

SPATIAL TRANSCRIPTOMICS FOR ANTIGEN-RECEPTORS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Patent Application No.

- 5 63/033,568, filed on June 2, 2020. The contents of that application are incorporated herein by reference in its entirety.

BACKGROUND

- Cells within a tissue have differences in cell morphology and/or function due to varied analyte levels (e.g., gene and/or protein expression) within the different cells. The specific position of a cell within a tissue (e.g., the cell's position relative to neighboring cells or the cell's position relative to the tissue microenvironment) can affect, e.g., the cell's morphology, differentiation, fate, viability, proliferation, behavior, signaling, and cross-talk with other cells in the tissue.

- Spatial heterogeneity has been previously studied using techniques that typically provide data for a handful of analytes in the context of intact tissue or a portion of a tissue (e.g., tissue section), or provide significant analyte data from individual, single cells, but fails to provide information regarding the position of the single cells from the originating biological sample (e.g., tissue).

- Understanding spatial heterogeneity in the context of immune cell clonotypes (e.g., T-cell receptor, B-cell receptor) within an intact biological sample, or a portion thereof, can give insight into which cells or cell-types specific T-cell or B-cell clonotypes may be interacting. Single-cell methods can identify clonotype populations, but fail to link the spatial organization of immune cell clonotypes within a biological sample.

SUMMARY

- A fundamental understanding of spatial heterogeneity with respect to T-cell receptor (TCR) and B-cell receptor (BCR) clonotypes within a biological sample is needed to understand which cells a TCR or BCR may interacting with, the identity of TCR and/or BCR clonotypes in a given biological sample, or the identity of TCR and/or BCR clonotypes that are autoreactive in different autoimmune disorders. Numerous single-cell sequencing approaches can identify TCR and BCR clonotypes from a biological sample, however, at present methods are needed to link TCR and BCR sequences to spatial locations within a biological sample. Additionally, identifying the clonal regions, that is, regions defined by the

places where variable (V), diverse (D), and joining (J) segments join to form the complementarity determining regions, including CDR1, CDR2, and CDR3, which provide specificity to the TCRs and/or BCRs, is important in understanding the TCR and BCR biological interactions. By coupling clonal information to spatial information it is possible to understand which T-cell and B-cell clonotypes may be specifically interacting with given cell types within a biological sample.

Provided herein are methods for determining the presence and/or abundance of an immune cell clonotype at a location in a biological sample. Some embodiments of any of the methods described herein include capturing transcripts to identify an immune cell clonotype. Some embodiments of any of the methods herein include generating a nucleic acid library from captured transcripts. Some embodiments of any of the methods described herein include enriching analytes of interest in the nucleic acid library, including analytes to identify an immune cell clonotype.

Provided herein are methods for determining the presence and/or abundance of an immune cell receptor at a location in a biological sample. Some embodiments of any of the methods described herein include capturing analytes to identify an immune cell receptor. Some embodiments of any of the methods described herein include generating a nucleic acid library from captured analytes. Some embodiments of any of the methods described here include enriching analytes of interest in the nucleic acid library, including analytes to identify an immune cell receptor.

Thus provided herein are methods for determining the presence and/or abundance of an immune cell clonotype at a location in a biological sample, the method including: (a) contacting a biological sample with an array including a plurality of capture probes, where a capture probe of the plurality of capture probes includes (i) a spatial barcode and (ii) a capture domain that binds to a nucleic acid encoding an immune cell receptor of the immune cell clonotype; and (b) determining (i) all or a portion of the sequence of the spatial barcode or a complement thereof, and (ii) all or a portion of the sequence of the nucleic acid encoding the immune cell receptor or a complement thereof, and using the determined sequences of (i) and (ii) to determine the presence and/or abundance of the immune cell clonotype at a location in the biological sample.

In some embodiments, the immune cell clonotype is a T cell clonotype. In some embodiments, the T cell clonotype is a T cell receptor alpha chain. In some embodiments, the capture domain binds to a nucleic acid sequence encoding a constant region of the T cell receptor alpha chain. In some embodiments, step (b) includes determining a sequence

encoding CDR3 of the T cell receptor alpha chain. In some embodiments, step (b) includes determining a sequence encoding one or both of CDR1 and CDR2 of the T cell receptor alpha chain. In some embodiments, step (b) includes determining a sequence encoding a full-length variable domain of the T cell receptor alpha chain.

- 5 In some embodiments, the immune cell receptor is a T cell receptor beta chain. In some embodiments, the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the T cell receptor beta chain. In some embodiments, step (b) includes determining a sequence encoding CDR3 of the T cell receptor beta chain. In some embodiments, step (b) includes determining a sequence encoding one or both of CDR1 and
- 10 CDR2 of the T cell receptor beta chain. In some embodiments, step (b) includes determining a sequence encoding a full-length variable domain of the T cell receptor beta chain.

- In some embodiments, the immune cell clonotype is a B cell clonotype. In some embodiments, the B cell clonotype is an immunoglobulin kappa light chain. In some embodiments, the capture domain binds specifically to a nucleic acid sequence encoding a
- 15 constant region of the immunoglobulin kappa light chain. In some embodiments, step (b) includes determining a sequence encoding CDR3 of the immunoglobulin kappa light chain. In some embodiments, step (b) includes determining a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin kappa light chain. In some embodiments, step (b) includes determining a sequence encoding a full-length variable domain of the
- 20 immunoglobulin kappa light chain. In some embodiments, the B cell clonotype is an immunoglobulin lambda light chain. In some embodiments, the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin lambda light chain. In some embodiments, step (b) includes determining a sequence encoding CDR3 of the immunoglobulin lambda light chain. In some embodiments, step (b) includes
- 25 determining a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin lambda light chain. In some embodiments, step (b) includes determining a sequence encoding a full-length variable domain of the immunoglobulin lambda light chain. In some embodiments, the B cell clonotype is an immunoglobulin heavy chain. In some embodiments, the capture domain binds specifically to a nucleic acid sequence encoding a constant region
- 30 of the immunoglobulin heavy chain. In some embodiments, step (b) includes determining a sequence encoding CDR3 of the immunoglobulin heavy chain. In some embodiments, step (b) includes determining a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin heavy chain. In some embodiments, step (b) includes determining a sequence encoding a full-length variable domain of the immunoglobulin heavy chain.

In some embodiments, the capture domain binds a poly(A) sequence of a nucleic acid encoding an immune cell clonotype. In some embodiments, the capture domain binds to a nucleic acid sequence encoding a T cell clonotype. In some embodiments, the T cell clonotype is a T cell receptor alpha chain, a T cell receptor beta chain, and combinations thereof. In some embodiments, step (b) includes determining: a sequence encoding CDR3 of the T cell receptor alpha chain, a sequence encoding CDR3 of the T cell receptor beta chain, and combinations thereof. In some embodiments, step (b) includes determining: a sequence encoding one or both of CDR1 and CDR2 of the T cell receptor alpha chain, a sequence encoding one or both of CDR1 and CDR2 of the T cell receptor beta chain, and combinations thereof. In some embodiments, step (b) includes determining: a sequence encoding a full-length variable domain of the T cell receptor alpha chain, a sequence encoding a full-length variable domain of the T cell receptor beta chain, and combinations thereof.

In some embodiments, the capture domain binds to a nucleic acid encoding a B cell clonotype. In some embodiments, the B cell clonotype is an immunoglobulin kappa light chain, an immunoglobulin lambda light chain, an immunoglobulin heavy chain, and combinations thereof. In some embodiments, step (b) includes determining: a sequence encoding CDR3 of the immunoglobulin kappa light chain, a sequence encoding CDR3 of immunoglobulin lambda light chain, a sequence encoding CDR3 of the immunoglobulin heavy chain, and combinations thereof. In some embodiments, step (b) includes determining: a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin kappa light chain, a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin lambda light chain, a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin heavy chain, and combinations thereof. In some embodiments, step (b) includes determining: a sequence encoding a full-length variable domain of the immunoglobulin kappa light chain, a sequence encoding a full-length variable domain of the immunoglobulin lambda light chain, a sequence encoding a full-length variable domain of the immunoglobulin heavy chain, and combinations thereof.

In some embodiments, step (b) includes the capture probe using the nucleic acid encoding the immune cell receptor as a template, thereby generating an extended capture probe. In some embodiments, step (b) includes extending a 3' end of the capture probe.

In some embodiments, step (b) includes generating a second strand of nucleic acid that includes (i) a sequence that is complementary to all or a portion of the spatial barcode, and (ii) a sequence that corresponds to all or a portion of the sequence of the nucleic acid encoding the immune cell receptor.

In some embodiments, the capture probe includes a cleavage domain, a functional domain, a unique molecular identifier, or any combination thereof. In some embodiments, the capture probe includes a functional domain.

5 In some embodiments, step (b) includes generating a second strand of nucleic acid that includes (i) a sequence that is complementary to all or a portion of the functional domain, (ii) a sequence that is complementary to all or a portion of the spatial barcode, and (iii) a sequence that corresponds to all or a portion of the sequence of the nucleic acid encoding the immune cell receptor.

10 In some embodiments, the method includes enriching the nucleic acid encoding the immune cell receptor of the immune cell clonotype.

In some embodiments, enriching includes hybridizing a plurality of hybridization probes to the nucleic acid encoding the immune cell receptor of the immune cell clonotype, where a hybridization probe includes (i) a sequence complementary to a portion of the nucleic acid encoding the immune cell receptor and (ii) a binding moiety that interacts with a
15 capture moiety.

In some embodiments, the binding moiety includes biotin and the capture moiety includes streptavidin.

In some embodiments, enriching the nucleic acid encoding the immune cell receptor of the immune cell clonotype includes one or more blocking probes. In some embodiments,
20 the one or more blocking probes includes a sequence having at least 80% identity to SEQ ID NO: 639. In some embodiments, the one or more blocking probes includes a sequence having at least 80% identity to SEQ ID NO: 640.

In some embodiments, the method includes amplifying the nucleic acid encoding the immune cell receptor of the immune cell clonotype, or a complement thereof, using (i) a first
25 primer including all or a portion of the functional domain, where the functional domain is 5' to the spatial barcode, and (ii) a second primer including a sequence that is substantially complementary to a portion of a sequence encoding a variable region of the immune cell receptor.

In some embodiments, the method includes amplifying the nucleic acid encoding the
30 immune cell receptor of the immune cell clonotype, or a complement thereof, using (i) the first primer including all or a portion of the functional domain, where the functional domain is 5' to the spatial barcode, and (ii) a third primer including a sequence that is substantially complementary to a portion of the nucleic acid sequence encoding a variable region of the immune cell receptor, where the third primer is 5' to the second primer.

In some embodiments, the biological sample includes a tissue sample. In some embodiments, the tissue sample is a tissue section. In some embodiments, the tissue section is a fixed tissue section. In some embodiments, the fixed tissue section is a formalin-fixed paraffin-embedded tissue section. In some embodiments, the tissue section includes a tumor
5 region.

In some embodiments, the nucleic acid encoding the immune cell receptor includes RNA. In some embodiments, the RNA is mRNA. In some embodiments, the nucleic acid encoding the immune cell receptor includes DNA. In some embodiments, the DNA is genomic DNA.

10 In some embodiments, the method includes imaging the biological sample.

In some embodiments, the determining in step (b) includes sequencing (i) all or a portion of the sequence of the spatial barcode or a complement thereof, and (ii) all or a portion of the sequence of the nucleic acid encoding the immune cell receptor or a complement thereof.

15 In some embodiments, step (b) includes determining the presence of the immune cell clonotype at a location in the biological sample. In some embodiments, step (b) includes determining the abundance of the immune cell clonotype at a location in the biological sample. In some embodiments, step (b) includes determining the presence and abundance of the immune cell clonotype at a location in the biological sample. In some embodiments, step
20 (b) includes determining the presence of two or more immune cell clonotypes at a location in the biological sample. In some embodiments, step (b) includes determining the abundance of two or more immune cell clonotypes at a location in the biological sample. In some embodiments, step (b) includes determining the presence and abundance of two or more immune cell clonotypes at a location in the biological sample. In some embodiments, the
25 method includes comparing the two or more immune cell clonotypes. In some embodiments, the two or more immune cell clonotypes are each a B cell clonotype.

In some embodiments, the two or more immune cell clonotypes are each a T cell clonotype. In some embodiments, the two or more immune cell clonotypes comprise at least one T cell clonotype and at least one B cell clonotype.

30 Also provided herein are methods for determining the presence and/or abundance of an immune cell receptor at a location in a biological sample, the method including: (a) contacting a biological sample with an array including a plurality of capture probes, where a capture probe of the plurality of capture probes includes (i) a spatial barcode and (ii) a capture domain that specifically binds to a nucleic acid encoding an immune cell receptor;

and (b) determining (i) all or a portion of the sequence of the spatial barcode or a complement thereof, and (ii) all or a portion of the sequence of the nucleic acid encoding the immune cell receptor or a complement thereof, and using the determined sequences of (i) and (ii) to determine the presence and/or abundance of the immune cell receptor at a location in the
5 biological sample.

In some embodiments, the immune cell receptor is a T cell receptor alpha chain. In some embodiments, the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the T cell receptor alpha chain. In some embodiments, step (b) includes determining a sequence encoding CDR3 of the T cell receptor alpha chain. In some
10 embodiments, step (b) includes determining a sequence encoding one or both of CDR1 and CDR2 of the T cell receptor alpha chain. In some embodiments, step (b) includes determining a sequence encoding a full-length variable domain of the T cell receptor alpha chain. In some embodiments, the immune cell receptor is a T cell receptor beta chain. In some embodiments, the capture domain binds specifically to a nucleic acid sequence encoding a constant region
15 of the T cell receptor beta chain. In some embodiments, step (b) includes determining a sequence encoding CDR3 of the T cell receptor beta chain. In some embodiments, step (b) includes determining a sequence encoding one or both of CDR1 and CDR2 of the T cell receptor beta chain. In some embodiments, step (b) includes determining a full-length variable domain of the T cell receptor beta chain. In some embodiments, the immune cell
20 receptor is an immunoglobulin kappa light chain. In some embodiments, the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin kappa light chain. In some embodiments, step (b) includes determining a sequence encoding CDR3 of the immunoglobulin kappa light chain. In some embodiments, step (b) includes determining a sequence encoding one or both of CDR1 and CDR2 of the
25 immunoglobulin kappa light chain. In some embodiments, step (b) includes determining a sequence encoding a full-length variable domain of the immunoglobulin kappa light chain. In some embodiments, the immune cell receptor is an immunoglobulin lambda light chain. In some embodiments, the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin lambda light chain. In some embodiments,
30 step (b) includes determining a sequence encoding CDR3 of the immunoglobulin lambda light chain. In some embodiments, step (b) includes determining a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin lambda light chain. In some embodiments, step (b) includes determining a sequence encoding a full-length variable domain of the immunoglobulin lambda light chain. In some embodiments, the immune cell receptor is an

immunoglobulin heavy chain. In some embodiments, the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin heavy chain. In some embodiments, step (b) includes determining a sequence encoding CDR3 of the immunoglobulin heavy chain. In some embodiments, step (b) includes determining a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin heavy chain. In some embodiments, step (b) includes determining a sequence encoding a full-length variable domain of the immunoglobulin heavy chain.

In some embodiments, the capture domain binds a poly(A) sequence of a nucleic acid encoding an immune cell receptor. In some embodiments, the immune cell receptor is a T cell receptor alpha chain, a T cell receptor beta chain, and combinations thereof. In some embodiments, step (b) includes determining: a sequence encoding CDR3 of the T cell receptor alpha chain, a sequence encoding CDR3 of the T cell receptor beta chain, and combinations thereof. In some embodiments, step (b) includes determining: a sequence encoding one or both of CDR1 and CDR2 of the T cell receptor alpha chain, a sequence encoding one or both of CDR1 and CDR2 of the T cell receptor beta chain, and combinations thereof. In some embodiments, step (b) includes determining: a sequence encoding a full-length variable domain of the T cell receptor alpha chain, a sequence encoding a full-length variable domain of the T cell receptor beta chain, and combinations thereof.

In some embodiments, the immune cell receptor is a B cell receptor an immunoglobulin kappa light chain, an immunoglobulin lambda light chain, an immunoglobulin heavy chain, and combinations thereof. In some embodiments, step (b) includes determining: a sequence encoding CDR3 of the immunoglobulin kappa light chain, a sequence encoding CDR3 of immunoglobulin lambda light chain, a sequence encoding CDR3 of the immunoglobulin heavy chain, and combinations thereof. In some embodiments, step (b) includes determining: a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin kappa light chain, a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin lambda light chain, a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin heavy chain, and combinations thereof. In some embodiments, step (b) includes determining: a sequence encoding a full-length variable domain of the immunoglobulin kappa light chain, a sequence encoding a full-length variable domain of the immunoglobulin lambda light chain, a sequence encoding a full-length variable domain of the immunoglobulin heavy chain, and combinations thereof.

In some embodiments, step (b) includes extending an end of the capture probe using the nucleic acid encoding the immune cell receptor as a template, thereby generating an

extended capture probe. In some embodiments, step (b) includes extending a 3' end of the capture probe.

In some embodiments, step (b) includes generating a second strand of nucleic acid that includes (i) a sequence that is complementary to all or a portion of the spatial barcode, and (ii) a sequence that corresponds to all or a portion of the sequence of the nucleic acid encoding the immune cell receptor.

In some embodiments, the capture probe includes a cleavage domain, a functional domain, a unique molecular identifier, or any combination thereof. In some embodiments, the capture probe includes a functional domain.

In some embodiments, step (b) includes generating a second strand of nucleic acid that includes (i) a sequence that is complementary to all or a portion of the functional domain, (ii) a sequence that is complementary to all or a portion of the spatial barcode, and (iii) a sequence that corresponds to all or a portion of the sequence of the nucleic acid encoding the immune cell receptor.

In some embodiments, the method includes enriching the nucleic acid encoding the immune cell receptor. In some embodiments, enriching includes hybridizing a plurality of hybridization probes to the nucleic acid encoding the immune cell receptor, where a hybridization probe includes (i) a sequence complementary to a portion of the nucleic acid encoding the immune cell receptor and (ii) a binding moiety that interacts with a capture moiety. In some embodiments, the binding moiety includes biotin and the capture moiety includes streptavidin. In some embodiments, enriching the nucleic acid encoding the immune cell receptor of the immune cell receptor includes one or more blocking probes. In some embodiments, the one or more blocking probes includes a sequence having at least 80% identity to SEQ ID NO: 639. In some embodiments, the one or more blocking probes includes a sequence having at least 80% identity to SEQ ID NO: 640.

In some embodiments, the method includes amplifying the nucleic acid encoding an immune cell receptor, or a complement thereof, using (i) a first primer including all or a portion of the functional domain, where the functional domain is 5' to the spatial barcode in the second strand of nucleic acid, and (ii) a second primer including a sequence that is substantially complementary to a portion of a sequence encoding a variable region of the immune cell receptor.

In some embodiments, the method includes amplifying the nucleic acid encoding the immune cell receptor, or a complement thereof, using (i) the first primer including all or a portion of the functional domain, where the functional domain is 5' to the spatial barcode,

and (ii) a third primer including a sequence that is substantially complementary to a portion of the nucleic acid sequence encoding a variable region of the immune cell receptor, where the third primer is 5' to the second primer.

5 In some embodiments, the biological sample includes a tissue sample. In some embodiments, the tissue sample is a tissue section. In some embodiments, the tissue section is a fixed tissue section. In some embodiments, the fixed tissue section is a formalin-fixed paraffin-embedded tissue section. In some embodiments, the tissue section includes a tumor region.

10 In some embodiments, the nucleic acid encoding the immune cell receptor includes RNA. In some embodiments, the RNA is mRNA. In some embodiments, the nucleic acid encoding the immune cell receptor includes DNA. In some embodiments, the DNA is genomic DNA.

In some embodiments, the method includes, prior to step (b), contacting the biological sample with ribosomal RNA depletion probes and mitochondrial RNA depletion probes.

15 In some embodiments, the method includes imaging the biological sample.

In some embodiments, the determining in step (b) includes sequencing (i) all or a portion of the sequence of the spatial barcode or a complement thereof, and (ii) all or a portion of the sequence of the nucleic acid encoding the immune cell receptor or a complement thereof.

20 In some embodiments, step (b) includes determining the presence of the immune cell receptor at a location in the biological sample. In some embodiments, step (b) includes determining the abundance of the immune cell receptor at a location in the biological sample. In some embodiments, step (b) includes determining the presence and abundance of the immune cell receptor at a location in the biological sample. In some embodiments, step (b)
25 includes determining the presence of two or more immune cell receptors at a location in the biological sample. In some embodiments, step (b) includes determining the abundance of two or more immune cell receptors at a location in the biological sample. In some embodiments, step (b) includes determining the presence and abundance of two or more immune cell receptors at a location in the biological sample. In some embodiments, the method includes
30 comparing the two or more immune cell receptors. In some embodiments, the two or more immune cell clonotypes are each an immune cell receptor of a B cell. In some embodiments, the two or more immune cell clonotypes are each an immune cell receptor of a T cell. In some embodiments, the two or more immune cell clonotypes comprise at least one immune cell receptor of a T cell and at least one immune cell receptor from a B cell.

Also provided herein are arrays including a plurality of capture probes, where a capture probe of the plurality of capture probes includes (i) a spatial barcode and (ii) a capture domain that binds to a nucleic acid encoding an immune cell receptor of an immune cell clonotype.

5 In some embodiments, the immune cell clonotype is a T cell clonotype. In some embodiments, the immune cell receptor is a T cell receptor alpha chain. In some embodiments, the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the T cell receptor alpha chain. In some embodiments, the immune cell receptor is a T cell receptor beta chain. In some embodiments, the capture domain binds
10 specifically to a nucleic acid sequence encoding a constant region of the T cell receptor beta chain.

 In some embodiments, the immune cell clonotype is a B cell clonotype. In some embodiments, the immune cell receptor is an immunoglobulin kappa light chain. In some embodiments, the capture domain binds specifically to a nucleic acid sequence encoding a
15 constant region of the immunoglobulin kappa light chain. In some embodiments, the immune cell receptor is an immunoglobulin lambda light chain. In some embodiments, the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin lambda light chain. In some embodiments, the immune cell receptor is an immunoglobulin heavy chain. In some embodiments, the capture domain binds specifically
20 to a nucleic acid sequence encoding a constant region of the immunoglobulin heavy chain.

