e.g.*, a production micro-chamber, acquiring an mRNA loaded capture substrate.

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

In an example, the isolated cell reaction site, *e.g.*, cell isolation micro-chamber, comprises a lysing reagent, *e.g.*, a detergent. In an example, the cell is lysed by heat or an enzyme. In an example, the capture substrate comprises a moiety (*e.g.*, an oligonucleotide) which binds mRNA, *e.g.*, an oligo(dT).

In an example, the method further comprises releasing the mRNA loaded capture substrate from the isolated cell reaction site, *e.g.*, the cell isolation micro-chamber. In an example, the releasing step is performed in the presence of a poly(dA) or poly(dT) oligonucleotide, *e.g.*, to reduce cross-binding of non-captured mRNA.

In an example, the mRNA loaded capture substrate is transferred from the isolated cell reaction site, *e.g.*, the cell isolation micro-chamber, to the isolated production reaction site, *e.g.*, the production micro-chamber.

In an example, the method further comprises releasing the nucleic acid sequence from the isolated production reaction site, *e.g.*, the production micro-chamber. In an example, the method further comprises amplifying the nucleic acid sequence. In an example, amplification of the nucleic acid sequence occurs outside the isolated production reaction site, *e.g.*, the production micro-chamber, *e.g.*, after the nucleic acid is released from the isolated production reaction site, *e.g.*, the production micro-chamber. In an example, amplification of the nucleic acid sequence occurs at the isolated production reaction site, *e.g.*, the production micro-chamber.

In an example, the method further comprises sequencing all or a portion of the nucleic acid sequence.

In an example, the method further comprises inserting all or a portion of nucleic acid sequence into a vector. In an example, the vector supplies an additional AC element or BC element not included in the nucleic acid sequence. In an example, the method further comprises expressing the vector.

In an example, the method further comprises expressing the nucleic acid sequence to produce a polypeptide comprising a segment that encodes an AC element of the ACVR, *e.g.*, an ACVRS, and a segment that encodes a BC element of the BCVR, *e.g.*, a BCVRS. In an example, the BC element is N-terminal to the AC element in the polypeptide. In an example, the AC element is C-terminal to the BC element in the polypeptide.

In an example, the method further comprises contacting the polypeptide with an antigen. In an example, the method further comprises determining if the polypeptide binds the antigen, *in vitro*, *ex vivo*, or *in vivo*, *e.g.*, by a method or assay described herein.

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In an example, the disclosure features a method of making a nucleic acid sequence comprising a sequence that encodes a TCR α chain element (AC element) of 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:

10 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;

15 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 ACVR or a BCVR from a cell other than the cell (*e.g.*, a different cell);

20 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);

25 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 TCR α chain (AC) strand, wherein the AC strand is a strand of a TCR α chain double-stranded cDNA (AC ds cDNA) comprising a segment that encodes an AC element of the ACVR from the cell, *e.g.*, a TCR α chain variable region sequence (ACVRS); and ii) a TCR β chain (BC) strand, wherein the BC strand is a strand of a TCR β chain double-stranded cDNA (BC ds cDNA) comprising a segment that encodes a BC element of the BCVR from the cell, *e.g.*, a TCR β chain variable region sequence (BCVRS),

30 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); and

e) covalent linking, *e.g.*, ligation, of the AC strand to the BC strand.

35 In an example, 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 example, each of the steps a)-e) is performed in accordance with a method described herein.

In an example, 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 example, 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 example, each of the steps a)-f) is performed in accordance with a method described herein.

In an example, 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 example, 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 example, 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 example, 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 example, 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 example, 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).

In an example, 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 example, the library is a display library. In an example, 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 example, the library is a phage display library. In an example, the library is a yeast display library. In an example, the library is a mammalian display library.

In an example, 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.

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

In an example, 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 example, 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.

In an example, 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.

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

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

In an example, 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 example, 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 example, the universal priming sequence is different from the sequence that is complementary to at least a portion of the first member. In an example, the second member comprises a sequence for homologous recombination (*e.g.*, in a yeast or mammalian cell).