 In some embodiments, the capture probe includes a cleavage domain, a functional domain, a unique molecular identifier, or any combination thereof.

 Also provided herein are kits any one of the arrays described herein; one or more hybridization probes, where a hybridization probe includes (i) a sequence substantially
25 complementary to a nucleic acid encoding an immune cell receptor and (ii) a binding moiety that interacts with a capturing moiety; and one or more blocking probes.

 All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, patent application, or item of information was specifically and individually indicated to be
30 incorporated by reference. To the extent publications, patents, patent applications, and items of information incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

Where values are described in terms of ranges, it should be understood that the description includes the disclosure of all possible sub-ranges within such ranges, as well as specific numerical values that fall within such ranges irrespective of whether a specific numerical value or specific sub-range is expressly stated.

5 The term “each,” when used in reference to a collection of items, is intended to identify an individual item in the collection but does not necessarily refer to every item in the collection, unless expressly stated otherwise, or unless the context of the usage clearly indicates otherwise.

Various embodiments of the features of this disclosure are described herein. However, 10 it should be understood that such embodiments are provided merely by way of example, and numerous variations, changes, and substitutions can occur to those skilled in the art without departing from the scope of this disclosure. It should also be understood that various alternatives to the specific embodiments described herein are also within the scope of this disclosure.

15 **DESCRIPTION OF DRAWINGS**

The following drawings illustrate certain embodiments of the features and advantages of this disclosure. These embodiments are not intended to limit the scope of the appended claims in any manner. Like reference symbols in the drawings indicate like elements.

FIG. 1 is a schematic diagram showing an example of a barcoded capture probe, as
20 described herein.

FIG. 2 shows an exemplary workflow for spatial transcriptomics for antigen-receptors.

FIG. 3A shows an exemplary capture probe with a capture sequence complementary to a constant region of an analyte.

25 **FIG. 3B** shows an exemplary capture probe with a poly(dT) capture domain.

FIG. 4 shows an exemplary enrichment strategy with a Read1 primer and a primer(s) complementary to a variable region of an analyte.

FIG. 5 shows an exemplary sequencing strategy with a ligated sequencing handle (P5) and a custom sequencing primer complementary to a portion of the constant region of an

30 analyte.

FIG. 6 shows a graph showing the number of unique clonotypes detected from lymph node (LN) and spleen (SP) tissues.

FIG. 7A shows an exemplary graph of the number of unique T-cell receptor A (TRA) and T-cell receptor B (TRB) clonotypes detected with a targeted capture probe compared with a poly(dT) capture probe.

FIG. 7B shows an exemplary graph of the number of unique IG heavy chain clonotypes (e.g., A, G, M and E clonotypes) detected with a targeted capture probe compared with a poly(dT) capture probe.

FIG. 8 shows an exemplary graph of the number of unique IG clonotypes detected in tonsil tissue on a spatial array (Vis) compared with single cell (SS2) analysis, with or without an enrichment strategy.

FIG. 9 shows an exemplary graph of the number of unique IG clonotypes detected in lymph node (LN) tissue on a spatial array with and without an enrichment strategy. Lymph node and spleen (SP) non-enrichment samples serve as controls.

FIG. 10A shows H&E stained tonsil tissue.

FIG. 10B shows a gene expression library generated from the tonsil tissue in **FIG. 10A**.

FIG. 11 shows single-cell clustering analysis of the T-cell receptor and B-cell receptor clonotypes present in a breast tumor sample.

FIG. 12 shows an exemplary capture probe with a poly(dT) capture domain (top) followed by reverse transcription to generate cDNA of an analyte.

FIG. 13 shows cDNA libraries for either B-cell receptors (BCR), T-cell receptors (TCR), or other analytes and pools of BCR and TCR with enrichment hybridization probes.

FIG. 14 shows hybridization of the BCR and TCR specific enrichment hybridization probes to their respective targets in the cDNA library.

FIG. 15 shows hybridization of blocking oligonucleotides targeting various domains present in the cDNA library.

FIG. 16A shows replicate tonsil sections (top and bottom) and detection of BCR and TCR clonotype count (left) and BCR and TCR unique molecular identifier count (right).

FIG. 16B shows a graph showing the total number of unique clonotypes found in the replicate tonsil sections from **FIG. 16A**.

FIG. 16C shows a graph showing the clonotype count split by IGH isotype found in the replicate tonsil sections from **FIG. 16A**.

FIG. 17A shows H&E stained tonsil tissue (left), CD20 spatial expression (middle), and CD138 expression (control).

FIG. 17B shows spatial expression in tonsil tissue of the heavy chain IGH constant gene (top) including IGHM, IGHG1, IGHA1, and IGHD and the light chain (bottom) including IGKC and IGLC2.

FIG. 17C shows T-cell specific spatial expression for CD3E, TRBC1, TRABC2, and TRAC in tonsil tissue.

FIG. 18A shows a detected IG clone expression (IGKC) restricted to about one B-cell follicle of in tonsil tissue in replicate experiments.

FIG. 18B shows a detected IG clone expression (IGLC) restricted to a B-cell follicle in tonsil tissue in replicate experiments.

FIG. 18C shows detected IG clone expression (IGLV3-1, IGLJ2, IGLC2/IGLC3) with expression not restricted to B-cell follicles in tonsil tissue in replicate experiments.

FIG. 18D shows detected IG clone expression (IGHM) in single B-cell follicles in tonsil tissue in replicate experiments.

FIG. 18E shows detected IG clone expression (IGHA) expression outside B-cell follicles in tonsil tissue in replicate experiments.

FIG. 18F shows a representative T-cell clone expression (TRB) distributed outside of B-cell follicles in tonsil tissue in replicate experiments.

FIG. 18G shows a representative T-cell clone expression (TRA) distributed outside of B-cell follicles in tonsil tissue in replicate experiments.

FIG. 19A shows clonotype distribution in replicate breast tumor samples (Tumor D1, Tumor D2) and clonotype count (left) and UMI count (right).

FIG. 19B is a graph showing total clonotype count of the replicate breast tumor samples shown in **FIG. 19A**.

FIG. 19C is a graph showing clonotype count split by IGH isotype of the replicate breast tumor samples shown in **FIG. 19A**.

FIG. 20 shows the distribution of a representative IGH clonotypes of the replicate breast tumor samples shown in **FIG. 19A**.

FIG. 21A shows spatial patterning of paired IG receptors (IGHG2 and IGK) (left) in breast tumor tissue and single-cell RNA-seq (right).

FIG. 21B shows spatial patterning of paired IG receptors (IGHG1 and IGK) (left) in breast tumor tissue and single-cell RNA-seq (right).

FIG. 21C shows spatial patterning of paired IG receptors (IGHG2 and IGK) (left) in breast tumor tissue and single-cell RNA-seq (right).

FIG. 22 shows an exemplary nested PCR strategy for additional TCR enrichment.

DETAILED DESCRIPTION

A fundamental understanding of spatial heterogeneity with respect to T-cell receptor (TCR) and B-cell receptor (BCR) clonotypes within a biological sample is needed to understand which cells a TCR or BCR may be interacting with, the identity of TCR and/or BCR clonotypes in a given biological sample, or the identity of TCR and/or BCR clonotypes that are autoreactive in different autoimmune disorders. Numerous single-cell sequencing approaches can identify TCR and BCR clonotypes from a biological sample, however, at present methods are need to link TCR and BCR sequences to spatial locations within a biological sample. Additionally, identifying the clonal regions, that is, regions defined by the places where variable (V), diverse (D), and joining (J) segments join to from the complementarity determining regions, including CDR1, CDR2, and CDR3, which provide specificity to the TCRs and/or BCRs, is needed to help determine biological interactions. By coupling clonal information to spatial information it is possible to understand which T-cell and B-cell clonotypes may be specifically interacting with given cell types within a biological sample.

Provided herein are methods for determining the presence and/or abundance of an immune cell clonotype at a location in a biological sample. Some embodiments of any of the methods described herein include capturing transcripts to identify an immune cell clonotype. Some embodiments of any of the methods herein include generating a nucleic acid library from captured transcripts. Some embodiments of any of the methods described herein include enriching analytes of interest in the nucleic acid library, including analytes to identify an immune cell clonotype.

Provided herein are methods for determining the presence and/or abundance of an immune cell receptor at a location in a biological sample. Some embodiments of any of the methods described herein include capturing analytes to identify an immune cell receptor. Some embodiments of any of the methods described herein include generating a nucleic acid library from captured analytes. Some embodiments of any of the methods described here include enriching analytes of interest in the nucleic acid library, including analytes to identify an immune cell receptor.

Spatial analysis methodologies and compositions described herein can provide a vast amount of analyte and/or expression data for a variety of analytes within a biological sample at high spatial resolution, while retaining native spatial context. Spatial analysis methods and

compositions can include, e.g., the use of a capture probe including a spatial barcode (e.g., a nucleic acid sequence that provides information as to the location or position of an analyte within a cell or a tissue sample (e.g., mammalian cell or a mammalian tissue sample) and a capture domain that is capable of binding to an analyte (e.g., a protein and/or a nucleic acid) produced by and/or present in a cell. Spatial analysis methods and compositions can also include the use of a capture probe having a capture domain that captures an intermediate agent for indirect detection of an analyte. For example, the intermediate agent can include a nucleic acid sequence (e.g., a barcode) associated with the intermediate agent. Detection of the intermediate agent is therefore indicative of the analyte in the cell or tissue sample.

Non-limiting aspects of spatial analysis methodologies and compositions are described in U.S. Patent Nos. 10,774,374, 10,724,078, 10,480,022, 10,059,990, 10,041,949, 10,002,316, 9,879,313, 9,783,841, 9,727,810, 9,593,365, 8,951,726, 8,604,182, 7,709,198, U.S. Patent Application Publication Nos. 2020/239946, 2020/080136, 2020/0277663, 2020/024641, 2019/330617, 2019/264268, 2020/256867, 2020/224244, 2019/194709, 2019/161796, 2019/085383, 2019/055594, 2018/216161, 2018/051322, 2018/0245142, 2017/241911, 2017/089811, 2017/067096, 2017/029875, 2017/0016053, 2016/108458, 2015/000854, 2013/171621, WO 2018/091676, WO 2020/176788, Rodriques et al., Science 363(6434):1463-1467, 2019; Lee et al., Nat. Protoc. 10(3):442-458, 2015; Trejo et al., PLoS ONE 14(2):e0212031, 2019; Chen et al., Science 348(6233):aaa6090, 2015; Gao et al., BMC Biol. 15:50, 2017; and Gupta et al., Nature Biotechnol. 36:1197-1202, 2018; the Visium Spatial Gene Expression Reagent Kits User Guide (e.g., Rev C, dated June 2020), and/or the Visium Spatial Tissue Optimization Reagent Kits User Guide (e.g., Rev C, dated July 2020), both of which are available at the 10x Genomics Support Documentation website, and can be used herein in any combination. Further non-limiting aspects of spatial analysis methodologies and compositions are described herein.

Some general terminology that may be used in this disclosure can be found in Section (I)(b) of WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663. Typically, a “barcode” is a label, or identifier, that conveys or is capable of conveying information (e.g., information about an analyte in a sample, a bead, and/or a capture probe). A barcode can be part of an analyte, or independent of an analyte. A barcode can be attached to an analyte. A particular barcode can be unique relative to other barcodes. For the purpose of this disclosure, an “analyte” can include any biological substance, structure, moiety, or component to be analyzed. The term “target” can similarly refer to an analyte of interest.

In some embodiments, the analyte is an immune cell receptor. In some embodiments, the immune cell receptor is a B cell receptor. In some embodiments, the B cell receptor is an immunoglobulin kappa light chain. In some embodiments, the variable region of the analyte includes a CDR3 region of the immunoglobulin kappa light chain. In some embodiments, the variable region of the analyte includes one or both of CDR1 and CDR2 of the immunoglobulin kappa light chain. In some embodiments, the variable region of the analyte includes a full-length variable domain of the immunoglobulin kappa light chain.

In some embodiments, the B cell receptor is an immunoglobulin lambda light chain. In some embodiments, the variable region of the analyte includes a CDR3 of the immunoglobulin lambda light chain. In some embodiments, the variable region of the analyte includes one or both of CDR1 and CDR2 of the immunoglobulin lambda light chain. In some embodiments, the variable region of the analyte includes a full-length variable domain of the immunoglobulin lambda light chain.

In some embodiments, the B cell receptor is an immunoglobulin heavy chain. In some embodiments, the variable region of the analyte includes a CDR3 of the immunoglobulin heavy chain. In some embodiments, the variable region of the analyte includes one or both of CDR1 and CDR2 of the immunoglobulin heavy chain. In some embodiments, the variable region of the analyte includes a full-length variable domain of the immunoglobulin heavy chain.

In some embodiments, the immune cell receptor is a T cell receptor. In some embodiments, the T cell receptor is a T cell receptor alpha chain. In some embodiments, the variable region of the analyte includes a CDR3 of the T cell receptor alpha chain. In some embodiments, the variable region of the analyte includes one or both of CDR1 and CDR2 of the T cell receptor alpha chain. In some embodiments, the variable region of the analyte includes a full-length variable domain of the T cell receptor alpha chain.

In some embodiments, the T cell receptor is a T cell receptor beta chain. In some embodiments, the variable region of the analyte includes a CDR3 of the T cell receptor beta chain. In some embodiments, the variable region of the analyte includes one or both of CDR1 and CDR2 of the T cell receptor beta chain. In some embodiments, the variable region of the analyte further includes a full-length variable domain of the T cell receptor beta chain.

Analytes can be broadly classified into one of two groups: nucleic acid analytes, and non-nucleic acid analytes. Examples of non-nucleic acid analytes include, but are not limited to, lipids, carbohydrates, peptides, proteins, glycoproteins (N-linked or O-linked), lipoproteins, phosphoproteins, specific phosphorylated or acetylated variants of proteins,

amidation variants of proteins, hydroxylation variants of proteins, methylation variants of proteins, ubiquitylation variants of proteins, sulfation variants of proteins, viral proteins (e.g., viral capsid, viral envelope, viral coat, viral accessory, viral glycoproteins, viral spike, etc.), extracellular and intracellular proteins, antibodies, and antigen binding fragments. In some
5 embodiments, the analyte(s) can be localized to subcellular location(s), including, for example, organelles, e.g., mitochondria, Golgi apparatus, endoplasmic reticulum, chloroplasts, endocytic vesicles, exocytic vesicles, vacuoles, lysosomes, etc. In some embodiments, analyte(s) can be peptides or proteins, including without limitation antibodies and enzymes. Additional examples of analytes can be found in Section (I)(c) of WO
10 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663. In some embodiments, an analyte can be detected indirectly, such as through detection of an intermediate agent, for example, a ligation product or an analyte capture agent (e.g., an oligonucleotide-conjugated antibody), such as those described herein.

A “biological sample” is typically obtained from the subject for analysis using any of
15 a variety of techniques including, but not limited to, biopsy, surgery, and laser capture microscopy (LCM), and generally includes cells and/or other biological material from the subject. In some embodiments, a biological sample can be a tissue section. In some embodiments, a biological sample can be a fixed and/or stained biological sample (e.g., a fixed and/or stained tissue section). Non-limiting examples of stains include histological
20 stains (e.g., hematoxylin and/or eosin) and immunological stains (e.g., fluorescent stains). In some embodiments, a biological sample (e.g., a fixed and/or stained biological sample) can be imaged. Biological samples are also described in Section (I)(d) of WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663.

In some embodiments, a biological sample is permeabilized with one or more
25 permeabilization reagents. For example, permeabilization of a biological sample can facilitate analyte capture. Exemplary permeabilization agents and conditions are described in Section (I)(d)(ii)(13) or the Exemplary Embodiments Section of WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663.

Array-based spatial analysis methods involve the transfer of one or more analytes
30 from a biological sample to an array of features on a substrate, where each feature is associated with a unique spatial location on the array. Subsequent analysis of the transferred analytes includes determining the identity of the analytes and the spatial location of the analytes within the biological sample. The spatial location of an analyte within the biological

sample is determined based on the feature to which the analyte is bound (e.g., directly or indirectly) on the array, and the feature's relative spatial location within the array.

A "capture probe" refers to any molecule capable of capturing (directly or indirectly) and/or labelling an analyte (e.g., an analyte of interest) in a biological sample. In some embodiments, the capture probe is a nucleic acid or a polypeptide. In some embodiments, the capture probe includes a barcode (e.g., a spatial barcode and/or a unique molecular identifier (UMI)) and a capture domain). In some embodiments, a capture probe can include a cleavage domain and/or a functional domain (e.g., a primer-binding site, such as for next-generation sequencing (NGS)). See, e.g., Section (II)(b) (e.g., subsections (i)-(vi)) of WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663. Generation of capture probes can be achieved by any appropriate method, including those described in Section (II)(d)(ii) of WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663.

In some embodiments, more than one analyte type (e.g., nucleic acids and proteins) from a biological sample can be detected (e.g., simultaneously or sequentially) using any appropriate multiplexing technique, such as those described in Section (IV) of WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663.

In some embodiments, detection of one or more analytes (e.g., protein analytes) can be performed using one or more analyte capture agents. As used herein, an "analyte capture agent" refers to an agent that interacts with an analyte (e.g., an analyte in a biological sample) and with a capture probe (e.g., a capture probe attached to a substrate or a feature) to identify the analyte. In some embodiments, the analyte capture agent includes: (i) an analyte binding moiety (e.g., that binds to an analyte), for example, an antibody or antigen-binding fragment thereof; (ii) analyte binding moiety barcode; and (iii) an analyte capture sequence. As used herein, the term "analyte binding moiety barcode" refers to a barcode that is associated with or otherwise identifies the analyte binding moiety. As used herein, the term "analyte capture sequence" refers to a region or moiety configured to hybridize to, bind to, couple to, or otherwise interact with a capture domain of a capture probe. In some cases, an analyte binding moiety barcode (or portion thereof) may be able to be removed (e.g., cleaved) from the analyte capture agent. Additional description of analyte capture agents can be found in Section (II)(b)(ix) of WO 2020/176788 and/or Section (II)(b)(viii) U.S. Patent Application Publication No. 2020/0277663.

There are at least two methods to associate a spatial barcode with one or more neighboring cells, such that the spatial barcode identifies the one or more cells, and/or

contents of the one or more cells, as associated with a particular spatial location. One method is to promote analytes or analyte proxies (e.g., intermediate agents) out of a cell and towards a spatially-barcoded array (e.g., including spatially-barcoded capture probes). Another method is to cleave spatially-barcoded capture probes from an array and promote the spatially-barcoded capture probes towards and/or into or onto the biological sample.

FIG. 1 is a schematic diagram showing an exemplary capture probe, as described herein. As shown, the capture probe **102** is optionally coupled to a feature **101** by a cleavage domain **103**, such as a disulfide linker. The capture probe can include a functional sequence **104** that are useful for subsequent processing. The functional sequence **104** can include all or a part of sequencer specific flow cell attachment sequence (e.g., a P5 or P7 sequence), all or a part of a sequencing primer sequence, (e.g., a R1 primer binding site, a R2 primer binding site), or combinations thereof. The capture probe can also include a spatial barcode **105**. The capture probe can also include a unique molecular identifier (UMI) sequence **106**. While **FIG. 1** shows the spatial barcode **105** as being located upstream (5') of UMI sequence **106**, it is to be understood that capture probes wherein UMI sequence **106** is located upstream (5') of the spatial barcode **105** is also suitable for use in any of the methods described herein. The capture probe can also include a capture domain **107** to facilitate capture of a target analyte. In some embodiments, the capture probe comprises one or more additional functional sequences that can be located, for example between the spatial barcode **105** and the UMI sequence **106**, between the UMI sequence **106** and the capture domain **107**, or following the capture domain **107**. The capture domain can have a sequence complementary to a sequence of a nucleic acid analyte. The capture domain can have a sequence complementary to a connected probe described herein. The capture domain can have a sequence complementary to a capture handle sequence present in an analyte capture agent. The capture domain can have a sequence complementary to a splint oligonucleotide. Such splint oligonucleotide, in addition to having a sequence complementary to a capture domain of a capture probe, can have a sequence of a nucleic acid analyte, a sequence complementary to a portion of a connected probe described herein, and/or a capture handle sequence described herein.

The functional sequences can generally be selected for compatibility with any of a variety of different sequencing systems, e.g., Ion Torrent Proton or PGM, Illumina sequencing instruments, PacBio, Oxford Nanopore, etc., and the requirements thereof. In some embodiments, functional sequences can be selected for compatibility with non-commercialized sequencing systems. Examples of such sequencing systems and techniques, for which suitable functional sequences can be used, include (but are not limited to) Ion

Torrent Proton or PGM sequencing, Illumina sequencing, PacBio SMRT sequencing, and Oxford Nanopore sequencing. Further, in some embodiments, functional sequences can be selected for compatibility with other sequencing systems, including non-commercialized sequencing systems.

5 In some embodiments, the spatial barcode **105** and functional sequences **104** is common to all of the probes attached to a given feature. In some embodiments, the UMI sequence **106** of a capture probe attached to a given feature is different from the UMI sequence of a different capture probe attached to the given feature.

 In some cases, capture probes may be configured to prime, replicate, and
10 consequently yield optionally barcoded extension products from a template (e.g., a DNA or RNA template, such as an analyte or an intermediate agent (e.g., a ligation product or an analyte capture agent), or a portion thereof), or derivatives thereof (see, e.g., Section (II)(b)(vii) of WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663 regarding extended capture probes). In some cases, capture probes may be
15 configured to form ligation products with a template (e.g., a DNA or RNA template, such as an analyte or an intermediate agent, or portion thereof), thereby creating ligation products that serve as proxies for a template.

 As used herein, an “extended capture probe” refers to a capture probe having additional nucleotides added to the terminus (e.g., 3’ or 5’ end) of the capture probe thereby
20 extending the overall length of the capture probe. For example, an “extended 3’ end” indicates additional nucleotides were added to the most 3’ nucleotide of the capture probe to extend the length of the capture probe, for example, by polymerization reactions used to extend nucleic acid molecules including templated polymerization catalyzed by a polymerase (e.g., a DNA polymerase or a reverse transcriptase). In some embodiments, extending the
25 capture probe includes adding to a 3’ end of a capture probe a nucleic acid sequence that is complementary to a nucleic acid sequence of an analyte or intermediate agent specifically bound to the capture domain of the capture probe. In some embodiments, the capture probe is extended using reverse transcription. In some embodiments, the capture probe is extended using one or more DNA polymerases. The extended capture probes include the sequence of
30 the capture probe and the sequence of the spatial barcode of the capture probe.

 In some embodiments, extended capture probes are amplified (e.g., in bulk solution or on the array) to yield quantities that are sufficient for downstream analysis, e.g., via DNA sequencing. In some embodiments, extended capture probes (e.g., DNA molecules) act as templates for an amplification reaction (e.g., a polymerase chain reaction).

Additional variants of spatial analysis methods, including in some embodiments, an imaging step, are described in Section (II)(a) of WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663. Analysis of captured analytes (and/or intermediate agents or portions thereof), for example, including sample removal, extension of
5 capture probes, sequencing (e.g., of a cleaved extended capture probe and/or a cDNA molecule complementary to an extended capture probe), sequencing on the array (e.g., using, for example, in situ hybridization or in situ ligation approaches), temporal analysis, and/or proximity capture, is described in Section (II)(g) of WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663. Some quality control measures are described in
10 Section (II)(h) of WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663.

Spatial information can provide information of biological and/or medical importance. For example, the methods and compositions described herein can allow for: identification of one or more biomarkers (e.g., diagnostic, prognostic, and/or for determination of efficacy of a
15 treatment) of a disease or disorder; identification of a candidate drug target for treatment of a disease or disorder; identification (e.g., diagnosis) of a subject as having a disease or disorder; identification of stage and/or prognosis of a disease or disorder in a subject; identification of a subject as having an increased likelihood of developing a disease or disorder; monitoring of progression of a disease or disorder in a subject; determination of
20 efficacy of a treatment of a disease or disorder in a subject; identification of a patient subpopulation for which a treatment is effective for a disease or disorder; modification of a treatment of a subject with a disease or disorder; selection of a subject for participation in a clinical trial; and/or selection of a treatment for a subject with a disease or disorder.

Spatial information can provide information of biological importance. For example,
25 the methods and compositions described herein can allow for: identification of transcriptome and/or proteome expression profiles (e.g., in healthy and/or diseased tissue); identification of multiple analyte types in close proximity (e.g., nearest neighbor analysis); determination of up- and/or down-regulated genes and/or proteins in diseased tissue; characterization of tumor microenvironments; characterization of tumor immune responses; characterization of cells
30 types and their co-localization in tissue; and identification of genetic variants within tissues (e.g., based on gene and/or protein expression profiles associated with specific disease or disorder biomarkers).

Typically, for spatial array-based methods, a substrate functions as a support for direct or indirect attachment of capture probes to features of the array. A “feature” is an entity that

acts as a support or repository for various molecular entities used in spatial analysis. In some embodiments, some or all of the features in an array are functionalized for analyte capture. Exemplary substrates are described in Section (II)(c) of WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663. Exemplary features and geometric attributes of an array can be found in Sections (II)(d)(i), (II)(d)(iii), and (II)(d)(iv) of WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663.

Generally, analytes and/or intermediate agents (or portions thereof) can be captured when contacting a biological sample with a substrate including capture probes (e.g., a substrate with capture probes embedded, spotted, printed, fabricated on the substrate, or a substrate with features (e.g., beads, wells) comprising capture probes). As used herein, “contact,” “contacted,” and/or “contacting,” a biological sample with a substrate refers to any contact (e.g., direct or indirect) such that capture probes can interact (e.g., bind covalently or non-covalently (e.g., hybridize)) with analytes from the biological sample. Capture can be achieved actively (e.g., using electrophoresis) or passively (e.g., using diffusion). Analyte capture is further described in Section (II)(e) of WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663.

In some cases, spatial analysis can be performed by attaching and/or introducing a molecule (e.g., a peptide, a lipid, or a nucleic acid molecule) having a barcode (e.g., a spatial barcode) to a biological sample (e.g., to a cell in a biological sample). In some embodiments, a plurality of molecules (e.g., a plurality of nucleic acid molecules) having a plurality of barcodes (e.g., a plurality of spatial barcodes) are introduced to a biological sample (e.g., to a plurality of cells in a biological sample) for use in spatial analysis. In some embodiments, after attaching and/or introducing a molecule having a barcode to a biological sample, the biological sample can be physically separated (e.g., dissociated) into single cells or cell groups for analysis. Some such methods of spatial analysis are described in Section (III) of WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663.

In some cases, spatial analysis can be performed by detecting multiple oligonucleotides that hybridize to an analyte. In some instances, for example, spatial analysis can be performed using RNA-templated ligation (RTL). Methods of RTL have been described previously. See, e.g., Credle *et al.*, *Nucleic Acids Res.* 2017 Aug 21;45(14):e128. Typically, RTL includes hybridization of two oligonucleotides to adjacent sequences on an analyte (e.g., an RNA molecule, such as an mRNA molecule). In some instances, the oligonucleotides are DNA molecules. In some instances, one of the oligonucleotides includes at least two ribonucleic acid bases at the 3' end and/or the other oligonucleotide includes a

phosphorylated nucleotide at the 5' end. In some instances, one of the two oligonucleotides includes a capture domain (e.g., a poly(A) sequence, a non-homopolymeric sequence). After hybridization to the analyte, a ligase (e.g., SplintR ligase) ligates the two oligonucleotides together, creating a ligation product. In some instances, the two oligonucleotides hybridize to sequences that are not adjacent to one another. For example, hybridization of the two oligonucleotides creates a gap between the hybridized oligonucleotides. In some instances, a polymerase (e.g., a DNA polymerase) can extend one of the oligonucleotides prior to ligation. After ligation, the ligation product is released from the analyte. In some instances, the ligation product is released using an endonuclease (e.g., RNase H). The released ligation product can then be captured by capture probes (e.g., instead of direct capture of an analyte) on an array, optionally amplified, and sequenced, thus determining the location and optionally the abundance of the analyte in the biological sample.

During analysis of spatial information, sequence information for a spatial barcode associated with an analyte is obtained, and the sequence information can be used to provide information about the spatial distribution of the analyte in the biological sample. Various methods can be used to obtain the spatial information. In some embodiments, specific capture probes and the analytes they capture are associated with specific locations in an array of features on a substrate. For example, specific spatial barcodes can be associated with specific array locations prior to array fabrication, and the sequences of the spatial barcodes can be stored (e.g., in a database) along with specific array location information, so that each spatial barcode uniquely maps to a particular array location.

Alternatively, specific spatial barcodes can be deposited at predetermined locations in an array of features during fabrication such that at each location, only one type of spatial barcode is present so that spatial barcodes are uniquely associated with a single feature of the array. Where necessary, the arrays can be decoded using any of the methods described herein so that spatial barcodes are uniquely associated with array feature locations, and this mapping can be stored as described above.

When sequence information is obtained for capture probes and/or analytes during analysis of spatial information, the locations of the capture probes and/or analytes can be determined by referring to the stored information that uniquely associates each spatial barcode with an array feature location. In this manner, specific capture probes and captured analytes are associated with specific locations in the array of features. Each array feature location represents a position relative to a coordinate reference point (e.g., an array location, a

fiducial marker) for the array. Accordingly, each feature location has an “address” or location in the coordinate space of the array.

Some exemplary spatial analysis workflows are described in the Exemplary Embodiments section of WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663. See, for example, the Exemplary embodiment starting with “In some non-limiting examples of the workflows described herein, the sample can be immersed...” of WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663. See also, e.g., the Visium Spatial Gene Expression Reagent Kits User Guide (e.g., Rev C, dated June 2020), and/or the Visium Spatial Tissue Optimization Reagent Kits User Guide (e.g., Rev C, dated July 2020).

In some embodiments, spatial analysis can be performed using dedicated hardware and/or software, such as any of the systems described in Sections (II)(e)(ii) and/or (V) of WO 2020/176788 and/or U.S. Patent Application Publication No. 2020/0277663, or any of one or more of the devices or methods described in Sections *Control Slide for Imaging, Methods of Using Control Slides and Substrates for, Systems of Using Control Slides and Substrates for Imaging, and/or Sample and Array Alignment Devices and Methods, Informational labels* of WO 2020/123320.

Suitable systems for performing spatial analysis can include components such as a chamber (e.g., a flow cell or sealable, fluid-tight chamber) for containing a biological sample. The biological sample can be mounted for example, in a biological sample holder. One or more fluid chambers can be connected to the chamber and/or the sample holder via fluid conduits, and fluids can be delivered into the chamber and/or sample holder via fluidic pumps, vacuum sources, or other devices coupled to the fluid conduits that create a pressure gradient to drive fluid flow. One or more valves can also be connected to fluid conduits to regulate the flow of reagents from reservoirs to the chamber and/or sample holder.

The systems can optionally include a control unit that includes one or more electronic processors, an input interface, an output interface (such as a display), and a storage unit (e.g., a solid state storage medium such as, but not limited to, a magnetic, optical, or other solid state, persistent, writeable and/or re-writeable storage medium). The control unit can optionally be connected to one or more remote devices via a network. The control unit (and components thereof) can generally perform any of the steps and functions described herein. Where the system is connected to a remote device, the remote device (or devices) can perform any of the steps or features described herein. The systems can optionally include one or more detectors (e.g., CCD, CMOS) used to capture images. The systems can also

optionally include one or more light sources (e.g., LED-based, diode-based, lasers) for illuminating a sample, a substrate with features, analytes from a biological sample captured on a substrate, and various control and calibration media.

5 The systems can optionally include software instructions encoded and/or implemented in one or more of tangible storage media and hardware components such as application specific integrated circuits. The software instructions, when executed by a control unit (and in particular, an electronic processor) or an integrated circuit, can cause the control unit, integrated circuit, or other component executing the software instructions to perform any of the method steps or functions described herein.

10 In some cases, the systems described herein can detect (e.g., register an image) the biological sample on the array. Exemplary methods to detect the biological sample on an array are described in PCT Application No. 2020/061064 and/or U.S. Patent Application Serial No. 16/951,854.

15 Prior to transferring analytes from the biological sample to the array of features on the substrate, the biological sample can be aligned with the array. Alignment of a biological sample and an array of features including capture probes can facilitate spatial analysis, which can be used to detect differences in analyte presence and/or level within different positions in the biological sample, for example, to generate a three-dimensional map of the analyte presence and/or level. Exemplary methods to generate a two- and/or three-dimensional map of the analyte presence and/or level are described in PCT Application No. 2020/053655 and spatial analysis methods are generally described in WO 2020/061108 and/or U.S. Patent Application Serial No. 16/951,864.

25 In some cases, a map of analyte presence and/or level can be aligned to an image of a biological sample using one or more fiducial markers, e.g., objects placed in the field of view of an imaging system which appear in the image produced, as described in the *Substrate Attributes* Section, *Control Slide for Imaging* Section of WO 2020/123320, PCT Application No. 2020/061066, and/or U.S. Patent Application Serial No. 16/951,843. Fiducial markers can be used as a point of reference or measurement scale for alignment (e.g., to align a sample and an array, to align two substrates, to determine a location of a sample or array on a substrate relative to a fiducial marker) and/or for quantitative measurements of sizes and/or distances.

Spatial Transcriptomics for Antigen Receptors

A fundamental understanding of spatial heterogeneity with respect to T-cell receptor (TCR) and B-cell receptor (BCR) clonotypes within a biological sample is needed to understand multiple facets of their functionality, including, for example, which cells a particular TCR or BCR may be interacting with within the biological sample, the identity of TCR and/or BCR clonotypes in a given biological sample, and/or the identity of TCR and/or BCR clonotypes that are autoreactive in different autoimmune disorders. Numerous single-cell sequencing approaches can identify TCR and BCR clonotypes from a biological sample, however, at present methods are needed to link TCR and BCR sequences to spatial locations within a biological sample. Additionally, identifying the clonal regions, that is, regions defined by the places where variable (V), diverse (D), and joining (J) segments join to form the complementarity determining regions, including CDR1, CDR2, and CDR3, which provide specificity to the TCRs and/or BCRs, would greatly benefit the scientific arts. By coupling clonal information to spatial information it is possible to understand which T-cell and B-cell clonotypes may be specifically interacting with given cell types within a biological sample.

However, capturing analytes encoding immune cell receptors can provide unique challenges. For example, spatially capturing the TCR and BCR gene components with sufficient efficiency to profile the majority of clonotypes in a given tissue is difficult. Capturing analytes encoding immune cell receptors with conventional short-read sequencing methods can result in a loss of sequenced regions that are more than about 1 kb away from the point where sequencing starts. Linking separate TCR or BCR gene components that together form a complete receptor using sequencing data from spots containing multiple different cells are challenges addressed by the methods described herein.

Methods described herein are utilized to analyze the various sequences of TCRs and BCRs from immune cells, for example, various clonotypes. In some embodiments, the methods are used to analyze the sequence of a TCR alpha chain, a TCR beta chain, a TCR delta chain, a TCR gamma chain, or any fragment thereof (e.g., variable regions including V(D)J or VJ regions, constant regions, transmembrane regions, fragments thereof, combinations thereof, and combinations of fragments thereof). In some embodiments, the methods described herein can be used to analyze the sequence of a B cell receptor heavy chain, B cell receptor light chain, or any fragment thereof (e.g., variable regions including V(D)J or VJ regions, constant regions, transmembrane regions, fragments thereof, combinations thereof, and combinations of fragments thereof).

Analytes

The analyte sequences present in the nucleic acid library (e.g., nucleic acid library generated from single-cells or from a biological sample on an array) can be captured from a biological sample (e.g., any of the biological samples described herein). In some
5 embodiments, the biological sample is a tissue sample. In some embodiments, the tissue sample is a tissue section. In some embodiments, the tissue section is a fixed tissue section. In some embodiments, the fixed tissue section is formalin-fixed paraffin-embedded tissue section. In some embodiments, the tissue section is a fresh, frozen tissue section.

The analytes to be detected can be any of the analytes described herein. Analytes can
10 include a nucleic acid molecule with a nucleic acid sequence encoding at least a portion of a V(D)J sequence of an immune cell receptor (e.g., a TCR or BCR). In some embodiments, the analyte is RNA. In some embodiments, the RNA is mRNA. In some embodiments, the analyte is DNA. In some embodiments, the DNA is genomic DNA. In some embodiments, the analytes are analytes encoding immune cell receptors. In some embodiments, analytes
15 encoding immune cell receptors identify clonotype populations from a biological sample.

In some embodiments, analytes include a constant region, such as a constant region present in analytes encoding immune cell receptors. In some embodiments, analytes include a variable region, such as analytes encoding immune cell receptors. In some embodiments, analytes encoding immune cell receptors identify clonotype populations present in a
20 biological sample.

In some embodiments, the analyte is an immune cell receptor. In some embodiments, the immune cell receptor is a B cell receptor. In some embodiments, the B cell receptor is an immunoglobulin kappa light chain. In some embodiments, the variable region of the analyte includes a CDR3 region of the immunoglobulin kappa light chain. In some embodiments, the
25 variable region of the analyte includes one or both of CDR1 and CDR2 of the immunoglobulin kappa light chain. In some embodiments, the variable region of the analyte includes a full-length variable domain of the immunoglobulin kappa light chain.

In some embodiments, the B cell receptor is an immunoglobulin lambda light chain. In some embodiments, the variable region of the analyte includes a CDR3 of the
30 immunoglobulin lambda light chain. In some embodiments, the variable region of the analyte includes one or both of CDR1 and CDR2 of the immunoglobulin lambda light chain. In some embodiments, the variable region of the analyte includes a full-length variable domain of the immunoglobulin lambda light chain.

In some embodiments, the B cell receptor is an immunoglobulin heavy chain. In some embodiments, the variable region of the analyte includes a CDR3 of the immunoglobulin heavy chain. In some embodiments, the variable region of the analyte includes one or both of CDR1 and CDR2 of the immunoglobulin heavy chain. In some embodiments, the variable
5 region of the analyte includes a full-length variable domain of the immunoglobulin heavy chain.

In some embodiments, the immune cell receptor is a T cell receptor. In some embodiments, the T cell receptor is a T cell receptor alpha chain. In some embodiments, the variable region of the analyte includes a CDR3 of the T cell receptor alpha chain. In some
10 embodiments, the variable region of the analyte includes one or both of CDR1 and CDR2 of the T cell receptor alpha chain. In some embodiments, the variable region of the analyte includes a full-length variable domain of the T cell receptor alpha chain.

In some embodiments, the T cell receptor is a T cell receptor beta chain. In some embodiments, the variable region of the analyte includes a CDR3 of the T cell receptor beta
15 chain. In some embodiments, the variable region of the analyte includes one or both of CDR1 and CDR2 of the T cell receptor beta chain. In some embodiments, the variable region of the analyte further includes a full-length variable domain of the T cell receptor beta chain.

Capturing analytes encoding immune cell receptors

20 Provided herein are methods for determining the presence and/or abundance of an immune cell clonotype at a location in a biological sample, the method including (a) contacting a biological sample with an array including a plurality of capture probes, wherein a capture probe of the plurality of capture probes includes (i) a spatial barcode and (ii) a capture domain that specifically binds to a nucleic acid encoding an immune cell receptor of
25 the immune cell clonotype, and, (b) determining (i) all or a portion of the sequence of the spatial barcode or a complement thereof, and (ii) all or a portion of the sequence of the nucleic acid encoding the immune cell receptor or a complement thereof, and using the determined sequences of (i) and (ii) to determine the presence and/or abundance of the immune cell clonotype at a location in the biological sample.

30 Also provided herein are methods for determining the presence and/or abundance of an immune cell receptor at a location in a biological sample, the method including (a) contacting a biological sample with an array including a plurality of capture probes, wherein a capture probe of the plurality of capture probes includes (i) a spatial barcode and (ii) a capture domain that specifically binds to a nucleic acid encoding an immune cell receptor;

and (b) determining (i) all or a portion of the sequence of the spatial barcode or a complement thereof, and (ii) all or a portion of the sequence of the nucleic acid encoding the immune cell receptor or a complement thereof, and using the determined sequences of (i) and (ii) to determine the presence and/or abundance of the immune cell receptor at a location in the
5 biological sample.

Also provided herein are methods for determining the presence and/or abundance of an immune cell clonotype at a location in a biological sample, the method including (a) contacting a biological sample with an array including a plurality of capture probes, wherein a capture probe of the plurality of capture probes includes (i) a spatial barcode and (ii) a
10 capture domain that binds to a nucleic acid encoding an immune cell receptor of the immune cell clonotype; (b) determining (i) all or a portion of the sequence of the spatial barcode or a complement thereof, and (ii) all or a portion of the sequence of the nucleic acid encoding the immune cell receptor or a complement thereof, and using the determined sequences of (i) and (ii) to determine the presence and/or abundance of the immune cell clonotype at a location in
15 the biological sample.

Also provided herein are methods for determining the presence and/or abundance of an immune cell receptor at a location in a biological sample, the method including (a) contacting a biological sample with an array including a plurality of capture probes, wherein a capture probe of the plurality of capture probes includes (i) a spatial barcode and (ii) a
20 capture domain that binds to a nucleic acid encoding an immune cell receptor and (b) determining (i) all or a portion of the sequence of the spatial barcode or a complement thereof, and (ii) all or a portion of the sequence of the nucleic acid encoding the immune cell receptor or a complement thereof, and using the determined sequences of (i) and (ii) to determine the presence and/or abundance of the immune cell receptor at a location in the
25 biological sample.

In some embodiments of determining the presence and/or abundance of an immune cell clonotype or an immune cell receptor at a location in a biological sample, step (b) includes extending an end of the capture probe using the nucleic acid encoding the immune cell receptor as a template, thereby generating an extended capture probe. In some
30 embodiments, extending an end of the capture probe includes using a reverse transcriptase (e.g., any of the reverse transcriptases described herein). In some embodiments, step (b) includes extending a 3' end of the capture probe. In some embodiments, step (b) includes generating a second strand of nucleic acid that includes (i) a sequence that is complementary

to all or a portion of the spatial barcode, and (ii) a sequence that corresponds to all or a portion of the sequence of the nucleic acid encoding the immune cell receptor.

In some embodiments of determining the presence and/or abundance of an immune cell clonotype or an immune cell receptor at a location in a biological sample, the capture probe includes a cleavage domain, a functional domain, a unique molecular identifier, or any combination thereof. In some embodiments, the capture probe includes a functional domain. In some embodiments, the capture domain includes a poly(T) sequence. In some embodiments, the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the T cell receptor alpha chain. In some embodiments, the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the T cell receptor beta chain. In some embodiments, the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin kappa light chain. In some embodiments, the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin lambda light chain. In some embodiments, the capture probe binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin heavy chain.

Variable region primer enrichment

As demonstrated in the Examples, analytes encoding immune cell receptors were captured and identified with capture domains designed to specifically bind a constant region of a particular immune cell receptor from a biological sample. However, such a strategy does not capture analytes other than analytes encoding immune cell receptors. An additional and alternative approach can include using one or more variable region (V-region) specific primer sets to amplify analytes encoding immune cell receptors (e.g., TCRs and/or BCRs) from nucleic acid libraries generated from poly(T) captured total cDNA libraries, thus allowing sequencing into CDR regions (e.g., CDR3 region) from the 5' end of an amplicon. An advantage of this approach would be the simultaneous detection of lymphocyte clonality alongside global spatial gene expression. An additional consideration is capturing full IGH complexity (e.g., IGH isotypes, e.g., IGHA1-2, IGHG1-4, IGHM, IGHD, and IGHE) without paired end sequencing reads through the CDR3 region. Additional receptor diversity is added to the BCR throughout development and may be difficult to distinguish from sequencing errors with only a single CDR3 read. Additionally, some analytes encoding immune cell receptors are known to be in low abundance (See e.g., Tu, A.A., et al., TCR sequencing paired with massively parallel 3' RNAseq reveals clonotypic T cell signatures, *Nature*

Immunology, 20, 1692-1699 (2019); Singh M., et al., High-throughput long-read single cell sequencing reveals the clonal and transcriptional landscape of lymphocytes. *Nature Communications*, 10, 3120 (2019), both of which are incorporated herein by reference in their entireties). Thus, for example, variable region primer enrichment can provide an alternate
5 method to enrich for analytes encoding immune cell receptors from arrays with capture probes including a poly(T) capture domain, followed by one or more amplification reactions (e.g., PCR).