In an example, 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 example, 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 example, 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 example, 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 example, the disclosure features a method of making a nucleic acid sequence comprising a sequence that encodes a 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 the DCVR are matched, the method comprising:

a) acquiring an isolated production reaction site, *e.g.*, a production micro-chamber, comprising:

i) an γ chain (GC) strand, wherein the GC strand is a strand of an γ chain double-stranded cDNA (GC ds cDNA) comprising a segment that encodes a GC element of the GCVR from a cell, *e.g.*, an γ chain variable region sequence (GCVRS); and

ii) a δ chain (DC) strand, wherein the DC strand is a strand of a δ chain ds cDNA (DC ds cDNA) comprising a segment that encodes a DC element of the DCVR from the cell, *e.g.*, a δ chain variable region sequence (DCVRS), and

b) covalent linking, *e.g.*, ligation, of the first strand to the second strand, 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, *e.g.*, a different T cell),

thereby 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 the DCVR are matched.

In an example, the GC element comprises, or consists of, a GCVRS, or a functional fragment thereof (*e.g.*, an antigen binding fragment thereof). In an example, the DC element comprises, or consists of, a DCVRS, or a functional fragment thereof (*e.g.*, an antigen binding fragment thereof).

In an example, the GC ds cDNA comprises a segment that encodes a GCVRS. In an example, the DC ds cDNA comprises a segment that encodes a DCVRS. In an example, the GC ds cDNA comprises a segment that encodes a GCVRS, and the DC ds cDNA comprises a segment that encodes a DCVRS.

In an example, the cell is an immune cell, *e.g.*, a T cell, *e.g.*, a human T cell. In an example, the cell is a mammalian cell or an avian cell.

In an example, the nucleic acid sequence is configured such that, when expressed, the GC element and the DC element (*e.g.*, the GCVRS and 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 an example, the antigen binding molecule, *e.g.*, a TCR γ chain and/or a δ chain, is functional *in vitro*, *ex vivo*, or *in vivo*, *e.g.*, as determined by a method or assay described herein.

In an example, acquiring an isolated production reaction site, *e.g.*, a production micro-chamber, 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 a GCVR from a cell; and (ii) a second ds cDNA comprising a strand complementary to a second mRNA encoding a DCVR from the cell (the cDNA loaded capture substrate), and

b) 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.

In an example, the GC ds cDNA is identical, or substantially identical, to the first ds cDNA. For example, the sense strand of the GC 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 GC 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 example, the DC ds cDNA is identical, or substantially identical, to the second ds cDNA. For example, the sense strand of the DC 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 DC 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 example, the GC strand is a sense strand. In an example, the DC strand is a sense strand. In an example, the GC strand is an antisense strand. In an example, the DC strand is an antisense strand. In an example, both the GC strand and the DC strand are sense strands. In an example, both the GC strand and the DC strand are antisense strands.

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

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

In an example, 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 cDNA comprising a segment that encodes a GC element of the GCVR of the cell, *e.g.*, a GCVRS, and
5 a second cDNA comprising a segment that encodes a DC element of the DCVR of the cell, *e.g.*, a DCVRS.

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

In an example, a cDNA strand that is complementary to a first mRNA that encodes a GCVR from a cell is made by reverse transcription of the first mRNA. In an example, a cDNA strand that is complementary to a second mRNA that encodes a DCVR from a cell is made by reverse transcription of the second mRNA.

In an example, the reverse transcription takes place in the isolated production reaction site, *e.g.*, a production-micro chamber. In an example, the reverse transcription takes place in an isolated cell reaction site, *e.g.*, a cell isolation micro-chamber. In an example, the reverse transcription takes place outside the isolated production reaction site, *e.g.*, a production micro-chamber, or outside an isolated cell reaction site, *e.g.*, a cell isolation micro-chamber. In an example, the reverse
15 transcription takes place outside the isolated production reaction site, *e.g.*, a production-micro chamber, and outside an isolated cell reaction site, *e.g.*, a cell isolation micro-chamber. In an example, the reverse transcription takes place outside an isolated reaction site, *e.g.*, outside a micro-chamber.
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In an example, the amplification comprises 20 or fewer cycles, *e.g.*, 15 or fewer, 14 or fewer, 13 or fewer, 12 or fewer, 11 or fewer, 10 or fewer, 9 or fewer, 8 or fewer, 7 or fewer, 6 or fewer, or 5 or fewer cycles.
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In an example, the reverse transcription and/or amplification uses one or more primers, *e.g.*, comprising a sequence specific for a GCVRS and/or a DCVRS.