In some embodiments of any of the spatial methods described herein, step (b) further includes generating a second strand of nucleic acid that includes (i) a sequence that is
10 complementary to all or a portion of the functional domain, (ii) a sequence that is complementary to all or a portion of the spatial barcode, and (iii) a sequence that corresponds to all or a portion of the sequence of the nucleic acid encoding the immune cell receptor. In some embodiments, step (b) further includes amplifying the second strand of the nucleic acid using (i) a first primer including all or a portion of the functional domain, wherein the
15 functional domain is 5' to the spatial barcode in the second strand of nucleic acid, and (ii) a second primer including a sequence that is substantially complementary to a portion of a sequence encoding a variable region of the immune cell receptor.

In some embodiments, more than one second primer including a sequence substantially complementary to a portion of the sequence encoding the variable region of the
20 immune cell receptor is used. For example, a nested PCR strategy can be used where a first amplification product is generated with a variable region primer and a primer substantially complementary to the functional domain 5' to the spatial barcode, followed by a second, a third, or a fourth round of amplification using a second, a third, or a fourth variable region primer internal to the first region variable region primer (e.g., 5' to the first variable region
25 primer)(for example, see **FIG. 22**). It will be understood to a person of ordinary skill in the art that additional rounds of amplification require an internal (e.g., 5') located variable region primer in subsequent amplification rounds.

Hybridization Probes and Blocking Probes

30 In some embodiments, targeted enrichment of cDNAs of interest are enriched from cDNA derived libraries generated from captured analytes (e.g., immune cell analytes). For example, a pool of hybridization probes to an analyte of interest, or a complement thereof, can be designed. In some embodiments, about 10 to about 500 hybridization probes, about 25 to about 450 hybridization probes, about 50 to about 400 hybridization probes, about 75 to

about 350 hybridization probes, or about 100 to 300 hybridization probes can be designed for hybridizing to an analyte of interest, or a complement thereof. In some embodiments, the hybridization probes can include an additional moiety, such as a binding moiety, (e.g., biotin) capable of binding another moiety, such as a capture moiety, (e.g., streptavidin). Thus, in
5 some embodiments, one or more hybridization probes (e.g., including an additional moiety, such as biotin) hybridize to the analyte of interest, or complement thereof, in the cDNA library and the total cDNA library is processed on streptavidin beads, for example. The biotin moieties of the hybridization probes specifically bind the streptavidin molecules, thereby enriching for the analytes of interest, or complements thereof. Hybridization probes
10 can be designed to be complementary to any analyte or its complementary sequence, including, for example, analytes encoding immune cell analytes.

In some embodiments, enriching analytes of interest includes the use of blocking probes. Blocking probes can be added to the cDNA library before, after, or concurrently with hybridization probes. In some embodiments, blocking probes reduce background (e.g., non-
15 specific binding events) when enriching for targets within the cDNA library. In some embodiments, blocking probes can be about 5, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, about 50, about 55, about 60, about 65, about 70, about 75, about 80, about 85, about 90, about 95, about 100, about 105, about 110, about 115, about 120, about 125, about 130, about 135, about 140, about 145, or about 150 nucleotides long.
20 In some embodiments, blocking probes are designed specifically to domains present in one or more members of the cDNA library. In some embodiments, one blocking probe is added to the cDNA library. In some embodiments, two or more blocking probes (e.g., different blocking probes). In some embodiments, 3, 4, 5 or more different blocking probes are added to the cDNA library (e.g., blocking probes having a different sequence). In some
25 embodiments, the blocking probe comprises SEQ ID NO: 639. In some embodiments, the blocking probe comprises SEQ ID NO: 640. In some embodiments, the blocking probe comprises at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 639. In some embodiments, the blocking probe comprises at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least
30 97%, at least 98%, or at least 99% identity to SEQ ID NO: 640.

Identifying Immune Cell Receptors

In some embodiments of determining the presence and/or abundance of an immune cell clonotype at a location in a biological sample, determining in step (b) includes

sequencing (i) all or a portion of the sequence of the spatial barcode or a complement thereof, and (ii) all or a portion of the sequence of the nucleic acid encoding the immune cell receptor or a complement thereof. Any of the sequencing methods described herein can be used. In some embodiments, step (b) includes determining the presence of the immune cell clonotype at a location in the biological sample. In some embodiments, step (b) includes determining the abundance of the immune cell clonotype at a location in the biological sample. In some embodiments, step (b) includes determining the presence and abundance of the immune cell clonotype at a location in the biological sample. In some embodiments, step (b) includes determining the presence of two or more immune cell clonotypes at a location in the biological sample. In some embodiments, step (b) includes determining the abundance of two or more immune cell clonotypes at a location in the biological sample. In some embodiments, step (b) includes determining the presence and abundance of two or more immune cell clonotypes at a location in the biological sample. In some embodiments, the method includes comparing the two or more immune cell clonotypes. In some embodiments, the two or more immune cell clonotypes are each a B cell clonotype. In some embodiments, the two or more immune cell clonotypes are each a T cell clonotype. In some embodiments, the two or more immune cell clonotypes include at least one T cell clonotype and at least one B cell clonotype.

In some embodiments of determining the presence and/or abundance of an immune cell receptor at a location in a biological sample, the determining in step (b) includes sequencing (i) all or a portion of the sequence of the spatial barcode or a complement thereof, and (ii) all or a portion of the sequence of the nucleic acid encoding the immune cell receptor or a complement thereof. In some embodiments, step (b) includes determining the presence of the immune cell receptor at a location in the biological sample. In some embodiments, step (b) includes determining the abundance of the immune cell receptor at a location in the biological sample. In some embodiments, step (b) includes determining the presence and abundance of the immune cell receptor at a location in the biological sample. In some embodiments, step (b) includes determining the presence of two or more immune cell receptors at a location in the biological sample. In some embodiments, step (b) includes determining the abundance of two or more immune cell receptors at a location in the biological sample. In some embodiments, step (b) includes determining the presence and abundance of two or more immune cell receptors at a location in the biological sample. In some embodiments, the method includes comparing the two or more immune cell receptors. In some embodiments, the two or more immune cell clonotypes are each an immune cell

receptor of a B cell. In some embodiments, two or more immune cell clonotypes are each an immune cell receptor of a T cell. In some embodiments, two or more immune cell clonotypes include at least one immune cell receptor of a T cell and at least one immune cell receptor from a B cell.

- 5 In some embodiments of determining the presence and/or abundance of an immune cell clonotype or an immune cell receptor at a location in a biological sample, includes prior to step (b), contacting the biological sample with ribosomal RNA depletion probes and/or mitochondrial RNA depletion probes. In some embodiments, the biological sample is imaged. In some embodiments, the biological sample is stained.

10

Arrays and Kits

- Provided herein are arrays including a plurality of capture probes, where a capture probe of the plurality of capture probes includes (i) a spatial barcode and (ii) a capture domain that specifically binds to a nucleic acid encoding an immune cell receptor of an
- 15 immune cell clonotype. In some arrays, the immune cell clonotype is a T cell clonotype. In some arrays, the immune cell receptor is a T cell receptor alpha chain. In some arrays, the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the T cell receptor alpha chain. In some arrays, the immune cell receptor is a T cell receptor beta chain. In some arrays, the capture domain binds specifically to a nucleic acid sequence
- 20 encoding a constant region of the T cell receptor beta chain. In some arrays, the immune cell clonotype is a B cell clonotype. In some arrays, the immune cell receptor is an immunoglobulin kappa light chain. In some arrays, the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin kappa light chain. In some arrays, the immune cell receptor is an immunoglobulin lambda light chain. In some
- 25 arrays, the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin lambda light chain. In some arrays, the immune cell receptor is an immunoglobulin heavy chain. In some arrays, the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin heavy chain. In some arrays, the capture probe includes a cleavage domain, a functional domain, a unique
- 30 molecular identifier, or any combination thereof.

Also provided herein are kits including an array (e.g., any of the arrays described herein) and one or more hybridization probes, wherein a hybridization probe includes (i) a sequence substantially complementary to a nucleic acid encoding an immune cell receptor

and (ii) a binding moiety that interacts with a capturing moiety and one or more blocking probes.

Also provided herein are kits, including an array of any of the arrays described herein and one or both of ribosomal RNA depletion probes and mitochondrial RNA depletion probes.

Targeted RNA depletion allows for depletion or removal of one or more species of undesirable RNA molecules (e.g., ribosomal RNA and/or mitochondrial RNA), thereby reducing the pool and concentration of undesirable RNA molecules in the sample which could interfere with desired target detection (e.g., detection of mRNA). To achieve depletion, one or more probes are designed that hybridize to one or more undesirable RNA molecules. For example, in one embodiment, probes can be administered to a biological sample that selectively hybridize to ribosomal RNA (rRNA), thereby reducing the pool and concentration of rRNA in the sample. In one embodiment, probes can be administered to a biological sample that selectively hybridize to mitochondria RNA (mtRNA), thereby reducing the pool and concentration of mtRNA in the sample. Subsequent application of capture probes to the sample can result in improved capture of other types of RNA due to a reduction in undesirable RNA (e.g., down-selected RNA) present in the sample.

Upon depletion of the undesirable RNA, the sample will contain an enriched population of the RNA target of interest (e.g., an mRNA target). In some embodiments, the undesirable RNA comprises less than 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1%, or any range therein, of the total RNA in the sample after depletion of the undesirable RNA (i.e., less than 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1%, or any range therein compared to a sample that undergoes no depletion step). Consequently, the enriched population of the RNA target of interest may comprise at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, or 80%, or any range therein, of the total RNA in the sample.

As used herein, the term “undesirable RNA molecule”, or “undesirable RNA”, refers to an undesired RNA that is the target for depletion from the biological sample. In some embodiments, examples of the undesirable RNA include, but are not limited to, messenger RNA (mRNA), ribosomal RNA (rRNA), mitochondrial RNA (mtRNA), transfer RNA (tRNA), microRNA (miRNA), and viral RNA. In some embodiments, the undesirable RNA can be a transcript (e.g., present in a tissue section). The undesirable RNA can be small (e.g.,

less than 200 nucleic acid bases in length) or large (e.g., RNA greater than 200 nucleic acid bases in length).

In some embodiments, the undesirable RNA molecule includes 5.8S ribosomal RNA (rRNA), 5S rRNA, transfer RNA (tRNA), microRNA (miRNA), a small nucleolar RNA (snoRNAs), Piwi-interacting RNA (piRNA), tRNA-derived small RNA (tsRNA), and small rDNA-derived RNA (srRNA), or mitochondrial RNA (mtRNA). In some embodiments, the undesirable RNA molecule includes an RNA molecule that is added (e.g., transfected) into a sample (e.g., a small interfering RNA (siRNA)). The undesirable RNA can be double-stranded RNA or single-stranded RNA. In embodiments where the undesirable RNA is double-stranded it is processed as a single-stranded RNA prior to depletion. In some embodiments, the undesirable RNA can be circular RNA. In some embodiments, the undesirable RNA can be a bacterial rRNA (e.g., 16S rRNA or 23S rRNA). In some embodiments, the undesirable RNA is from *E. coli*.

In some embodiments, the undesirable RNA molecule is rRNA. In some embodiments, the rRNA is eukaryotic rRNA. In some embodiments, the rRNA is cytoplasmic rRNA. In some embodiments, the rRNA is mitochondrial rRNA. Cytoplasmic rRNAs include, for example, 28S, 5.8S, 5S and 18S rRNAs. Mitochondrial rRNAs include, for example, 12S and 16S rRNAs. The rRNA may also be prokaryotic rRNA, which includes, for example, 5S, 16S, and 23S rRNA. The sequences for rRNAs are well known to those skilled in the art and can be readily found in sequence databases such as GenBank or may be found in the literature. For example, the sequence for the human 18S rRNA can be found in GenBank as Accession No. M10098 and the human 28S rRNA as Accession No. M11167.

In some embodiments, the undesirable RNA molecule is mitochondrial RNA. Mitochondrial RNAs include, for example, 12S rRNA (encoded by MT-RNR1), and 16S rRNA (encoded by MT-RNR2), RNAs encoding electron transport chain proteins (e.g., NADH dehydrogenase, coenzyme Q- cytochrome c reductase / cytochrome b, cytochrome c oxidase, ATP synthase, or humanin), and tRNAs (encoded by MT-TA, MT-TR, MT-TN, MT-TD, MT-TC, MT-TE, MT-TQ, MT-TG, MT-TH, MT-TI, MT-TL1, MT-TL2, MT-TK, MT-TM, MT-TF, MT-TP, MT-TS1, MT-TS2, MT-TT, MT-TW, MT-TY, or MT-TV).

In some embodiments, the one or more undesirable RNA depletion probes is a DNA probe. In some embodiments, the DNA probe includes a single-stranded DNA oligonucleotide having a sequence partially or completely complementary to an undesirable RNA and specifically hybridizes to the undesirable RNA. In some embodiments, the one or more undesirable RNA depletion probes are at least 70%, at least 75%, at least 80%, at least

85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to one or more undesirable RNA molecules. In some embodiments, the one or more undesirable RNA depletion probes is 100% (i.e., completely) complementary to one or more undesirable RNA molecules.

5 In some embodiments, probes used herein have been described in Morlan et al., PLoS One. 2012;7(8):e42882, which is incorporated by reference in its entirety. In some embodiments, probes used herein have been described in U.S. Appl. Publ. No. 2011/0111409, which is incorporated by reference in its entirety. In some embodiments, probes used herein have been described in Adiconis et al., Nat Methods. 2013 Jul;10(7):623-
10 9, which is incorporated by reference in its entirety.

The DNA probe can be produced by techniques known in the art. For example, in some embodiments, a DNA probe is produced by chemical synthesis, by in vitro expression from recombinant nucleic acid molecules, or by in vivo expression from recombinant nucleic acid molecules. The undesirable RNA depletion probe may also be produced by amplification
15 of the undesirable RNA, e.g., RT-PCR, asymmetric PCR, or rolling circle amplification.

Examples

Example 1: Analyte Capture and Enrichment Strategies

FIGs. 3A and 3B show two different capture probes with different exemplary capture
20 strategies to capture analytes encoding immune cell receptors in a biological sample. **FIG. 3A** shows “targeted” capture where the capture domain is substantially complementary to the constant region of the analyte encoding an immune cell receptor to be detected. Targeted capture increases the likelihood that the portion of interest in the variable domain, CDR3, is retained during library preparation. Alternatively, **FIG. 3B** shows poly(A) capture with a
25 poly(T) capture domain. A poly(T) capture domain can capture other analytes, including analytes encoding immune cell receptors within the biological sample.

FIG. 4 shows an exemplary analyte enrichment strategy following analyte capture on the array. The portion of the immune cell analyte of interest includes the sequence of the V(D)J region. The CDR sequences are also important because these sequences define the
30 immune cell receptor’s binding specificity. As described herein, a poly(T) capture probe captures an analyte encoding an immune cell receptor, an extended capture probe is generated by a reverse transcription reaction, and a second strand is generated. The resulting nucleic acid library can be enriched by the exemplary scheme shown in **FIG. 4**, where an

amplification reaction including a Read 1 primer complementary to the Read 1 sequence of the capture probe and a primer complementary to a portion of the variable region of the immune cell analyte, can enrich the library via PCR. The enriched library can be further enriched by nested primers complementary to a portion of the variable region internal (e.g., 5') to the initial variable region primer for practicing nested PCR.

FIG. 5 shows a sequencing strategy with a primer specific complementary to the sequencing flow cell attachment sequence (e.g., P5) and a custom sequencing primer complementary to a portion of the constant region of the analyte. This sequencing strategy targets the constant region to obtain the sequence of the CDR regions, including CDR3, while concurrently or sequentially sequencing the spatial barcode (BC) and/or unique molecular identifier (UMI) of the capture probe. By capturing the sequence of a spatial barcode, UMI and a V(D)J region the receptor is not only determined, but its spatial location and abundance within a cell or tissue is also identified.

15 **Example 2 – Capture of Analytes Encoding Immune Cell Receptors**

FIG. 6 shows the number of unique clonotypes detected for TRA, TRB, IGH, IGK, and IGL on an array for both lymph node tissue (LN, black) and spleen tissue (SP, gray). It is contemplated that the lack of detected clonotypes found may be the result of inefficient or decreased TRAC/TRBC/IGH transcript capture or decreased sequencing of the variable region (e.g., CDR3 region) due to its distance from the sequencing domain (e.g. Read 1 sequencing domain). A greater abundance of unique clonotypes were detected for IGK and IGL, which may be due in part to the shorter constant regions present in these clonotypes relative to the constant regions present in TRAC, TRAB, and IGH transcripts.

FIGs. 7A-B show the number of unique clonotypes detected for TRA and TRB (**FIG. 7A**) and the number of unique clonotypes detected for IGA, IGHG, IGHM, and IGHE (**FIG. 7B**). The data show that targeted capture (gray bars) yields a higher number of TRA and TRB clonotypes (**FIG. 7A**) than poly(T) capture as demonstrated by the lack of clonotypes detected. Similarly, targeted capture of IGHA, IGHG, and IGHM yielded a higher number of unique clonotypes detected than poly(T) capture, as demonstrated by the lack of clonotypes detected. Thus the data demonstrate that targeted capture of analytes encoding immune cell receptors is possible for some analytes, but may not be sufficient for other analytes encoding immune cell receptors (e.g., IGHE).

As discussed, undetectable levels of T-cell receptor and B-cell receptor transcripts were captured with poly(T) capture domains as shown in **FIGs. 7A-B**. Targeted capture,

however, requires custom capture domains for each analyte encoding an immune cell receptor and does not allow for the simultaneous capture of analytes other than targeted analytes encoding immune cell receptors.

- 5 A strategy to detect whether analytes encoding immune cell receptors were captured was investigated and includes using poly(T) capture sequences in combination with PCR amplification performed on full length cDNA from several different sources, including lymph node tissue and tonsil tissue (Table 1).

Table 1.

Lymph Node (LN) spatial library	n=6
Tonsil spatial library	n=2
Tonsil SmartSeq2 (SS2) (single-cell) RNAseq (positive control)	n=1

10

The tonsil SS2 sample was derived from the same tonsil as the tonsil spatial libraries and adapted from Picelli et al., Full-length RNA-seq from single cells using Smart-seq2, 9, 171-181, *Nature* (2014), and used as a positive control and without PCR enrichment.

- 15 To begin, 0.5 ng of each library in Table 1 as input material was run in triplicate for each sample and PCR reaction (TRB, IGHG, and IGHM), except for one LN (#9) and the Tonsil SS2 bulk sample, which were run in duplicate and once, respectively. The PCR primers targeted: a) the constant region of either TRB, IGHG, or IGHM (Table 2), and b) the variable segments for TRB (Balazs, A.B., et al., Isolation of unknown rearranged T-cell receptors from single cells WO 2011/008502, which is incorporated herein by reference in its entirety) and IGH (Vázquez, B., et al., High-Quality Library Preparation for NGS-Based Immunoglobulin Germline Gene Inference and Repertoire Expression Analysis, *Frontiers Immunol*, 10, 660 (2019), which is incorporated herein by reference in its entirety). The constant primers were selected based on their proximity to the CDR3 region and testing of various primers for each target was performed in PCR optimization experiments. Both the forward and reverse primers were tagged with partial P5 and P7 domains that allowed subsequent Truseq indexing for Illumina sequencing. PCR was performed using the KAPA HiFi Hotstart ready mix according to the manufacturer's instructions with 30 amplification cycles.
- 25

Table 2.

Constant Primer Sequence

TRB SEQ ID NO: 1 TCTGATGGCTCAAACACAGC
 IGHG SEQ ID NO: 2 GCCAGGGGGAAGACCGATGGG
 IGHM SEQ ID NO: 3 CACGCTGCTCGTATCCGA

5

Table 3.