In an example, the reverse transcription and/or amplification comprises using two or more primers that mediate the production of the GC ds cDNA, wherein at least one primer comprises a nucleotide modification, and wherein at least one primer does not comprise a nucleotide modification. In an example, the amplification comprises using two or more primers that mediate the production of the DC ds cDNA, wherein at least one primer comprises a nucleotide modification, and wherein at least one primer does not comprise a nucleotide modification.
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In an example, at least one primer comprises a nucleotide modification, *e.g.*, which reduces, *e.g.*, inhibits, DNA synthesis, *e.g.*, by a DNA polymerase. In an example, at least one
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primer does not comprise a nucleotide modification, *e.g.*, which reduces, *e.g.*, inhibits, DNA synthesis, *e.g.*, by a DNA polymerase.

In an example, the nucleotide modification inhibits a DNA polymerase from extending the DNA. Without wishing to be bound by theory, it is believed that in an example, any chemical entity that reduces (5 *e.g.*, blocks) DNA polymerase extension can be used in accordance with the methods described herein.

In an example, the nucleotide modification is an insertion of a spacer to the primer, *e.g.*, between two adjacent nucleotides in the primer. In an example, the spacer is a flexible spacer. In an example, the spacer is a carbon spacer (*e.g.*, $-(CH_2)_n-$, wherein $n=3, 4, 5, 6, 7, 8, 9, 10$, or more), two or more (*e.g.*, three, four, five, six, seven, eight, nine, ten, or more) abasic nucleotides, or a polyethylene glycol (PEG) spacer. In 10 an example, the spacer is a PEG spacer. In an example, the nucleotide modification is 2'-O-methyl, 2'-OH, 2'-NH₂, or uracil, *e.g.*, to a ribose.

In an example, the nucleotide modification is located internally or at the 3' end of the primer. In an example, at least one primer comprises (i) a first member; (ii) a second member; and optionally (iii) a third member, *e.g.*, comprising a nucleotide modification described herein, *e.g.*, located between (i) and (ii).

15 In an example, the first member is capable of annealing with the second member. In an example, the first member is capable of annealing with the second member in the same primer, *e.g.*, through intra-molecular hybridization, *e.g.*, to form 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 another example, the first member is capable of annealing hybridizing with the second member in a different primer, *e.g.*, through inter-molecular 20 hybridization, *e.g.*, to form 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. Without wishing to be bound by theory, it is believed that in an example, there are at least two secondary structures that the modified primers can form and facilitate reduction (*e.g.*, prevention) of competition to substrate (*e.g.*, bead) capture. For example, the secondary structure can be a hairpin-like structure formed by intra-molecular hybridization (within the same primer), or 25 the secondary structure can be a duplex structure formed by inter-molecular hybridization (between two different primers).

In an example, the first member comprises a sequence that is complementary to the sequence of an oligonucleotide attached to the capture substrate. In an example, 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) 30 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 example, 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 example, the universal priming sequence is different from the sequence that is complementary to

at least a portion of the first member. In an example, the second member comprises a sequence for homologous recombination (*e.g.*, in a yeast or mammalian cell).

In an example, at least one primer comprises a sequence encoding at least a portion of a linker sequence, or a complementary sequence thereof. In an example, the primer that comprises a sequence encoding at least a portion of a linker sequence, or a complementary sequence thereof, is phosphorylated, *e.g.*, 5' phosphorylated. Without wishing to be bound by theory, it is believed that in an example, any sequence with the general properties of flexibility (*e.g.*, facilitated by glycine) and hydrophilicity can work effectively in accordance with the methods described herein. Exemplary linkers can generally have overrepresentation of one or more of Gly, Ser, Thr, or Ala and underrepresentation of hydrophobic residues, *e.g.*, one or more of Trp, Tyr, Phe, Cys, Met, Leu, or Ile. The length of the primer may vary, *e.g.*, 3-50 amino acid residues (*e.g.*, 5-45, 10-40, 15-35, 20-30, 10-20, 10-30, 20-40, or 30-40 amino acid residues). In an example, the linker sequence comprises, or consists of, ((Gly)*m*-Ser)*n*, where *m*=3, 4, 5, or more and *n*=1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more. In an example, the linker sequence comprises, or consists of, (Gly-Gly-Gly-Gly-Ser)*n*, where *n*=1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more.

In an example, the primer is a primer described herein, *e.g.*, in Examples.

In an example, the reverse transcription, the amplification, or both, occurs in a solution in the isolated production reaction site, *e.g.*, production micro-chamber. In an example, the reverse transcription, the amplification, or both, does not occur on the substrate (*e.g.*, bead). For example, the reverse transcription, the amplification, or both, can occur on in a solution within a droplet.

In an example, the GC ds cDNA comprises a 5' overhang, *e.g.*, a 5' overhang that is capable of hybridizing to an oligonucleotide attached to a capture substrate. In an example, the GC ds cDNA comprises a blunt end, *e.g.*, a blunt end comprising a 5' phosphate. In an example, the DC ds cDNA comprises a 5' overhang, *e.g.*, a 5' overhang that is capable of hybridizing to an oligonucleotide attached to a capture substrate. In an example, the DC ds cDNA comprises a blunt end, *e.g.*, a blunt end comprising a 5' phosphate. In an example, the GC ds cDNA and the DC ds cDNA comprise sticky ends, *e.g.*, both have 5' overhangs.

In an example, the GC strand and the DC strand are covalently linked, *e.g.*, ligated, to produce a single stranded nucleic acid sequence, wherein the GC and DC strands are both sense strands or both antisense strands. In an example, a denatured GC strand of the GC ds cDNA to a denatured DC strand of the DC ds cDNA are covalently linked, *e.g.*, ligated, wherein the GC and DC strands are both sense strands or both antisense strands. In an example, the GC strand is present in the GC ds cDNA and the DC strand is present in the DC ds cDNA, and wherein the GC ds cDNA and the DC ds cDNA are covalently linked, *e.g.*, ligated, *e.g.*, to produce a double stranded nucleic acid sequence.

In an example, the covalent linking, *e.g.*, ligation, occurs in the isolated production reaction site. In an example, the isolated production reaction site, *e.g.*, a production micro-

chamber, or the isolated linkage reaction site, *e.g.*, a linkage micro-chamber, comprises a reagent that is capable of covalently linking, *e.g.*, ligating, the GC and DC strands or the GC and DC ds cDNAs. In an example, the isolated production reaction site, *e.g.*, a production micro-chamber comprises an enzyme that covalently couples the GC and DC strands or the GC and DC ds cDNAs. In an example, the enzyme is a ligase, *e.g.*, a thermal stable ligase. In an example, the covalent linking comprises ligase thermocycling.

In an example, the covalent linking, *e.g.*, ligation, occurs in a site different from the isolated production reaction site, *e.g.*, occurs in an isolated linkage reaction site, *e.g.*, a linkage micro-chamber. In an example, the GC strand and the DC strand are transferred from the isolated production site to the isolated linkage reaction site, *e.g.*, a linkage micro-chamber, and the covalent linking occurs in the isolated linkage reaction site, *e.g.*, a linkage micro-chamber. In an example, the isolated linkage reaction site, *e.g.*, a linkage micro-chamber, comprises a reagent that is capable of covalently linking, *e.g.*, ligating, the GC and DC strands or the GC and DC ds cDNAs. In an example, the isolated linkage reaction site, *e.g.*, a linkage micro-chamber, comprises an enzyme that covalently couples the GC and DC strands or the GC and DC ds cDNAs. In an example, the enzyme is a ligase, *e.g.*, a thermal stable ligase. In an example, the covalent linking comprises ligase thermocycling.

In an example, the covalent linking, *e.g.*, ligation, comprises: (a) heating the isolated linkage reaction site, *e.g.*, the linkage micro-chamber, under conditions (*e.g.*, at 95°C) that allow denaturation of the GC strand and the DC strand; (b) cooling the isolated linkage reaction site, *e.g.*, the linkage micro-chamber, under conditions (*e.g.*, at 50-65°C) that allow hybridization of the splint oligonucleotide to the GC strand and the DC strand; (c) maintaining the isolated linkage reaction site, *e.g.*, the linkage micro-chamber, under conditions (*e.g.*, at 45-65°C) that allow ligation of the GC strand and the DC strand (*e.g.*, formation of phosphodiester bond between the GC strand and the DC strand); and (d) repeating steps (a), (b), and (c) sequentially for 2, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, or more cycles.