Variable Region Primer	Sequence
<u>TCRa V inner pool</u>	
TCRaV17	SEQ ID NO: 4 CAACAGGGAGAAGAGGATCCTCAGGCC
10 TCRaV1-2	SEQ ID NO: 5 GGACAAAAACATTGACCAGCCCACTGAGAT
TCRaV10	SEQ ID NO: 6 AAAAACCAGTGGAGCAGAGTCCTCAGTCC
TCRaV12-1	SEQ ID NO: 7 CAACGGAAGGAGGTGGAGCAGGATC
TCRaV12-2	SEQ ID NO: 8 CAACAGAAGGAGGTGGAGCAGAATTCTGG
TCRaV12-3	SEQ ID NO: 9 CAACAGAAGGAGGTGGAGCAGGATCCT
15 TCRaV13-1	SEQ ID NO: 10 GAGAATGTGGAGCAGCATCCTTCAACC
TCRaV13-2	SEQ ID NO: 11 GAGAGTGTGGGGTGCATCTTCTTACC
TCRaV14D4	SEQ ID NO: 12 CAGAAGATAACTCAAACCCAACCAGGAATGTTT
TCRaV16	SEQ ID NO: 13 CAGAGAGTGACTCAGCCCGAGAAGCTC
TCRaV18	SEQ ID NO: 14 GACTCGGTACCCAGACAGAAGGCC
20 TCRaV19	SEQ ID NO: 15 CAGAAGGTAATCAAGCGCAGACTGAAATTTCT
TCRaV2	SEQ ID NO: 16 AAGGACCAAGTGTTCAGCCTTCCACAGTG
TCRaV20	SEQ ID NO: 17 GAAGACCAGGTGACGCAGAGTCCCG
TCRaV21	SEQ ID NO: 18 AAACAGGAGGTGACGCAGATTCTTGC
TCRaV22	SEQ ID NO: 19 ATACAAGTGGAGCAGAGTCTCCAGACCTGA
25 TCRaV23DV6	SEQ ID NO: 20 CAACAGAAGGAGAAAAAGTGACCAGCAGCA
TCRaV24	SEQ ID NO: 21 ATACTGAACGTGGAACAAAAGTCTCAGTCACTG
TCRaV25	SEQ ID NO: 22 CAACAGGTAATGCAAATTCCTCAGTACCAGC
TCRaV26-1	SEQ ID NO: 23 AAGACCACCCAGCCCCCTCC
TCRaV26-2	SEQ ID NO: 24 AAGACCACACAGCCAAATTCAATGGAGAGTAAC
30 TCRaV27	SEQ ID NO: 25 CAGCTGTGGAGCAGAGCCCTCAGT
TCRaV29DV5	SEQ ID NO: 26 CAACAGAAGAATGATGACCAGCAAGTTAAGCAA
TCRaV3	SEQ ID NO: 27 CAGTCAGTGGCTCAGCCGGAAGATC
TCRaV30	SEQ ID NO: 28 CAACAACCAAGTGACAGATCCTCAAGCC
TCRaV34	SEQ ID NO: 29 CAAGAAGTGGAGCAGAGTCTCAGTCTTG
35 TCRaV35	SEQ ID NO: 30 CAACAGCTGAATCAGAGTCTCAATCTATGTTTATC
TCRaV36DV7	SEQ ID NO: 31 GAAGACAAGGTGGTACAAAGCCCTCTATCTCTG
TCRaV38-2DV8	SEQ ID NO: 32 CAGACAGTCACTCAGTCTCAACCAGAGATGTCT
TCRaV39	SEQ ID NO: 33 GAGCTGAAAGTGGAACAAAACCTCTGTTC
TCRaV4	SEQ ID NO: 34 AAGACCACCCAGCCCATCTCCATG
40 TCRaV40	SEQ ID NO: 35 AATTCAGTCAAGCAGACGGGCCAAATAAC
TCRaV41	SEQ ID NO: 36 GCCAAAAATGAAGTGGAGCAGAGTCTCTC
TCRaV5	SEQ ID NO: 37 GAGGATGTGGAGCAGAGTCTTTCTCCTGAGTG
TCRaV6	SEQ ID NO: 38 CAAAAGATAGAACAGAATTCCGAGGCCCTG
TCRaV7	SEQ ID NO: 39 GAAAACCAGGTGGAGCACAGCCCTC
45 TCRaV8-1	SEQ ID NO: 40 CAGTCTGTGAGCCAGCATAACCACCAC
TCRaV8-2	SEQ ID NO: 41 CAGTCGGTGACCCAGCTTGACAGC
TCRaV8-3	SEQ ID NO: 42 CAGTCAGTGACCCAGCCTGACATCCAC
TCRaV8-4	SEQ ID NO: 43 CAGTCGGTGACCCAGCTTGCCAG
TCRaV8-6	SEQ ID NO: 44 CAGTCTGTGACCCAGCTTGACAGCCA
50 TCRaV8-7	SEQ ID NO: 45 CAGTCGGTGACCCAGCTTGATGGC
TCRaV9-1	SEQ ID NO: 46 GATTCAGTGGTCCAGACAGAAGGCCAAGT
TCRaV9-2	SEQ ID NO: 47 AATTCAGTGACCCAGATGGAAGGGCC
<u>TCRb V Inner Pool</u>	
55 TCRb_JM_V2	SEQ ID NO: 48 GAACCTGAAGTCACCCAGACTCCCAGC

	TCRb_JM_V3-1	SEQ ID NO: 49 GCTGTTTCCCAGACTCCAAAATACCTGGTC
	TCRb_JM_V4-1	SEQ ID NO: 50 GAAGTTACCCAGACACCAAAACACCTGGTC
	TCRb_JM_V5-1	SEQ ID NO: 51 GGAGTCACTCAAACCTCCAAGATATCTGATCAAAAC
	TCRb_JM_V6-1	SEQ ID NO: 52 GGTGTCACTCAGACCCCAAAATTCCAG
5	TCRb_JM_V7-1	SEQ ID NO: 53 GGAGTCTCCCAGTCCCTGAGACACAAGG
	TCRb_JM_V4-2	SEQ ID NO: 54 GGAGTTACGCAGACACCAAGACACCTGG
	TCRb_JM_V6-2	SEQ ID NO: 55 GGTGTCACTCAGACCCCAAAATTCCG
	TCRb_JM_V7-2	SEQ ID NO: 56 GGAGTCTCCCAGTCCCCCAGTAACAAG
	TCRb_JM_V6-4	SEQ ID NO: 57 GGGATCACCCAGGCACCAACATCTC
10	TCRb_JM_V7-3	SEQ ID NO: 58 GGAGTCTCCCAGACCCCCAGTAACAAG
	TCRb_JM_V5-3	SEQ ID NO: 59 GGAGTCACCCAAAGTCCCACACACCT
	TCRb_JM_V9	SEQ ID NO: 60 GGAGTCACACAAAACCCCAAGCACCT
	TCRb_JM_V10-1	SEQ ID NO: 61 GAAATCACCCAGAGCCCAAGACACAAGA
	TCRb_JM_V11-1	SEQ ID NO: 62 GAAGTTGCCCAGTCCCCCAGATATAAGATTA
15	TCRb_JM_V10-2	SEQ ID NO: 63 GGAATCACCCAGAGCCCAAGATACAAGAT
	TCRb_JM_V11-2	SEQ ID NO: 64 GGAGTTGCCCAGTCTCCCAGATATAAGATTATAGAG
	TCRb_JM_V7-4	SEQ ID NO: 65 GGAGTCTCCCAGTCCCCAAGGTACAAAG
	TCRb_JM_V7-5	SEQ ID NO: 66 GGAGTCTCCCAGTCCCCAAGGTACGA
	TCRb_JM_V6-7	SEQ ID NO: 67 GGTGTCACTCAGACCCCAAAATTCCAC
20	TCRb_JM_V7-6	SEQ ID NO: 68 GGAGTCTCCCAGTCTCCCAGGTACAAAGTC
	TCRb_JM_V6-8	SEQ ID NO: 69 GGTGTCACTCAGACCCCAAAATTCCACAT
	TCRb_JM_V7-8	SEQ ID NO: 70 GGAGTCTCCCAGTCCCCCTAGGTACAAAGTC
	TCRb_JM_V5-8	SEQ ID NO: 71 GGAGTCACACAAAGTCCCACACACCTGA
	TCRb_JM_V7-9	SEQ ID NO: 72 GGAGTCTCCAGAACCCCAAGACACAAG
25	TCRb_JM_V13	SEQ ID NO: 73 GGAGTCATCCAGTCCCCAAGACATCTGAT
	TCRb_JM_V12-3	SEQ ID NO: 74 GGAGTTATCCAGTCAACCCCGCCATG
	TCRb_JM_V12-4	SEQ ID NO: 75 GGAGTTATCCAGTCAACCCCGGCAC
	TCRb_JM_V12-5	SEQ ID NO: 76 AGAGTCACCCAGACACCAAGGCACAAG
	TCRb_JM_V14	SEQ ID NO: 77 GGAGTTACTAGTCTCCCAGCCACAGC
30	TCRb_JM_V15	SEQ ID NO: 78 ATGGTCATCCAGAACCCCAAGATACCAGGTT
	TCRb_JM_V17	SEQ ID NO: 79 GAGCCTGGAGTCAGCCAGACCCC
	TCRb_JM_V18	SEQ ID NO: 80 GGCCTCATGCAGAACCCCAAGACAC
	TCRb_JM_V19	SEQ ID NO: 81 GGAATCACTCAGTCCCCAAAGTACCTGTTC
	TCRb_JM_V20-1	SEQ ID NO: 82 GCTGTCGTCTCTCAACATCCGAGCTG
35	TCRb_JM_V22	SEQ ID NO: 83 ATTCAGCTCACTGGGGCTGGATG
	TCRb_JM_V23-1	SEQ ID NO: 84 AAAGTCACACAGACTCCAGGACATTTGGTCA
	TCRb_JM_V24-1	SEQ ID NO: 85 GATGTTACCCAGACCCCAAGGAATAGGATC
	TCRb_JM_V25-1	SEQ ID NO: 86 GACATCTACCAGACCCCAAGATACCTTGTATAGG
	TCRb_JM_V26	SEQ ID NO: 87 GTAGTTACACAATTCCCAAGACACAGAATCATTTGG
40	TCRb_JM_V27	SEQ ID NO: 88 CAAGTGACCCAGAACCCCAAGATACCTCATC

IGH V pool

	IGH_MTPX_1	SEQ ID NO: 89 GGTGGCAGCAGTCACAGATGCCTACTC
	IGH_MTPX_2	SEQ ID NO: 90 GGTGGCAGCAGCCACAGGTGCCCACTC
45	IGH_MTPX_3	SEQ ID NO: 91 GGTGGCAGCAGCTACAGGTGTCCAGTC
	IGH_MTPX_4	SEQ ID NO: 92 GGTGGSAGCAGCAACARGWGCCCCACTC
	IGH_MTPX_5	SEQ ID NO: 93 GCTGGCTGTAGCTCCAGGTGCTCACTC
	IGH_MTPX_6	SEQ ID NO: 94 CCTGCTGCTGACCAYCCCTTCMTGGGTCTTGTC
	IGH_MTPX_7	SEQ ID NO: 95 CCTGCTACTGACTGTCCCGTCCTGGGTCTTATC
50	IGH_MTPX_8	SEQ ID NO: 96 GGGTTTTTCCTCGTTGCTCTTTTAAAGAGGTGTCCAGTG
	IGH_MTPX_9	SEQ ID NO: 97 GGGTTTTTCCTTGTTGCTATTTTAAAAGGTGTCCARTG
	IGH_MTPX_10	SEQ ID NO: 98 GGATTTTTCCTTGCTGCTATTTTAAAAGGTGTCCAGTG
	IGH_MTPX_11	SEQ ID NO: 99 GGGTTTTTCCTTKTKGCTATWTTAGAAGGTGTCCAGTG
	IGH_MTPX_12	SEQ ID NO: 100 GGTGGCRGCTCCCAGATGGGTCCTGTGTC
55	IGH_MTPX_13	SEQ ID NO: 101 CTGGCTGTTCTCCAAGGAGTCTGTG
	IGH_MTPX_14	SEQ ID NO: 102 GGCCTCCCATGGGGTGTCTGTGTC
	IGH_MTPX_15	SEQ ID NO: 103 GGTGGCAGCAGCAACAGGTGCCCACT
	IGH_MTPX_16	SEQ ID NO: 104 ATGGAACTGGGGCTCCGCTGGGTTTTC
	IGH_MTPX_17	SEQ ID NO: 105 ATGGACTGCACCTGGAGGATCCTCCTC
60	IGH_MTPX_18	SEQ ID NO: 106 TGGCTGAGCTGGGTTTYCCTTGTTGC

	IGH_MTPX_19	SEQ ID NO: 107 GGAGTTKGGGCTGMGCTGGGTTTTCC
	IGH_MTPX_20	SEQ ID NO: 108 GCACCTGTGGTTTTTCCTCCTGCTGGTG
	IGH_MTPX_21	SEQ ID NO: 109 CACCTGTGGTTCTCCTCCTSCTGG
	IGH_MTPX_22	SEQ ID NO: 110 CCAGGATGGGGTCAACCGCCATCCTC
5	IGH_MTPX_23	SEQ ID NO: 111 CAGAGGACTCACCATGGAGTTTGGGCTGAG
	IGH_MTPX_24	SEQ ID NO: 112 GGA CT CACCATGGAGTTGGGACTGAGC
	IGH_MTPX_25	SEQ ID NO: 113 GGGCTGAGCTGGCTTTTTCTTGTGGC
	TSO Sequence	SEQ ID NO: 114 AAGCAGTGGTATCAACGCAGAGTACATGGG
10	TSO Sequence Portion	SEQ ID NO: 115 TCTGCGTTGATACCACT

CDR3

	TRAV1-2	SEQ ID NO: 116 gaaggagctccagatgaaagactctgcctc
	TRAV2	SEQ ID NO: 117 gttctcttcacgcgtgctcatcctccaggt
15	TRAV3	SEQ ID NO: 118 ctgtgagcgactccgctttgtacttctgt
	TRAV4	SEQ ID NO: 119 ttatccctgccgacagaaagtcagcactc
	TRAV5	SEQ ID NO: 120 aaggataaacatctgtctctgcgcattgcag
	TRAV6	SEQ ID NO: 121 ttgttcatacacagcctccagcctgca
	TRAV7	SEQ ID NO: 122 tacattacagccgtgcagcctgaagattcag
20	TRAV8-1	SEQ ID NO: 123 aatctgaggaaaccctctgtgcagtgaggt
	TRAV8-2	SEQ ID NO: 124 gaaacctccttcacctgacgaaacctca
	TRAV8-3	SEQ ID NO: 125 caatctgaggaaaccctctgtgcattggag
	TRAV8-4	SEQ ID NO: 126 cacctgacgaaaccctcagccatagagc
	TRAV8-6	SEQ ID NO: 127 gga aaccctcagtcataataagcgacacgg
25	TRAV8-7	SEQ ID NO: 128 gaggaaacctcaacccatgtgagtgatgc
	TRAV9-1	SEQ ID NO: 129 ggaaggaacaaaggtttgaagccatgtaccg
	TRAV9-2	SEQ ID NO: 130 tccacttggagaaaggctcagttcaaggt
	TRAV10	SEQ ID NO: 131 gcagacacaaagcaagctctctgcacatc
	TRAV12-1	SEQ ID NO: 132 gccagccagtataattccctgctcatcaga
30	TRAV12-2	SEQ ID NO: 133 gccagccagtattgttctctgctcatcaga
	TRAV12-3	SEQ ID NO: 134 ggtttacagcacaggtcgataaatccagca
	TRAV13-1	SEQ ID NO: 135 gccaaacatttctccctgcacatcacagag
	TRAV13-2	SEQ ID NO: 136 tctgcaaattgcagctactcaacctggaga
	TRAV14DV4	SEQ ID NO: 137 gccaacctgtcatctccgttcacaactg
35	TRAV16	SEQ ID NO: 138 gaccttaacaaaggcgagacatctttccacc
	TRAV17	SEQ ID NO: 139 gtcacgcttgacacttccagaaaagcagt
	TRAV18	SEQ ID NO: 140 cctatcaagagtgcagttcctccacctg
	TRAV19	SEQ ID NO: 141 ggaacttcagaaatccaccagttcctca
	TRAV20	SEQ ID NO: 142 agaaggaaagcttctgcacatcacagcc
40	TRAV21	SEQ ID NO: 143 caagtggagactaatgcctcgtggata
	TRAV22	SEQ ID NO: 144 gactgtcgtacggaacgctacagcttatt
	TRAV23DV6	SEQ ID NO: 145 tgccaagcagttctcatcgcatatcatgga
	TRAV24	SEQ ID NO: 146 gccactttaataccaaggagggttacagc
	TRAV25	SEQ ID NO: 147 cacatcacagcccccagactacagatgta
45	TRAV26-1	SEQ ID NO: 148 tcatcacagaagacagaaagtcagcacct
	TRAV26-2	SEQ ID NO: 149 agaaagtcaggtaccttgatcctgcaccgt
	TRAV27	SEQ ID NO: 150 gttctctccatcactgcagcccagactg
	TRAV29DV5	SEQ ID NO: 151 aaagtccaagcacctctctctgcacattg
	TRAV30	SEQ ID NO: 152 ctgtaccttacggcctcccagctcagttac
50	TRAV34	SEQ ID NO: 153 gccagttggatgagaaaaagcagcaaggt
	TRAV35	SEQ ID NO: 154 gacctcaaatggaagactgactgctcagtt
	TRAV36DV7	SEQ ID NO: 155 tttcagatcctgaacatcacagccacca
	TRAV38-2DV8	SEQ ID NO: 156 cettcagttcaagatctcagactcacagc
	TRAV39	SEQ ID NO: 157 aatggcctcactgataccaaagcccgtc
55	TRAV40	SEQ ID NO: 158 ctccccattgtgaaatattcagtcagggt
	TRAV41	SEQ ID NO: 159 catacaggaaaagcacagctccctgcacat
	TRAV11	SEQ ID NO: 160 atatcgagcctctcatctgggagattcagc
	TRAV1-1	SEQ ID NO: 161 caggagctccagatgaaagactctgcctctt
60	TRAV8-5	SEQ ID NO: 162 acttcttccacttgaggaaacctcagtcga

Inner TRAV Primers

TRAV-Handle 1	SEQ ID NO: 163	gtgactggagttcagacgtgtgctctccgatctgaaggagctccagatgaaagactctgcctc
TRAV-Handle 2	SEQ ID NO: 164	gtgactggagttcagacgtgtgctctccgatctgttctctcatcgctctcatctccaggt
TRAV-Handle 3	SEQ ID NO: 165	gtgactggagttcagacgtgtgctctccgatcttctgtgagcgactccgtttgtactctgt
5 TRAV-Handle 4	SEQ ID NO: 166	gtgactggagttcagacgtgtgctctccgatctttatccctgccagacagaaagtcagcactc
TRAV-Handle 5	SEQ ID NO: 167	gtgactggagttcagacgtgtgctctccgatctaaagataaacatctgtctctgcgcatggag
TRAV-Handle 6	SEQ ID NO: 168	gtgactggagttcagacgtgtgctctccgatctttgttcatatcacagcctccagcctgca
TRAV-Handle 7	SEQ ID NO: 169	gtgactggagttcagacgtgtgctctccgatcttacctacagccgtgcagcctgaagattcag
TRAV-Handle 8	SEQ ID NO: 170	gtgactggagttcagacgtgtgctctccgatctaatctgaggaaacccctctgtcagtgagggt
10 TRAV-Handle 9	SEQ ID NO: 171	gtgactggagttcagacgtgtgctctccgatctgaaacctcttccacctgacgaaacctca
TRAV-Handle 10	SEQ ID NO: 172	gtgactggagttcagacgtgtgctctccgatctcaatctgaggaaacccctctgtcattggag
TRAV-Handle 11	SEQ ID NO: 173	gtgactggagttcagacgtgtgctctccgatctcacctgacgaaacctcagcccatatgagc
TRAV-Handle 12	SEQ ID NO: 174	gtgactggagttcagacgtgtgctctccgatctgaaacctcagtcacataaagcgacacgg
TRAV-Handle 13	SEQ ID NO: 175	gtgactggagttcagacgtgtgctctccgatctgaggaaacctcaacctatgtgagtgtgc
15 TRAV-Handle 14	SEQ ID NO: 176	gtgactggagttcagacgtgtgctctccgatctggaaggaaacaaagtttgaagccatgtaccg
TRAV-Handle 15	SEQ ID NO: 177	gtgactggagttcagacgtgtgctctccgatcttccacctggagaaaggtcagttcaagtgt
TRAV-Handle 16	SEQ ID NO: 178	gtgactggagttcagacgtgtgctctccgatctgcagacacaaagcgaagctctctgcacatc
TRAV-Handle 17	SEQ ID NO: 179	gtgactggagttcagacgtgtgctctccgatctgcagccagtatatttctctgtcatcaga
20 TRAV-Handle 18	SEQ ID NO: 180	gtgactggagttcagacgtgtgctctccgatctgcagccagtatgttctctgtcatcaga
TRAV-Handle 19	SEQ ID NO: 181	gtgactggagttcagacgtgtgctctccgatctgtttacagcacaggtcgataaatccagca
TRAV-Handle 20	SEQ ID NO: 182	gtgactggagttcagacgtgtgctctccgatctgccaacatttctccctgcacatcacagag
TRAV-Handle 21	SEQ ID NO: 183	gtgactggagttcagacgtgtgctctccgatcttctgcaattgcagctactcaacctggaga
TRAV-Handle 22	SEQ ID NO: 184	gtgactggagttcagacgtgtgctctccgatctgccaacctgtcatctccgtctcacaactg
25 TRAV-Handle 23	SEQ ID NO: 185	gtgactggagttcagacgtgtgctctccgatctgaccttaacaaagcgagacatcttctccacc
TRAV-Handle 24	SEQ ID NO: 186	gtgactggagttcagacgtgtgctctccgatctgtcacgttgacacttccaaagaaagcaggt
TRAV-Handle 25	SEQ ID NO: 187	gtgactggagttcagacgtgtgctctccgatctctatcaagagtgacagttctctccacctg
TRAV-Handle 26	SEQ ID NO: 188	gtgactggagttcagacgtgtgctctccgatctggaactccagaaatccaccagttctctca
TRAV-Handle 27	SEQ ID NO: 189	gtgactggagttcagacgtgtgctctccgatctagaaggaaagctttctgcacatcacagcc
30 TRAV-Handle 28	SEQ ID NO: 190	gtgactggagttcagacgtgtgctctccgatctcaagtggaagacttaattgcctcgtcgata
TRAV-Handle 29	SEQ ID NO: 191	gtgactggagttcagacgtgtgctctccgatctgactgtcgtacggaacgtctacagcttatt
TRAV-Handle 30	SEQ ID NO: 192	gtgactggagttcagacgtgtgctctccgatcttgccaagcagttctctatcgataatcatgga
TRAV-Handle 31	SEQ ID NO: 193	gtgactggagttcagacgtgtgctctccgatctgccactcttaataacaaaggagggttacagc
TRAV-Handle 32	SEQ ID NO: 194	gtgactggagttcagacgtgtgctctccgatctcacatcacagcccccagactacagatgta
35 TRAV-Handle 33	SEQ ID NO: 195	gtgactggagttcagacgtgtgctctccgatcttcacacagaagacagaaagtcagcacct
TRAV-Handle 34	SEQ ID NO: 196	gtgactggagttcagacgtgtgctctccgatctagaaggtccagtaccttgatcctgcaccgt
TRAV-Handle 35	SEQ ID NO: 197	gtgactggagttcagacgtgtgctctccgatctgttctctccacatcactgcagccagactg
TRAV-Handle 36	SEQ ID NO: 198	gtgactggagttcagacgtgtgctctccgatctaaagtccaagcactctctctgcacattg
TRAV-Handle 37	SEQ ID NO: 199	gtgactggagttcagacgtgtgctctccgatctctgtaccttacggcctcccagctcagttac
40 TRAV-Handle 38	SEQ ID NO: 200	gtgactggagttcagacgtgtgctctccgatctgccaagttggatgagaaaaagcagcaaatg
TRAV-Handle 39	SEQ ID NO: 201	gtgactggagttcagacgtgtgctctccgatctgacctcaaatggaagactgactgctcagtt
TRAV-Handle 40	SEQ ID NO: 202	gtgactggagttcagacgtgtgctctccgatcttttcagcatcctgaacatcacagccacca
TRAV-Handle 41	SEQ ID NO: 203	gtgactggagttcagacgtgtgctctccgatctccttcagctcaaatctcagactcacagc
TRAV-Handle 42	SEQ ID NO: 204	gtgactggagttcagacgtgtgctctccgatctaatggcctcacttgataccaaagcccgtc
45 TRAV-Handle 43	SEQ ID NO: 205	gtgactggagttcagacgtgtgctctccgatctctccccattgtgaaattatcagtcaggt
TRAV-Handle 44	SEQ ID NO: 206	gtgactggagttcagacgtgtgctctccgatctcatacaggaagcagacgtccctgcacat
TRAV-Handle 45	SEQ ID NO: 207	gtgactggagttcagacgtgtgctctccgatctatcgcagcctctcatctggagattcagc
TRAV-Handle 46	SEQ ID NO: 208	gtgactggagttcagacgtgtgctctccgatctcaggagctccagatgaaagactctgcctctt
TRAV-Handle 47	SEQ ID NO: 209	gtgactggagttcagacgtgtgctctccgatctactctccacttgaggaaacctcagtcaca
50 5' Sequence Handle LN2	SEQ ID NO: 210	gtgactggagttcagacgtgtgctctccgatct

Outer TRAV Primers

TRAV10*01_out	SEQ ID NO: 211	aaaaccaaagtgagcagagtcctcagtcctg
TRAV21*01_out	SEQ ID NO: 212	aaacaggaggtgacgcagattcctgcagctc
55 TRAV2*01_out	SEQ ID NO: 213	aaggaccaaggtttcagcctccacagtggc
TRAV8-6*02_out	SEQ ID NO: 214	accagcttgacagccaagtccctgtct
TRAV8-7*02_out	SEQ ID NO: 215	accagcttgatggccacatcactgtctct
TRAV8-4*01_out	SEQ ID NO: 216	accagcttgagccacgtctctg
TRAV19*01_out	SEQ ID NO: 217	actcaagcgcagactgaaattctgtgtgtg

	TRAV12-3*01_outer	SEQ ID NO: 218	agaaggagggtggagcaggatcctggacca
	TRAV6*01_outer	SEQ ID NO: 219	agaattccgaggctctgaacattcaggagggtaa
	TRAV16*01_outer	SEQ ID NO: 220	agagagtgactcagcccagagaagctcctct
	TRAV8-3*01_outer	SEQ ID NO: 221	agagcccagtcagtgaccagcctgac
5	TRAV8-5*01_outer	SEQ ID NO: 222	agagcccagtcagtgaccagcctgac
	TRAV27*01_outer	SEQ ID NO: 223	agctgctggagcagagccctcagtttc
	TRAV17*01_outer	SEQ ID NO: 224	agtcaacaggagagaagaggatcctcaggccttg
	TRAV18*01_outer	SEQ ID NO: 225	agtggagactcggttaccagacagaaggcc
	TRAV22*01_outer	SEQ ID NO: 226	agtggagcagagtcctccagacctgatttc
10	TRAV13-2*01_outer	SEQ ID NO: 227	agtgtggggctgcatctctaccctga
	TRAV24*01_outer	SEQ ID NO: 228	atactgaacgtggaaacaaagtcctcagtcactgcatg
	TRAV9-2*01_outer	SEQ ID NO: 229	attcagtgaccagatggaaggccagtgga
	TRAV26-1*01_outer	SEQ ID NO: 230	attgatgctaagaccacccagcccacctc
	TRAV12-2*01_outer	SEQ ID NO: 231	cagaaggagggtggagcagaattctggacccc
15	TRAV40*01_outer	SEQ ID NO: 232	cagcaattcagtcagcagacgggcca
	TRAV30*01_outer	SEQ ID NO: 233	ccaacaaccagtcgagagtcctcaagccg
	TRAV12-1*01_outer	SEQ ID NO: 234	cggaaaggagggtggagcaggatcctgga
	TRAV11-1*01_outer	SEQ ID NO: 235	ctacatacggcgagcagagtcctcattcctgag
	TRAV14/DV4*02_outer	SEQ ID NO: 236	ctcaaacccaaccaggaattctctgagcagga
20	TRAV4*01_outer	SEQ ID NO: 237	cttgtaagaccacccagcccatctccatggactc
	TRAV7*01_outer	SEQ ID NO: 238	gaaaccaggtggagcacagccctattttctg
	TRAV36/DV7*01_outer	SEQ ID NO: 239	gaagacaagggtgtacaagccctctatctctgtg
	TRAV20*01_outer	SEQ ID NO: 240	gaagaccaggtgacgcagagtcctccgag
	TRAV23/DV6*01_outer	SEQ ID NO: 241	gaccagcagcaggtgaaacaaagtcctcaat
25	TRAV41*01_outer	SEQ ID NO: 242	gagcagagtcctcagaacctgactgccc
	TRAV29/DV5*01_outer	SEQ ID NO: 243	gatgaccagcaagttaagcaaaattcaccatccct
	TRAV34*01_outer	SEQ ID NO: 244	gccaaagaactggagcagagtcctcagtc
	TRAV8-2*01_outer	SEQ ID NO: 245	gcccagtcggtgaccagcttgacag
	TRAV8-1*01_outer	SEQ ID NO: 246	gcccagtcgtgagccagcataaccaccac
30	TRAV26-2*01_outer	SEQ ID NO: 247	gcctgttcacttgcttgaaccatccac
	TRAV3*01_outer	SEQ ID NO: 248	gctcagtcagtggtcagccggaagatcagg
	TRAV1-2*01_outer	SEQ ID NO: 249	ggacaaaacattgaccagccactgagatgacagc
	TRAV1-1*01_outer	SEQ ID NO: 250	ggacaaaagccttgagcagccctctgaagtgc
	TRAV25*01_outer	SEQ ID NO: 251	ggacaaacaggtaatgcaaatctcagtcaccagcatg
35	TRAV13-1*01_outer	SEQ ID NO: 252	ggagagaatgtggagcagcatcctcaaccctg
	TRAV5*01_outer	SEQ ID NO: 253	ggagagagatgtggagcagagtccttctgagtg
	TRAV9-1*01_outer	SEQ ID NO: 254	ggagattcagtggtccagacagaaggccaagtg
	TRAV38-2/DV8*01_outer	SEQ ID NO: 255	gtctcaaccagagatgtctgtgcaggagg
	TRAV39*01_outer	SEQ ID NO: 256	gtggaacaaaacctctgttctgagcatgc
40	TRAV35*01_outer	SEQ ID NO: 257	gtggtcaacagctgaatcagagtcctcaatcta
	TRAV11*01_outer	SEQ ID NO: 258	gttccggcaggatccggggagaagact

CDR3

	TRBV10-1	SEQ ID NO: 259	gcctcctcccagacatctgtatatttctgcg
45	TRBV10-2	SEQ ID NO: 260	aatttccccctcactctggagtcagctacc
	TRBV10-3	SEQ ID NO: 261	gatttctcctcactctggagtcgctacc
	TRBV11-1	SEQ ID NO: 262	aggctcaaaggagtagactccactctcaaga
	TRBV11-2	SEQ ID NO: 263	caagatccagcctgcaaaagcttgaggact
	TRBV11-3	SEQ ID NO: 264	tagactccactctcaagatccagcctgcag
50	TRBV12-1	SEQ ID NO: 265	tggaaaccaggaggacttggccctatattct
	TRBV12-2	SEQ ID NO: 266	tcattctctactctgaagatccagcctgcag
	TRBV12-3	SEQ ID NO: 267	cattctccactctgaagatccagccctcag
	TRBV12-4	SEQ ID NO: 268	catcattctccactctgaagatccagccctc
	TRBV12-5	SEQ ID NO: 269	cagcagagatgcctgatgcaactttagcca
55	TRBV13	SEQ ID NO: 270	gaactgaacatgagctccttgagctggg
	TRBV14	SEQ ID NO: 271	ggaggattctggagtttatttctgtgccagc
	TRBV15	SEQ ID NO: 272	ttctgctttctgacatccgctcaccaggc
	TRBV16	SEQ ID NO: 273	gagatccaggctacgaagcttgaggattcag
	TRBV17	SEQ ID NO: 274	aacgtctccacgctgaagatccatccc
60	TRBV18	SEQ ID NO: 275	aggatccagcaggtagtgcgaggagattcg

	TRBV19	SEQ ID NO: 276	acccgacagctttctatctgtgccagta
	TRBV20-1	SEQ ID NO: 277	gtgccatcctgaagacagcagcttctaca
	TRBV2	SEQ ID NO: 278	cacaaagctggagcactcagccatgtac
	TRBV21-1	SEQ ID NO: 279	tcaggggacacagcactgtattctgtgcc
5	TRBV22-1	SEQ ID NO: 280	cacaccagccaaacagcttctgtactctgt
	TRBV23-1	SEQ ID NO: 281	aatcctgtctcagaaccggagacacg
	TRBV24-1	SEQ ID NO: 282	ccaaccagacagctcttctactctgtgccac
	TRBV25-1	SEQ ID NO: 283	cacatacctctcagtaacctctgtgccagca
	TRBV26	SEQ ID NO: 284	ccaaccagacatctgtgtatctctatgccagc
10	TRBV27	SEQ ID NO: 285	accagacctctctgtactctgtgccagca
	TRBV28	SEQ ID NO: 286	aaccagacatctgtactctgtgccagc
	TRBV29-1	SEQ ID NO: 287	acatgagccctgaagacagcagcatatctc
	TRBV3-1	SEQ ID NO: 288	agcttggtgactctgctgtgtattctgtg
	TRBV3-2	SEQ ID NO: 289	cttggtgactctgctgtgtattctgtgcc
15	TRBV4-1	SEQ ID NO: 290	cagccagaagactcagccctgtatctctg
	TRBV4-2	SEQ ID NO: 291	gccagaagactcggccctgtatctctgt
	TRBV4-3	SEQ ID NO: 292	tattccttcacctacacacctgcagccag
	TRBV5-1	SEQ ID NO: 293	agatgaatgtgagcaccttggagctgg
	TRBV5-2	SEQ ID NO: 294	tactgagtcacacacggagctaggggact
20	TRBV5-3	SEQ ID NO: 295	gttgctctgagatgaatgtgagtccttgg
	TRBV5-4	SEQ ID NO: 296	atagctctgagctgaatgtgaacgccttgg
	TRBV5-5	SEQ ID NO: 297	gagctgaatgtgaacgccttgttctgg
	TRBV5-6	SEQ ID NO: 298	aactatagctctgagctgaatgtgaacgcct
	TRBV5-7	SEQ ID NO: 299	agctgaatgtgaacgccttgttctagg
25	TRBV5-8	SEQ ID NO: 300	ctgaatgtgaacgccttggagctggagga
	TRBV6-1	SEQ ID NO: 301	gtccctcccagacatctgtgtactct
	TRBV6-2	SEQ ID NO: 302	gctgctccctcccaacatctgtgtact
	TRBV6-3	SEQ ID NO: 303	gctccctcccaacatctgtgtactctgt
	TRBV6-4	SEQ ID NO: 304	aacacagatgatttcccctcagttggc
30	TRBV6-5	SEQ ID NO: 305	gctgctccctcccagacatctgtgtact
	TRBV6-6	SEQ ID NO: 306	agttggctgctccctcccagacatctg
	TRBV6-7	SEQ ID NO: 307	tcagctgctccctcagacttctgtttac
	TRBV6-8	SEQ ID NO: 308	taaacacagaggatttccactcagcctgt
	TRBV6-9	SEQ ID NO: 309	agtcagctgctccctcccagacatctgtata
35	TRBV7-1	SEQ ID NO: 310	cagcaggggacttggctgtgtatctc
	TRBV7-2	SEQ ID NO: 311	gcaggaggactcggccgtgtatctc
	TRBV7-3	SEQ ID NO: 312	tctactctgaagatccagcgcacagagcg
	TRBV7-4	SEQ ID NO: 313	cacagagcaggggactcagctgtgtat
	TRBV7-5	SEQ ID NO: 314	atcttctccacctgaagatccagcgaca
40	TRBV7-6	SEQ ID NO: 315	ttctctgcagagagcctgaggatccat
	TRBV7-8	SEQ ID NO: 316	ctgagggatccgtctccacttgaagatcc
	TRBV7-9	SEQ ID NO: 317	ggcctaaggatcttctccaccttggaga
	TRBV8-1	SEQ ID NO: 318	ttccctcaacctggagctactagcacca
	TRBV8-2	SEQ ID NO: 319	ttgagcatttcccaatcctggcatccac
45	TRBV9	SEQ ID NO: 320	gggactcagcttctgtattctgtgccagca

Inner TRBV Primers

	TRBV-Handle 1	SEQ ID NO: 321	gtgactggagttcagacgtgtgctctccgatctgcctctcccagacatctgtatattctgcg
	TRBV-Handle 2	SEQ ID NO: 322	gtgactggagttcagacgtgtgctctccgatctaatctccctcactctggagtcagctacc
50	TRBV-Handle 3	SEQ ID NO: 323	gtgactggagttcagacgtgtgctctccgatctgatttctcctcactctggagtcgctacc
	TRBV-Handle 4	SEQ ID NO: 324	gtgactggagttcagacgtgtgctctccgatctaggctcaaaggagtagactccactctcaaga
	TRBV-Handle 5	SEQ ID NO: 325	gtgactggagttcagacgtgtgctctccgatctcaagatccagcctgcaaagcttgaggact
	TRBV-Handle 6	SEQ ID NO: 326	gtgactggagttcagacgtgtgctctccgatcttagactccactctcaagatccagcctgcag
55	TRBV-Handle 7	SEQ ID NO: 327	gtgactggagttcagacgtgtgctctccgatcttgaaccagggacttggcctatattct
	TRBV-Handle 8	SEQ ID NO: 328	gtgactggagttcagacgtgtgctctccgatcttcaattctactctgaagatccagcctgcag
	TRBV-Handle 9	SEQ ID NO: 329	gtgactggagttcagacgtgtgctctccgatctcattctccactctgaagatccagcctcag
	TRBV-Handle 10	SEQ ID NO: 330	gtgactggagttcagacgtgtgctctccgatctcatcttctccactctgaagatccagcctc
	TRBV-Handle 11	SEQ ID NO: 331	gtgactggagttcagacgtgtgctctccgatctcagcagatgccttgatgcaactttagcca
60	TRBV-Handle 12	SEQ ID NO: 332	gtgactggagttcagacgtgtgctctccgatctgaactgaacatgagctccttggagctggg

TRBV-Handle 13	SEQ ID NO: 333	gtgactggagttcagacgtgtgctctccgatctgaggattctggagtttattctgtgccagc
TRBV-Handle 14	SEQ ID NO: 334	gtgactggagttcagacgtgtgctctccgatcttctgctttctgacatccgctcaccaggc
TRBV-Handle 15	SEQ ID NO: 335	gtgactggagttcagacgtgtgctctccgatctgagatccaggctacgaagcttgaggattcag
5 TRBV-Handle 16	SEQ ID NO: 336	gtgactggagttcagacgtgtgctctccgatctaacgtctccacgtgaagatccatccc
TRBV-Handle 17	SEQ ID NO: 337	gtgactggagttcagacgtgtgctctccgatctaggatccagcaggtagtcgaggagattcg
TRBV-Handle 18	SEQ ID NO: 338	gtgactggagttcagacgtgtgctctccgatctaccgacagctttctatctctgtgccagta
TRBV-Handle 19	SEQ ID NO: 339	gtgactggagttcagacgtgtgctctccgatctgtgccatcctgaagacagcagcttctaca
TRBV-Handle 20	SEQ ID NO: 340	gtgactggagttcagacgtgtgctctccgatctcacaagctggaggactcagccatgtac
10 TRBV-Handle 21	SEQ ID NO: 341	gtgactggagttcagacgtgtgctctccgatctcaggggacacagcactgtatttctgtgcc
TRBV-Handle 22	SEQ ID NO: 342	gtgactggagttcagacgtgtgctctccgatctcacaccagccaacagctttgtacttctgt
TRBV-Handle 23	SEQ ID NO: 343	gtgactggagttcagacgtgtgctctccgatctaatcctgctcagaaccgggagacacg
TRBV-Handle 24	SEQ ID NO: 344	gtgactggagttcagacgtgtgctctccgatctccaaccagacagctctttacttctgtgccac
TRBV-Handle 25	SEQ ID NO: 345	gtgactggagttcagacgtgtgctctccgatctcacatacctctcagtaacctctgtgccagca
15 TRBV-Handle 26	SEQ ID NO: 346	gtgactggagttcagacgtgtgctctccgatctccaaccagacatctgtgtatctctatgccagc
TRBV-Handle 27	SEQ ID NO: 347	gtgactggagttcagacgtgtgctctccgatctaccagacctctgtacttctgtgccagca
TRBV-Handle 28	SEQ ID NO: 348	gtgactggagttcagacgtgtgctctccgatctaacagacatctatgtacctctgtgccagc
TRBV-Handle 29	SEQ ID NO: 349	gtgactggagttcagacgtgtgctctccgatctacatgagccctgaagacagcagcatatattctc
20 TRBV-Handle 30	SEQ ID NO: 350	gtgactggagttcagacgtgtgctctccgatctagcttggtgactctgtgtatttctgtg
TRBV-Handle 31	SEQ ID NO: 351	gtgactggagttcagacgtgtgctctccgatcttgggtgactctgtgtatttctgtgcc
TRBV-Handle 32	SEQ ID NO: 352	gtgactggagttcagacgtgtgctctccgatctcagccagaagactcagccctgtatctctg
TRBV-Handle 33	SEQ ID NO: 353	gtgactggagttcagacgtgtgctctccgatctgccagaagactcggccctgtatctctgt
TRBV-Handle 34	SEQ ID NO: 354	gtgactggagttcagacgtgtgctctccgatctattccttcacacacccctgcagccag
25 TRBV-Handle 35	SEQ ID NO: 355	gtgactggagttcagacgtgtgctctccgatctagatgaatgtgagcaccttgagctgg
TRBV-Handle 36	SEQ ID NO: 356	gtgactggagttcagacgtgtgctctccgatcttactgagtcacacacggactaggggact
TRBV-Handle 37	SEQ ID NO: 357	gtgactggagttcagacgtgtgctctccgatctgtgctctgagatgaatgtgagtgccctgg
TRBV-Handle 38	SEQ ID NO: 358	gtgactggagttcagacgtgtgctctccgatctatagctctgagctgaatgtgaacgccttgg
TRBV-Handle 39	SEQ ID NO: 359	gtgactggagttcagacgtgtgctctccgatctgagctgaatgtgaacgccttgtgtctgg
30 TRBV-Handle 40	SEQ ID NO: 360	gtgactggagttcagacgtgtgctctccgatctaatatagctctgagctgaatgtgaacgcct
TRBV-Handle 41	SEQ ID NO: 361	gtgactggagttcagacgtgtgctctccgatctagctgaatgtgaacgccttgtgttaggg
TRBV-Handle 42	SEQ ID NO: 362	gtgactggagttcagacgtgtgctctccgatctctgaatgtgaacgccttggagctggagga
TRBV-Handle 43	SEQ ID NO: 363	gtgactggagttcagacgtgtgctctccgatctgctccctcccagacatctgtgtacttct
TRBV-Handle 44	SEQ ID NO: 364	gtgactggagttcagacgtgtgctctccgatctgctccctcccacacatctgtgtacttct
35 TRBV-Handle 45	SEQ ID NO: 365	gtgactggagttcagacgtgtgctctccgatctgctccctcccacacatctgtgtacttctgt
TRBV-Handle 46	SEQ ID NO: 366	gtgactggagttcagacgtgtgctctccgatctaacacagatgatttcccctcacgttggc
TRBV-Handle 47	SEQ ID NO: 367	gtgactggagttcagacgtgtgctctccgatctgctgctccctcccagacatctgtgtactt
TRBV-Handle 48	SEQ ID NO: 368	gtgactggagttcagacgtgtgctctccgatctagtgtgctgctccctcccagacatctg
TRBV-Handle 49	SEQ ID NO: 369	gtgactggagttcagacgtgtgctctccgatcttcagctgctccctctcagactctgtttac
40 TRBV-Handle 50	SEQ ID NO: 370	gtgactggagttcagacgtgtgctctccgatcttaaacacagaggatttcccactcaggctggt
TRBV-Handle 51	SEQ ID NO: 371	gtgactggagttcagacgtgtgctctccgatctagtcagctgctccctcccagacatctgtata
TRBV-Handle 52	SEQ ID NO: 372	gtgactggagttcagacgtgtgctctccgatctcagcagggggacttggtctgtatctc
TRBV-Handle 53	SEQ ID NO: 373	gtgactggagttcagacgtgtgctctccgatctgcaggaggactcggccgtgtatctc
TRBV-Handle 54	SEQ ID NO: 374	gtgactggagttcagacgtgtgctctccgatcttactctgaagatccagcgcacagagcg
45 TRBV-Handle 55	SEQ ID NO: 375	gtgactggagttcagacgtgtgctctccgatctcacagagcagggggactcagctgtgtat
TRBV-Handle 56	SEQ ID NO: 376	gtgactggagttcagacgtgtgctctccgatctatcttttccacctgaagatccagcgcaca
TRBV-Handle 57	SEQ ID NO: 377	gtgactggagttcagacgtgtgctctccgatctttctctgcagagaggcctgaggatccat
TRBV-Handle 58	SEQ ID NO: 378	gtgactggagttcagacgtgtgctctccgatctctgaggatccgtctccactctgaagatcc
TRBV-Handle 59	SEQ ID NO: 379	gtgactggagttcagacgtgtgctctccgatctgacctaaaggatcttttccaccttgagaga
50 TRBV-Handle 60	SEQ ID NO: 380	gtgactggagttcagacgtgtgctctccgatctttccctcaacctggagctactatgcacca
TRBV-Handle 61	SEQ ID NO: 381	gtgactggagttcagacgtgtgctctccgatctttgagcatttcccacatctctggcaccac
TRBV-Handle 62	SEQ ID NO: 382	gtgactggagttcagacgtgtgctctccgatctgggactcagcttgtatttctgtgccagca
<u>Outer TRBV Primers</u>		
55 TRBV10-1_out	SEQ ID NO: 383	gctgaaatcaccagagcccaagacacaag
TRBV10-2_out	SEQ ID NO: 384	cacagagacagggaaggcaggtgacctga
TRBV10-3_out	SEQ ID NO: 385	gatgtggaatcaccagagcccaagacac
TRBV11-1_out	SEQ ID NO: 386	gccaggctgtggcttttgggtgactcta
TRBV11-2_out	SEQ ID NO: 387	ggcagagtgtggcttttgggtgcaatcct
60 TRBV11-3_out	SEQ ID NO: 388	ggcttttgggtgcaatcctatttctggccac