In an example, the GC strand and the DC strand are covalently linked, *e.g.*, ligated, in the presence of a splint oligonucleotide. In an example, the splint oligonucleotide is hybridized to a sequence comprising the junction of the GC strand and the DC strand, or a sequence complementary thereof, and forms a duplexed region at the site of ligation. In an example, the splint oligonucleotide comprises a modification (*e.g.*, an NH₂ group) that inhibits DNA synthesis, *e.g.*, by a DNA polymerase. In an example, the modification is at the 3' end of the splint oligonucleotide.

In an example, a strand complimentary to the covalently linked, *e.g.*, ligated, GC and DC strands is produced by amplification.

In an example, the method, *e.g.*, the step of covalent linkage, does not include a step of overlap extension polymerase chain reaction (OE-PCR), also known as splicing by overlap extension or splicing by overhang extension (SOE) PCR.

In an example, the method further comprises, prior to acquiring the isolated production reaction site, *e.g.*, a production micro-chamber, acquiring an mRNA loaded capture substrate.

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

In an example, the isolated cell reaction site, *e.g.*, cell isolation micro-chamber, comprises a lysing reagent, *e.g.*, a detergent. In an example, the cell is lysed by heat or an enzyme. In an example, the capture substrate comprises a moiety (*e.g.*, an oligonucleotide) which binds mRNA, *e.g.*, an oligo(dT).

In an example, the method further comprises releasing the mRNA loaded capture substrate from the isolated cell reaction site, *e.g.*, the cell isolation micro-chamber. In an example, the releasing step is performed in the presence of a poly(dA) or poly(dT) oligonucleotide, *e.g.*, to reduce cross-binding of non-captured mRNA.

In an example, the mRNA loaded capture substrate is transferred from the isolated cell reaction site, *e.g.*, the cell isolation micro-chamber, to the isolated production reaction site, *e.g.*, the production micro-chamber.

In an example, the method further comprises releasing the nucleic acid sequence from the isolated production reaction site, *e.g.*, the production micro-chamber. In an example, the method further comprises amplifying the nucleic acid sequence. In an example, amplification of the nucleic acid sequence occurs outside the isolated production reaction site, *e.g.*, the production micro-chamber, *e.g.*, after the nucleic acid is released from the isolated production reaction site, *e.g.*, the production micro-chamber. In an example, amplification of the nucleic acid sequence occurs at the isolated production reaction site, *e.g.*, the production micro-chamber.

In an example, the method further comprises sequencing all or a portion of the nucleic acid sequence.

In an example, the method further comprises inserting all or a portion of nucleic acid sequence into a vector. In an example, the vector supplies an additional GC element or DC element not included in the nucleic acid sequence. In an example, the method further comprises expressing the vector.

In an example, the method further comprises expressing the nucleic acid sequence to produce a polypeptide comprising a segment that encodes a GC element of the GCVR, *e.g.*, a GCVRS, and a segment that encodes a DC element of the DCVR, *e.g.*, a DCVRS. In an example,

the DC element is N-terminal to the GC element in the polypeptide. In an example, the GC element is C-terminal to the DC element in the polypeptide.

In an example, the method further comprises contacting the polypeptide with an antigen. In an example, the method further comprises determining if the polypeptide binds the antigen, *in vitro*,
5 *ex vivo*, or *in vivo*, *e.g.*, by a method or assay described herein.

In an example, the disclosure features a method of making a nucleic acid sequence comprising a sequence that encodes a TCR γ chain element (GC element) of TCR γ chain variable region (GCVR) and a TCR δ chain element (DC element) of a TCR δ chain variable region (DCVR),
10 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;

15 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 a GCVR or a DCVR from a cell other than the cell (*e.g.*, a different cell);

20 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);

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 TCR γ chain (GC) strand, wherein the GC strand is a strand of a TCR γ chain double-stranded cDNA (GC ds cDNA) comprising a segment that encodes a GC element of the GCVR from the cell, *e.g.*, a TCR γ chain variable region sequence (GCVRS); and ii) a TCR δ chain (DC) strand, wherein the DC strand is a strand of a TCR δ chain double-stranded cDNA (DC ds cDNA) comprising a segment that encodes a DC element of the
30 DCVR from the cell, *e.g.*, a TCR δ chain variable region sequence (DCVRS),

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); and

e) covalent linking, *e.g.*, ligation, of the GC strand to the DC strand.

35 In an example, 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 example, each of the steps a)-e) is performed in accordance with a method described herein.