	TRBV12-1_outer	SEQ ID NO: 389	gatgctggtttatccagtcacccaggcac
	TRBV12-2_outer	SEQ ID NO: 390	gtcacccaagcatgaggtgacagaaatggg
	TRBV12-3_outer	SEQ ID NO: 391	atgctggagttatccagtcacccgcc
	TRBV12-4_outer	SEQ ID NO: 392	gagttatccagtcacccggcacgaggt
5	TRBV12-5_outer	SEQ ID NO: 393	gctagagtcacccagacaccaaggcaca
	TRBV13_outer	SEQ ID NO: 394	gctgctggagtcacagtcacccaaga
	TRBV14_outer	SEQ ID NO: 395	gttactcagttcccccagccacagcgtaat
	TRBV15_outer	SEQ ID NO: 396	gttaccagtttggaaagccagtgaccct
	TRBV16_outer	SEQ ID NO: 397	gaagtcgccagactccaaaacatctgtc
10	TRBV17_outer	SEQ ID NO: 398	cagacacaaggtcaccaacatgggacagg
	TRBV18_outer	SEQ ID NO: 399	gtcatgtttactggtatcggcagctccca
	TRBV19_outer	SEQ ID NO: 400	atgccatgtactggtaccgacaggaccca
	TRBV20-1_outer	SEQ ID NO: 401	gtcgtctctcaacatccgagctgggttat
	TRBV2_outer	SEQ ID NO: 402	gaacctgaagtcacccagactcccagcca
15	TRBV21-1_outer	SEQ ID NO: 403	cacggacaccaaggtcacccagagacct
	TRBV22-1_outer	SEQ ID NO: 404	agctcactggggctggatgggatgtac
	TRBV23-1_outer	SEQ ID NO: 405	gccaagtacacagactccaggacattt
	TRBV24-1_outer	SEQ ID NO: 406	gtatcgacaagaccaggactgggcctac
	TRBV25-1_outer	SEQ ID NO: 407	gtgacatctaccagacccaagatact
20	TRBV26_outer	SEQ ID NO: 408	gtatcgacaggaccaggacttgactga
	TRBV27_outer	SEQ ID NO: 409	agcccaagtgaccagacccaagatac
	TRBV28_outer	SEQ ID NO: 410	ctcgtagatgtgaaagtaacccagagctcga
	TRBV29-1_outer	SEQ ID NO: 411	gatatctgtcaacgtggaacctccctgacg
	TRBV3-1_outer	SEQ ID NO: 412	ggtcacacagatgggaaacgacaagtcca
25	TRBV3-2_outer	SEQ ID NO: 413	ccgttcccagactccaaatctacgtgctc
	TRBV4-1_outer	SEQ ID NO: 414	gaagttaccagacacaaaaacacctggtc
	TRBV4-2_outer	SEQ ID NO: 415	gagttacgcagacaccaagacacctggtc
	TRBV4-3_outer	SEQ ID NO: 416	ggagttacgcagacaccaagacacctgg
	TRBV5-1_outer	SEQ ID NO: 417	gtgacactgagctgctccctatctctgg
30	TRBV5-2_outer	SEQ ID NO: 418	gaatcacccaagctccaagacacctgatc
	TRBV5-3_outer	SEQ ID NO: 419	ctggagtcacccaaagtcccacacacc
	TRBV5-4_outer	SEQ ID NO: 420	gactggagtcacccaaagtcccacacac
	TRBV5-5_outer	SEQ ID NO: 421	gtcccacacacctgatcaaacgagagga
	TRBV5-6_outer	SEQ ID NO: 422	tagtggagctggtgagtcacccaaagtcc
35	TRBV5-7_outer	SEQ ID NO: 423	ctgatcaaaacgagaggacagcacgtgac
	TRBV5-8_outer	SEQ ID NO: 424	gagtcacacaagtcacacacacctgatc
	TRBV6-1_outer	SEQ ID NO: 425	gtgaatgctggtgtcactcagaccccaaa
	TRBV6-2_outer	SEQ ID NO: 426	gaatgctggtgtcactcagaccccaaaat
	TRBV6-3_outer	SEQ ID NO: 427	gctggtgtcactcagaccccaaaattccg
40	TRBV6-4_outer	SEQ ID NO: 428	gatcacccaggcaccaacatctcagatcc
	TRBV6-5_outer	SEQ ID NO: 429	gctggtgtcactcagaccccaaaattcca
	TRBV6-6_outer	SEQ ID NO: 430	gctggtgtcactcagaccccaaaattccg
	TRBV6-7_outer	SEQ ID NO: 431	gaatgctggtgtcactcagaccccaaaat
	TRBV6-8_outer	SEQ ID NO: 432	gctggtgtcactcagaccccaaaattcca
45	TRBV6-9_outer	SEQ ID NO: 433	gaatgctggtgtcactcagaccccaaaat
	TRBV7-1_outer	SEQ ID NO: 434	gtgctggagtcctccagtccttgagaca
	TRBV7-2_outer	SEQ ID NO: 435	gtccccagtaacaaggtcacagagaagg
	TRBV7-3_outer	SEQ ID NO: 436	gacccccagtaacaaggtcacagagaagg
	TRBV7-4_outer	SEQ ID NO: 437	cagtcaccaaggtacaagtcgcaagag
50	TRBV7-5_outer	SEQ ID NO: 438	gtctccagtcaccaaggtacgaagtc
	TRBV7-6_outer	SEQ ID NO: 439	cacaggtgctggagtcctccagtcctc
	TRBV7-8_outer	SEQ ID NO: 440	gtgctggagtcctccagtcctcctagg
	TRBV7-9_outer	SEQ ID NO: 441	ctggagtcctccagaacccagacaca
	TRBV8-1_outer	SEQ ID NO: 442	gaggcagggatcagccagataccaagat
55	TRBV8-2_outer	SEQ ID NO: 443	gatgctgggatcacccagatgccaaaga
	TRBV9_outer	SEQ ID NO: 444	tggagtcacacaaccccaagcacctg

Hybridization Probe Pool

- Ig1 SEQ ID NO: 445
GAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACCTG
CCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGT
- 5 Ig2 SEQ ID NO: 446
GGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAA
GGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGACTACGAGA
- Ig3 SEQ ID NO: 447
CTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAGAGGGAGAAGTGCCCCACCTGCTCC
TCAGTTCAGCCTGACCCCTCCCATCCTTTGGCCTCTGACCCTTTTTCCACAGG
- 10 Ig4 SEQ ID NO: 448
GGACCTACCCCTATTGCGGTCCTCCAGCTCATCTTTCACCTACCCCCCTCCTCTCCTTGGCTTTA
ATTATGCTAATGTTGGAGGAGAATGAATAAATAAAGTGAATCTTTGCACCTGT
- Ig5 SEQ ID NO: 449
GTCAGCCCAAGGCCAACCCCACTGTCACTCTGTTCCCGCCCTCCTCTGAGGAGCTCCAAGCCAACA
15 AGGCCACACTAGTGTGTCTGATCAGTGACTTCTACCCGGGAGCTGTGACAGTGG
- Ig6 SEQ ID NO: 450
GACGCCCGAGCAGTGGAAGTCCACAGAAGCTACAGCTGCCAGGTACGCATGAAGGGAGCACC
GTGGAGAAGACAGTGGCCCTACAGAATGTTCATAGGTTCCCAACTCTAACCCAC
- 20 Ig7 SEQ ID NO: 451
CCACGGGAGCCTGGAGCTGCAGGATCCCAGGGGAGGGGTCTCTCTCCCATCCCAAGTCATCCAG
CCCTTCTCCCTGCACTCATGAAACCCCAATAAATATCCTCATTGACAACCAGAAA
- Ig8 SEQ ID NO: 452
GTCAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCGCCCTCCTCTGAGGAGCTTCAAGCCAACA
AGGCCACACTGGTGTGTCTCATAAGTGACTTCTACCCGGGAGCCGTGACAGTGG
- 25 Ig9 SEQ ID NO: 453
CGCCTGAGCAGTGGAAGTCCACAGAAGCTACAGCTGCCAGGTACGCATGAAGGGAGCACCGTG
GAGAAGACAGTGGCCCTACAGAATGTTCATAGGTTCTCAACCCTACCCCCAC
- Ig10 SEQ ID NO: 454
CACGGGAGACTAGAGCTGCAGGATCCCAGGGGAGGGGTCTCTCCTCCACCCCAAGGCATCAAGC
30 CCTTCTCCCTGCACTCAATAAACCTCAATAAATATTCTCATTGTCAATCAGAAA
- Ig11 SEQ ID NO: 455
GTCAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCAACCTCCTCTGAGGAGCTTCAAGCCAACA
AGGCCACACTGGTGTGTCTCATAAGTGACTTCTACCCGGGAGCCGTGACAGTGG
- Ig12 SEQ ID NO: 456
35 CGCCTGAGCAGTGGAAGTCCACAAAAGCTACAGCTGCCAGGTACGCATGAAGGGAGCACCGTG
GAGAAGACAGTGGCCCTACAGAATGTTCATAGGTTCTCATCCCTACCCCCAC
- Ig13 SEQ ID NO: 457
CACGGGAGACTAGAGCTGCAGGATCCCAGGGGAGGGGTCTCTCCTCCACCCCAAGGCATCAAGC
CCTTCTCCCTGCACTCAATAAACCTCAATAAATATTCTCATTGTCAATCAGAAA
- 40 Ig14 SEQ ID NO: 458
GTCAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCAACCTCCTCTGAGGAGCTTCAAGCCAACA
AGGCCACACTGGTGTGTCTCGTAAGTGACTTCAACCCGGGAGCCGTGACAGTGG
- Ig15 SEQ ID NO: 459
45 CCAGCAGCTACCTGAGCCTGACGCCCGAGCAGTGGAAGTCCACAGAAGCTACAGCTGCCGGGTC
ACGCATGAAGGGAGCACCGTGGAGAAGACAGTGGCCCTGCAGAATGCTCTTAGG

- Ig16 SEQ ID NO: 460
CCCCGACCCTCACCCACCCACAGGGGCCTGGAGCTGCAGGTTCCCAGGGGAGGGGTCTCTGCC
CCCATCCCAAGTCATCCAGCCCTTCTCAATAAAATATCCTCATCGTCAACGAGAAA
- 5 Ig17 SEQ ID NO: 461
GCATCCCCGACCAGCCCCAAGGTCTTCCCGCTGAGCCTCGACAGCACCCCCCAAGATGGGAACGT
GGTCGTGCGATGCCTGGTCCAGGGCTTCTTCCCCAGGAGCCACTCAGTGTGACC
- Ig18 SEQ ID NO: 462
TGGAGCGAAAGCGGACAGAACGTGACCGCCAGAACTTCCCACCTAGCCAGGATGCCTCCGGGGA
CCTGTACACCACGAGCAGCCAGCTGACCCTGCCGGCCACACAGTGGCCAGACGGC
- 10 Ig19 SEQ ID NO: 463
AAGTCCGTGACATGCCACGTGAAGCACTACACGAATTCCAGCCAGGATGTGACTGTGCCCTGCCG
AGTCCCCCACCTCCCCATGCTGCCACCCCCACTGTGCTGCACCGACCGGCC
- Ig20 SEQ ID NO: 464
CTCGAGGACCTGCTCTTAGGTTCAAGCGAACCTCACGTGCACACTGACCGGCCTGAGAGATGC
15 CTCTGGTGCCACCTTCACTGGACGCCCTCAAGTGGGAAGAGCGCTGTTCAAGGA
- Ig21 SEQ ID NO: 465
CCACCTGAGCGTGACCTCTGTGGCTGCTACAGCGTGTCAGTGTCTGCCTGGCTGTGCCAGCCA
TGGAAACCATGGGGAGACCTTCACTGCACTGCTGCCACCCCGAGTTGAAGACC
- 20 Ig22 SEQ ID NO: 466
CCACTAACCGCCAACATCACAAAATCCGGAAACACATTCCGGCCCCGAGGTCCACCTGCTGCCGCC
GCCGTGCGAGGAGCTGGCCCTGAACGAGCTGGTGACGCTGACGTGCCTGGCACGT
- Ig23 SEQ ID NO: 467
GGCTTCAGCCCCAAGGATGTGCTGGTTCGCTGGCTGCAGGGGTCACAGGAGCTGCCCCGCGAGAA
GTACCTGACTTGCGCATCCCGGCAGGAGCCCAGCCAGGGCACACCACCTACGCT
- 25 Ig24 SEQ ID NO: 468
GTAACCAGCATACTGCGCGTGGCAGCTGAGGACTGGAAGAAGGGGGAGACCTTCTCCTGCATGGT
GGGCCACGAGGCCCTGCCGCTGGCCTTACACAGAAGACCATCGACCGCATGGCG
- Ig25 SEQ ID NO: 469
GGCTCTTGCTGTGTTGCAGATTGGCAGATGCCGCCCTCCCTATGTGGTGCTGGACTTGCCGAGGAG
30 ACCCTGGAGGAGGAGACCCCCGGCGCCAACCTGTGGCCACCACCATCACCTTC
- Ig26 SEQ ID NO: 470
CTCACCTCTTCTGCTGAGCCTGTTCTATAGCACAGCACTGACCGTGACCAGCGTCCGGGGCCCA
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- 35 Ig27 SEQ ID NO: 471
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- Ig28 SEQ ID NO: 472
GCCTCCACACAGAGCCCATCCGTCTTCCCCTTGACCCGCTGCTGCAAAAACATTCCCTCCAATGCC
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- 40 Ig29 SEQ ID NO: 473
GTGACCTGGGACACAGGCTCCCTCAACGGGACAACCTATGACCTTACCAGCCACCACCCTCACGCTC
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- Ig30 SEQ ID NO: 474
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45 AGCGTCTGCTCCAGGGACTTACCCCGCCACCGTGAAGATCTTACAGTCGTCC

- Ig31 SEQ ID NO: 475
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- 5 Ig32 SEQ ID NO: 476
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- Ig33 SEQ ID NO: 477
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- 10 Ig34 SEQ ID NO: 478
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- Ig35 SEQ ID NO: 479
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15 CAGCGGCCCGCTGCTGCCCCGGAAGTCTATGCGTTTGCACGCCGGAGTGGCCG
- Ig36 SEQ ID NO: 480
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- 20 Ig37 SEQ ID NO: 481
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- Ig38 SEQ ID NO: 482
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- 25 Ig39 SEQ ID NO: 483
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- Ig40 SEQ ID NO: 484
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30 TCCACTGTTGCAATGACCCAGGAAGCTACCCCCAATAAACTGTGCCTGCTCAG
- Ig41 SEQ ID NO: 485
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- 35 Ig42 SEQ ID NO: 486
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- Ig43 SEQ ID NO: 487
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- 40 Ig44 SEQ ID NO: 488
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- Ig45 SEQ ID NO: 489
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- Ig46 SEQ ID NO: 490
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- 5 Ig47 SEQ ID NO: 491
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- Ig48 SEQ ID NO: 492
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- 10 Ig49 SEQ ID NO: 493
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- Ig50 SEQ ID NO: 494
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- Ig51 SEQ ID NO: 495
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- 20 Ig52 SEQ ID NO: 496
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- Ig53 SEQ ID NO: 497
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- 25 Ig54 SEQ ID NO: 498
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- Ig55 SEQ ID NO: 499
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30 TGCTGCCACACTGCTGGATTCTCGGGTGGAACTGGACCCGCAGGGACAGCCA
- Ig56 SEQ ID NO: 500
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- 35 Ig57 SEQ ID NO: 501
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- Ig58 SEQ ID NO: 502
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- 40 Ig59 SEQ ID NO: 503
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- Ig60 SEQ ID NO: 504
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- Ig61 SEQ ID NO: 505
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- 5 Ig62 SEQ ID NO: 506
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- Ig63 SEQ ID NO: 507
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- 10 Ig64 SEQ ID NO: 508
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- Ig65 SEQ ID NO: 509
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- Ig66 SEQ ID NO: 510
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- 20 Ig67 SEQ ID NO: 511
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- Ig68 SEQ ID NO: 512
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- 25 Ig69 SEQ ID NO: 513
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- Ig70 SEQ ID NO: 514
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30 TCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTC
- Ig71 SEQ ID NO: 515
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- 35 Ig72 SEQ ID NO: 516
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- Ig73 SEQ ID NO: 517
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- 40 Ig74 SEQ ID NO: 518
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- Ig75 SEQ ID NO: 519
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- Ig76 SEQ ID NO: 520
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- 5 Ig77 SEQ ID NO: 521
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- Ig78 SEQ ID NO: 522
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- 10 Ig79 SEQ ID NO: 523
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- Ig80 SEQ ID NO: 524
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15 GTGGACACAGCCAGAACTCATCACAGAGGCTGGCGTCCAGCCCCGGGTCACGTGCAG
- Ig81 SEQ ID NO: 525
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- 20 Ig82 SEQ ID NO: 526
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- Ig83 SEQ ID NO: 527
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- 25 Ig84 SEQ ID NO: 528
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- Ig85 SEQ ID NO: 529
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30 GGTGATCGCCTGCCTGGTCCAGGGCTTCTTCCCCAGGAGCCACTCAGTGTGACC
- Ig86 SEQ ID NO: 530
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- 35 Ig87 SEQ ID NO: 531
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- Ig88 SEQ ID NO: 532
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- 40 Ig89 SEQ ID NO: 533
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- Ig90 SEQ ID NO: 534
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- Ig91 SEQ ID NO: 535
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- 5 Ig92 SEQ ID NO: 536
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- Ig93 SEQ ID NO: 537
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- 10 Ig94 SEQ ID NO: 538
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- Ig95 SEQ ID NO: 539
15 CTGGCGGGTAAACCCACCCATGTCAATGTGTCTGTGTGTCATGGCGGAGGTGGACGGCACCTGCTA
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- Ig96 SEQ ID NO: 540
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- 20 Ig97 SEQ ID NO: 541
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- Ig98 SEQ ID NO: 542
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- 25 Ig99 SEQ ID NO: 543
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30 TACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCA
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- Ig101 SEQ ID NO: 545
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- 35 Ig102 SEQ ID NO: 546
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- Ig103 SEQ ID NO: 547
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- 40 Ig104 SEQ ID NO: 548
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- Ig106 SEQ ID NO: 550
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- 5 Ig107 SEQ ID NO: 551
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- Ig108 SEQ ID NO: 552
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- 10 Ig109 SEQ ID NO: 553
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- Ig110 SEQ ID NO: 554
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15 CTGCCCACACTGCTGGATTCTCGGGTGAACTGGACCCGCAGGGACAGCCAGCC
- Ig111 SEQ ID NO: 555
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- 20 Ig112 SEQ ID NO: 556
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- Ig113 SEQ ID NO: 557
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- 25 Ig114 SEQ ID NO: 558
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- Ig115 SEQ ID NO: 559
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- Ig116 SEQ ID NO: 560
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- 35 Ig117 SEQ ID NO: 561
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- Ig118 SEQ ID NO: 562
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- 40 Ig119 SEQ ID NO: 563
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- Ig121 SEQ ID NO: 565
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- 5 Ig122 SEQ ID NO: 566
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- Ig123 SEQ ID NO: 567
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- 10 Ig124 SEQ ID NO: 568
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- Ig125 SEQ ID NO: 569
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15 TACCCAGCGACATCGCCGTGGAGTGGGAGAGCAGCGGGCAGCCGGAGAACAAT
- Ig126 SEQ ID NO: 570
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- 20 Ig127 SEQ ID NO: 571
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- Ig128 SEQ ID NO: 572
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- 25 Ig129 SEQ ID NO: 573
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- Ig130 SEQ ID NO: 574
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- Ig131 SEQ ID NO: 575
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- 35 Ig132 SEQ ID NO: 576
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- Ig133 SEQ ID NO: 577
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- 40 Ig134 SEQ ID NO: 578
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- Ig135 SEQ ID NO: 579
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45 AAGGCTGGTGACCAGGTCCCAGGCGGGCAAGACTAGCCTTGGTGGGGCCTGAGGA