In an example, 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:

- 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 a GCVR from the cell and a second mRNA encoding a DCVR 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 a GCVR or a DCVR 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 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
20 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).
- 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 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;
- 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 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.

35 In an example, 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 example, each of the steps a)-f) is performed in accordance with a method described herein.

In an example, 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 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),

thereby making a library comprising a plurality of unique members.

10 In an example, 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 example, 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 example, 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 example, 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 example, 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 GCVRS, and a segment that encodes a DC element, *e.g.*, a DCVRS, 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 an GC element, *e.g.*, a GCVRS, and a segment that encodes a DC element, *e.g.*, a DCVRS, 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;

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;

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 example, 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 example, the disclosure features a library made by the method disclosed herein. In an example, the library is a display library. In an example, 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 example, the library is a phage display library. In an example, the library is a yeast display library. In an example, the library is a mammalian display library.

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

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

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

In an example, 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 example, 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 example, the universal priming sequence is different from the sequence that is complementary to at least a portion of the first member. In an example, the second member comprises a sequence for homologous recombination (*e.g.*, in a yeast or mammalian cell).

In an example, 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 example, 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 example, 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.

In an example, 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.

BRIEF DESCRIPTION OF THE DRAWINGS

5 **FIG. 1** depicts a number of ways of making 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 A1, B1 and C2 boxes indicate steps occurring in an isolated reaction site, particularly, in an isolated cell reaction site. The C3, D1, D2, D3, D4, D5 and D6 boxes
10 indicate steps occurring in an isolated reaction site, particularly, in an isolated production reaction site. The E1, E2 and E3 boxes indicate steps occurring in an isolated reaction site, particularly, in an isolated linkage reaction site. The C1 box indicates steps that need not occur in an isolated reaction site. As is discussed in the text, the isolated reaction sites are free of nucleic acid that would result in a mismatched HC and LC element.

15 **FIGS. 2A-2D** are a series of diagrams showing an exemplary method of making a nucleic acid 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. In **FIG. 2A**, a cell (*e.g.*, an immune cell, such as a B cell) is lysed and mRNAs encoding an HCVR and a matched LCVR are captured on a bead. In **FIG. 2B**, captured mRNAs are converted to cDNA by reverse transcription followed by
20 amplification by DNA polymerase (PCR) to create cDNA beads comprising matched pairs of HCVR and LCVR cDNAs. A self-annealing primer (*e.g.*, a primer comprising a first member and a second member capable of hybridizing to the first member, with the first and second members separated by a spacer, *e.g.*, a PEG spacer, and further comprising a sequence capable of hybridizing to an HCVR or
25 LCVR sequence) can be used for the reverse transcription reaction and/or DNA polymerase amplification. In **FIG. 2C**, matched LCVR and HCVR cDNA products can be fused using a ligase cycling reaction, in which matched pairs of LCVRs and HCVRs are brought together using a splint oligo comprising sequences capable of hybridizing to an end of each of the LCVR or HCVR sequences (*e.g.*, the 3' terminus of the LCVR and the 5' terminus of the HCVR). In **FIG. 2D**, the
30 fused LCVR/HCVR product can be amplified, *e.g.*, by PCR.

FIG. 3 is a polyacrylamide gel electrophoresis (PAGE) image showing that Taq ligase and Ampligase thermostable ligase (Amp; Lucigen) were capable of efficiently linking VH and VL product.

FIGS. 4A-4B are gel electrophoresis images showing that natively paired, linked VH + VL products for each of antibodies 4G2 and 9E10 were successfully produced by ligase cycling. In **FIG. 4A**,
35 denaturing PAGE of ligase cycling products showed that ligase-containing reactions yielded the linked VH + VL products for each of 4G2 and 9E10, as well as the individual VH and VL polynucleotides. The linked VH + VL products were not detected in reactions lacking ligase. In **FIG.**

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,

wherein the HC strand and the LC strand are both sense strands, or wherein the HC strand and the LC strand are both antisense strands; 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;

5 (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,

10 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

15 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
20 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;

25 (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,

30 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:

35 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

5 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;

10 (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

15 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:

20 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
25 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;

30 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

35 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. 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, wherein the HC strand and the LC strand are both sense strands, or wherein the HC strand and the LC strand are both antisense strands, 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, or more basepairs.

10. 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 both sense strands, or wherein the HC strand and the LC strand are both antisense strands, 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.