- Ig136 SEQ ID NO: 580
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- 5 Ig137 SEQ ID NO: 581
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- Ig138 SEQ ID NO: 582
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- 10 Ig139 SEQ ID NO: 583
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- Ig140 SEQ ID NO: 584
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15 CCGGGCACCCAGCATGGAATAAAGCACCCAGCGCTGCCCTGGGCCCCCTGCGA
- Ig141 SEQ ID NO: 585
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- 20 Ig142 SEQ ID NO: 586
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- Ig143 SEQ ID NO: 587
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TCTCCAAAGGCACAGGCCTCCTCAGTGCCCACTGCACAACCCAAGCAGAGGGCA
- 25 Ig144 SEQ ID NO: 588
GCCTCGCCAAGGCAACCACAGCCCCAGCCACCACCCGTAACACAGGAAGAGGAGGAGAAGAGAA
GAAGAAGGAGAAGGAGAAAGAGGAACAAGAAGAGAGAGAGACAAAGACACCAGAGT
- Ig145 SEQ ID NO: 589
GTCCGAGCCACACCCAGCCTCTTGGCGTCTACCTGCTAACCCCTGCAGTGCAGGACCTGTGGCTCC
30 GGGACAAAGCCACCTTCACCTGCTTCGTGGTGGGCAGTGACCTGAAGGATGCTC
- Ig146 SEQ ID NO: 590
ACCTGACCTGGGAGGTGGCTGGGAAGGTCCCCACAGGGGGCGTGGAGGAAGGGCTGCTGGAGCG
GCACAGCAACGGCTCCAGAGCCAGCACAGCCGTCTGACCCTGCCAGGTCCTTGT
- 35 Ig147 SEQ ID NO: 591
GGCCTCGTCTGACCTCCCCGAGGCGGCCTCGTGGCTCCTGTGTGAGGTGTCTGGCTTCTCGCCCCC
AACATCCTCCTGATGTGGCTGGAGGACCAGCGTGAGGTGAACACTTCTGGGTT
- Ig148 SEQ ID NO: 592
TGCCCCCGCACGCCCCCTCCACAGCCCAGGAGCACCACGTTCTGGGCCTGGAGTGTGCTGCGTGT
CCCAGCCCCGCCAGCCCTCAGCCAGCCACCTACACGTGTGTGGTCAGCCACGA
- 40 Ig149 SEQ ID NO: 593
GGACTCCCGGACTCTGCTCAACGCCAGCCGAGCCTAGAAGTCAGCTACCTGGCCATGACCCCCCT
GATCCCTCAGAGCAAGGATGAGAACAGCGATGACTACACGACCTTTGATGATGT
- Ig150 SEQ ID NO: 594
GGGCAGCCTGTGGACCACCCTGTCCACGTTTGTGGCCCTCTTCATCCTCACCCCTCCTCTACAGCGGC
45 ATTGTAACCTTTCATCAAGGTGAAGTAGCCCCAGAAGAGCAGGACGCCCTGTAC

- Ig151 SEQ ID NO: 595
CTGCAGAGAAGGGAAGCAGCCTCTGTACCTCATCTGTGGCTACCAGAGAGCAGAAAAGACCCACC
CTGGACTCTTCTGTGTGCAGGAAGATGCGCCAGCCCCTGCCCCGGCTCCCCTCT
- 5 Ig152 SEQ ID NO: 596
GTCCGCCACAGAACCCAGTCTTCTAGACCAGGGGGACGGGCACCCATCACTCCGCAGGCGAATCA
GAGCCCCCTGCCCCGGCCCTAACCCCTGTGCCTCCTTCCCATGCTTCCCCGAGA
- Ig153 SEQ ID NO: 597
GCCAGCTACACCCCTGCCCCGGCCCTAACCCCATGCCTCCTTCTGTGCTTCCCCAGAGCCAGCT
AGTCCCACCTGCAGCCCGTGGCCTCCCATAAACACACTTTGGTTCAITTC
- 10 Ig154 SEQ ID NO: 598
GGGAGTGCATCCGCCCCAACCCCTTTTCCCCCTCGTCTCCTGTGAGAATTCCCCGTGGATACGAGC
AGCGTGCCGTTGGCTGCCTCGCACAGGACTTCCTTCCCGACTCCATCACTTC
- Ig155 SEQ ID NO: 599
TCCTGGAAATACAAGAACAACCTCTGACATCAGCAGCACCCGGGGCTTCCCATCAGTCTGAGAGG
15 GGGCAAGTACGCAGCCACCTCACAGGTGCTGCTGCCTTCCAAGGACGTCATGCAG
- Ig156 SEQ ID NO: 600
GGCACAGACGAACACGTGGTGTGCAAAGTCCAGCACCCCAACGGCAACAAAGAAAAGAACGTGC
CTTTCCAGTGATTGCTGAGCTGCCTCCCAAAGTGAGCGTCTTCGTCCCACCCCGC
- 20 Ig157 SEQ ID NO: 601
GACGGCTTCTTCGGCAACCCCGCAAGTCCAAGCTCATCTGCCAGGCCACGGGTTTCAGTCCCCGG
CAGATTCAAGGTGTCTGGCTGCGCGAGGGGAAGCAGGTGGGGTCTGGCGTCACC
- Ig158 SEQ ID NO: 602
ACGGACCAGGTGCAGGCTGAGGGCAAAGAGTCTGGGCCCACGACCTACAAGGTGACCAGCACACT
GACCATCAAAGAGAGCGACTGGCTCGGCCAGAGCATGTTACCTGCCGCGTGAT
- 25 Ig159 SEQ ID NO: 603
CACAGGGGCTGACCTTCCAGCAGAATGCGTCTCTCATGTGTGTCCCCGATCAAGACACAGCCATC
CGGGTCTTCGCCATCCCCCATCCTTTGCCAGCATCTTCTCACCAAGTCCACC
- Ig160 SEQ ID NO: 604
AAGTTGACCTGCCTGGTCACAGACCTGACCACCTATGACAGCGTGACCATCTCTGGACCCGCCAG
30 AATGGCGAAGCTGTGAAAACCCACACCAACATCTCCGAGAGCCACCCCAATGCC
- Ig161 SEQ ID NO: 605
AGCGCCGTGGGTGAGGCCAGCATCTGCGAGGATGACTGGAATTCCGGGGAGAGGTTACGTGCAC
CGTGACCCACACAGACCTGCCCTCGCCACTGAAGCAGACCATCTCCCGGCCAAG
- Ig162 SEQ ID NO: 606
35 GGGGTGGCCCTGCACAGGCCCGATGTCTACTTGCTGCCACCAGCCCGGGAGCAGCTGAACCTGCG
GGAGTCGGCCACCATCAGTGCCTGGTGACGGGCTTCTCTCCCGCGGACGTCTTC
- Ig163 SEQ ID NO: 607
GTGCAGTGGATGCAGAGGGGGCAGCCCTTGTCGCCGAGAGAAGTATGTGACCAGCGCCCCAATGCC
TGAGCCCCAGGCCCCAGGCCGGTACTTCGCCCACAGCATCCTGACCGTGTCCGAA
- 40 Ig164 SEQ ID NO: 608
GAGGAATGGAACACGGGGGAGACCTACACCTGCGTGGTGGCCCATGAGGCCCTGCCAACAGGGT
CACCGAGAGGACCGTGGAACAAGTCCACCGAGGGGGAGGTGAGCGCCGACGAGGAG
- Ig165 SEQ ID NO: 609
45 GGCTTTGAGAACCTGTGGGCCACCGCCTCCACCTTCATCGTCTCTTCTCCTGAGCCTCTTCTACA
GTACCACCGTCACCTTGTCAAGGTGAAATGATCCCAACAGAAGAATATCGGA

- Ig166 SEQ ID NO: 610
GACCAGAGAGAGGAACTCAAAGGGGCGCTGCCTCCGGGTCTGGGGTCTGGCCTGCGTGGCCTGT
TGGCAGTGTTTCTTCTCCCGCCCGGCTCCAGTTGTGTGCTCTCACACAGGCT
- 5 Ig167 SEQ ID NO: 611
TCCTTCTCGACCGGCAGGGGCTGGCTGGCTTGCAGGCCACGAGGTGGGCTCTACCCACACTGCTT
TGCTGTGTATACGCTTGTGCCCCTGAAATAAATATGCACATTTTATCCATGAAA
- Ig168 SEQ ID NO: 612
TGCTGGCCTGCCCACAGGCTCGGGGCGGCTGGCCGCTCTGTGTGTGCATGCAAATAACCGTGTCA
ACGGGGTGAGATGTTGCATCTTATAAAATTAGAAATAAAAAGATCCATTCAAAA
- 10 Ig169 SEQ ID NO: 613
GCCACCCCTTGGTCACTCTGTTCCCGCCCTCCTCTGAGGAGCTCCAAGCCAACAAGGCCATGCTG
GTGTGTCTCATAAATGACTTCTACCCAGGAGCCATAGAAGGAAAATGGCACCTT
- Ig170 SEQ ID NO: 614
ATGCGGCCAGCAGCTACCTGAGCCTGACGCCCAGCAGTGAAGTCCCACAGAAGCTACAGCTGC
15 CAGGTCACGCACAAAGAAAGTACCATGGAGAAGACAATGGCCCATGCAGAATGTT
- Ig171 SEQ ID NO: 615
ACAAGGCCCACTGGTGTGTCTCATGAGTGACTTCTACCCGAGAGCCATGACAGTGGCCTGGAAG
ATAGATGGCATCACCATCACCCAGGTGTGGAGACCACCACCCCTCCAAACAGA
- Ig172 SEQ ID NO: 616
20 TATGCGGCCAGCAGCTACCTAAGACTGGCACCCGACAGTGAAGTCCCACAACCTCTACAGCTGC
CAGGTCACGCATGAAAGGAACACTGTGGAGAAGACAGTGGCCCCTGCAGAATGTT
- Ig173 SEQ ID NO: 617
GTCAAGCCCAAGGCTGCCCCATCGGTCACTCTGTTCCCGCCCTCCTCTGAGGAGCTTCAAGCCAACA
AGGCCCACTGGTGTGCCTGATCAGTGACTTCTACCCGGGAGCTGTGAAAGTGG
- 25 Ig174 SEQ ID NO: 618
GCGGCCAGCAGCTAGCTACCTGAGCCTGACGCTGAGCAGTGAAGTCCCACAGAAGCTACAGTT
GCCAGGTCACGCATGAAGGGAGCACCGTGGAGAAGACAGTGGCCCCTGCAGAATG
- TCR1 SEQ ID NO: 619
AGGACCTGAACAAGGTGTTCCACCCGAGGTGCTGTGTTTGAAGCCATCAGAAGCAGAGATCTCC
30 CACACCCAAAAGGCCACACTGGTGTGCCTGGCCACAGGCTTCTTCCCTGACCAG
- TCR2 SEQ ID NO: 620
TGGAGCTGAGCTGGTGGGTGAATGGGAAGGAGGTGCACAGTGGGGTCAGCACGGACCCGAGCC
CCTCAAGGAGCAGCCCGCCCTCAATGACTCCAGATACTGCCTGAGCAGCCGCTGA
- TCR3 SEQ ID NO: 621
35 GGGTCTCGGCCACCTTCTGGCAGAACCCCGCAACCACTTCCGCTGTCAAGTCCAGTTCTACGGGC
TCTCGGAGAATGACGAGTGGACCCAGGATAGGGCCAAACCCGTCACCCAGATCG
- TCR4 SEQ ID NO: 622
CTCGGTGTCTACCAGCAAGGGTCTGTCTGCCACCATCCTCTATGAGATCCTGCTAGGGAAGGC
CACCTGTATGCTGTGCTGGTCAGCGCCCTTGTGTTGATGGCCATGGTCAAGAG
- 40 TCR5 SEQ ID NO: 623
AAAGGATTTCTGAAGGCAGCCCTGGAAGTGGAGTTAGGAGCTTCTAACCCGTCATGGTTTCAATAC
ACATTCTTCTTTGCCAGCGCTTCTGAAGAGCTGCTCTCACCTCTCTGCATCCC
- TCR6 SEQ ID NO: 624
AATAGATATCCCCCTATGTGCATGCACACCTGCACACTCACGGCTGAAATCTCCCTAACCCAGGGG
45 GACCTTAGCATGCCTAAGTGAATAAACCAATAAAAATGTTCTGGTCTGGCCTGA

TCR7 SEQ ID NO: 625

AGGACCTGAAAAACGTGTTCCCAACCAAGGTCGCTGTGTTTGAGCCATCAGAAGCAGAGATCTCC
CACACCCAAAAGGCCACACTGGTGTGCCTGGCCACAGGCTTCTACCCCGACCACG

TCR8 SEQ ID NO: 626

5 TGGAGCTGAGCTGGTGGGTGAATGGGAAGGAGGTGCACAGTGGGGTCAGCACAGACCCGCAGCC
CCTCAAGGAGCAGCCCCGCCCTCAATGACTCCAGATACTGCCTGAGCAGCCGCCTGA

TCR9 SEQ ID NO: 627

GGGTCTCGGCCACCTTCTGGCAGAACCCCGCAACCACTTCCGCTGTCAAGTCCAGTTCTACGGGC
TCTCGGAGAATGACGAGTGGACCCAGGATAGGGCCAAACCTGTCAACCAGATCG

10 TCR10 SEQ ID NO: 628

ACCTCCGAGTCTTACCAGCAAGGGGTCCTGTCTGCCACCATCCTCTATGAGATCTTGCTAGGGAAG
GCCACCTTGATGCCGTGCTGGTCAGTGCCCTCGTGTCTGATGGCCATGGTCAAG

TCR11 SEQ ID NO: 629

15 AGAAAGGATTCCAGAGGCTAGCTCCAAAACCATCCAGGTCAATTCTTCATCCTCACCCAGGATTCT
CCTGTACCTGCTCCCAATCTGTGTTCCCTAAAAGTGATTCTCACTCTGCTTCTCA

TCR12 SEQ ID NO: 630

TCTCCTACTTACATGAATACTTCTCTTTTTTCTGTTTCCCTGAAGATTGAGCTCCCAACCCCAAG
TACGAAATAGGCTAAACCAATAAAAAATTGTGTGTTGGCCTGGTTGCATT

TCR13 SEQ ID NO: 631

20 ATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCT
GCCTATTCACCGATTTTGATTCTCAAACAAATGTGTCACAAAGTAAGGATTCTG

TCR14 SEQ ID NO: 632

ATGTGTATATCACAGACAAAACCTGTGCTAGACATGAGGTCTATGGACTTCAAGAGCAACAGTGCT
GTGGCCTGGAGCAACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAGCA

25 TCR15 SEQ ID NO: 633

TTATTCCAGAAGACACCTTCTTCCCCAGCCCAGAAAGTTCTGTGATGTCAAGCTGGTCGAGAAAA
GCTTTGAAACAGATACGAACCTAACTTTCAAAACCTGTCAGTGATTGGGTTCC

TCR16 SEQ ID NO: 634

30 GAATCCTCCTCCTGAAAGTGGCCGGGTTTAATCTGCTCATGACGCTGCGGCTGTGGTCCAGCTGAG
ATCTGCAAGATTGTAAGACAGCCTGTGCTCCCTCGCTCCTTCTCTGCATTGCC

TCR17 SEQ ID NO: 635

ACAGAGGGAACCTCTCCTACCCCAAGGAGGTGAAAGCTGCTACCACCTCTGTGCCCCCCCCGGCAA
TGCCACCAACTGGATCCTACCCGAATTTATGATTAAAGATTGCTGAAGAGCTGCCA

TCR18 SEQ ID NO: 636

35 AACACTGTGCCACCCCTCTGTTCCCTTATTGCTGCTTGTCACTGCCTGACATTCACGGCAGAGGC
AAGGCTGTGCAGCCTCCCTGGCTGTGCACATTCCCTCCTGTCCCCAGAGA

TCR19 SEQ ID NO: 637

CTGCCTCCGCCATCCCACAGATGATGGATCTTCAGTGGGTTCTCTTGGGCTCTAGGTCTGCAGAA
TGTTGTGAGGGGTTTATTTTTTTTAATAGTGTTCAAAAGAAATACATAGTAT

40 TCR20 SEQ ID NO: 638

TCTTCTTCTCAAGACGTGGGGGGAAATTATCTCATTATCGAGGCCCTGCTATGCTGTGTATCTGGGC
GTGTTGTATGTCCTGCTGCCGATGCCTTCATTAATAATGATTGGAAGAGCAGA

Blocking Oligonucleotides

[illegible]

5

After PCR enrichment, the PCR product was purified before the PCR indexing reaction. Quant-iT (ThermoFisher Scientific) analysis measured the DNA concentration of each sample, which was also normalized to the input material in the PCR indexing reaction (1.25 ng/reaction). Indexing PCR was performed as previously described (Stahl, P.L., et al., Visualization and analysis of gene expression in tissue sections by spatial transcriptomics, *Science*, 353(6294), 78-82, (2016)). In total, 24 unique indexes were used, with each cDNA library receiving a unique index (TRB, IGHG, and IGHM products from the same cDNA library received the same indexing since the TCR and BCR clonotypes can be distinguished from each other bioinformatically using the constant primer sequence). After purification, PCR reactions were pooled. The pooled PCR library was run on a gel and a large band at around 500 bp excised was gel-purified and sequenced (NovaSeq, 2x 150bp). The resulting data were de-multiplexed and the FastQ files were analyzed using MiXCR (Bolotin, D.A., et al., MiXCR: software for comprehensive adaptive immune profiling, *Nature Methods*, 12, 380-381 (2015), which is incorporated herein by reference in its entirety).

After PCR variable region primer enrichment, a number of TRB and IGH clonotypes in all prepared libraries were detected. For TRB, about 10,000 unique clonotypes were detected in spatial libraries prepared from tonsil tissue (data not shown) and between about 12,000 and about 25,000 unique clonotypes were detected in spatial libraries prepared from lymph node (data not shown). The positive control (SmartSeq2 RNAseq after PCR enrichment) yielded about 35,000 unique clonotypes. Variable region primer enrichment of the Smartseq2 library increased the TRB unique clonotype count over 35-fold, however, the SmartSeq2 library contained RNA extracted from two tonsil sections, whereas only a single tissue section was used for the spatial samples.

Similar results were observed for IGH detection with about 10,000 unique clonotypes detected from spatial libraries prepared from tonsil (**FIG. 8**) and between about 12,000 and about 25,000 unique clonotypes detected from spatial libraries prepared from the lymph node (**FIG. 9**). **FIGS. 8 and 9** also shows the number of unique clonotypes found in non-enriched samples (far right) and the data show the number of unique clonotypes found is less than the

V primer enriched samples. Approximately 10-fold higher IGHG clonotypes were detected relative to IGHM clonotypes similar to previous results for the SmartSeq2 RNAseq libraries (data not shown).

Variable region primer enrichment also resulted in a 4-fold increase in detected clonotypes for single-cell SmartSeq2 libraries. The clonotype increase observed after PCR variable primer enrichment of TRB relative to IGH is consistent with a known lower abundance of TRB transcripts in the cDNA library. For example, it is known that TRA/TRB transcript expression per T-cell is less relative to IGH/IGK/IGL expression per B-cell, and in particular, for plasma cells. Substantial, but not complete overlap, of IGH clonotypes between technical replicates detected in spatial libraries from tonsil tissue was observed. Similarly, substantial, but not complete, overlap of TRB clonotypes between technical replicates detected in spatial libraries from tonsil tissue was also observed. The data show that approximately half the clonotypes from a given technical replicate were detected in at least one or more of the other two technical replicates, which suggests many clonotypes were detected in a given technical replicate, but not all clonotypes were detected in each sample (data not shown). Approximately 10-20 fold increase in clonotype counts were detected with a poly(T) capture domain combined with PCR variable region primer enrichment relative to targeted capture, without variable region primer enrichment.

Collectively, these data show that PCR primer enrichment of analytes encoding immune cell receptors captured by poly(A) capture domains is possible. The use of a poly(A) capture domain allows for the simultaneous capture of analytes that do not encode for immune cell receptors and also does not require a custom array with targeted capture domains.

FIG. 10A shows H&E stained tonsil tissue on a spatial array and **FIG. 10B** shows the size distribution of spatial libraries prepared from tonsil tissue. The data shown in **FIGs. 10A-B** show the stained tonsil tissue and size distribution of the spatial libraries of the data included in this Example. Similar H&E staining was performed on breast tumor tissue on a spatial array and size distribution of spatial libraries were also prepared from breast tumor tissue (data not shown). **FIG. 11** shows clustering of B-cells and T-cells from the single-cell analysis performed in this Example. **FIG. 11** shows that while identifying populations of cells that include immune cell receptors, there is no connection to the spatial location of those cells within a biological sample. **FIG. 11** shows a single-cell analysis which is not designed to be a spatial representation of immune cells within a biological sample.

Example 3 –Enrichment of Analytes Encoding Immune Cell Receptors

FIG. 2 shows an exemplary workflow for the enrichment of T-cell receptor (TCR) analytes and B-cell receptor (BCR) analytes after capture on a spatial array. After capture of analytes (e.g., TCR and BCR analytes) cDNA is synthesized, followed by target enrichment and either library preparation and sequencing or further target enrichment via a semi-nested PCR for TCR analytes followed by Illumina sequencing and finally analysis.

Preparation of Visium Spatial Gene Expression Libraries

Sections of fresh-frozen breast tumor and tonsil tissue were sliced to 10 µm thickness and mounted onto slides from the Visium Spatial Gene Expression Slide & Reagent kit (10X Genomics). Sequencing libraries were prepared following the manufacturer's protocol (Document number CG000239 Rev A, 10x Genomics). Prior to imaging, coverslips were mounted on the slides according to the protocol's optional step "Coverslip Application & Removal". Tissue images were taken at 20x magnification using a Metafer Slide Scanning platform (MetaSystems) and raw images were stitched with VSlide software (MetaSystems). Adaptions of the protocol were made in that the Hematoxylin and Eosin (H&E) staining time was reduced to 4 minutes and tissue permeabilization was performed for 12 minutes.

Sequencing and data processing of Visium Spatial Gene Expression Libraries

Final sequencing libraries were sequenced on NextSeq2000 (Illumina) with a 28-10-10-150 setup (tonsil), or NovaSeq6000 (Illumina) with a 28-10-10-120 setup (breast tumor). 172M and 93M raw read pairs were obtained from tonsil-1 and tonsil-2, respectively, and 215M and 244M from breast tumor 1 and breast tumor 2, respectively.

Following demultiplexing of the bcl files, read 2 fastq files were trimmed using Cutadapt (Martin, M., Cutadapt removes adapter sequences from high-throughput sequencing reads, *EMBnet Journal*, 17(1) (2011)) to remove full-length or truncated template switch oligo (TSO) sequences from the 5' end (e.g., beginning of Read 2) and poly(A) homopolymers from the 3' end (e.g., end of read 2). The TSO sequence (SEQ ID NO: 114) (AAGCAGTGGTATCAACGCAGAGTACATGGG) was used as a non-internal 5' adapter with a minimum overlap of 5, meaning that partial matches (up to 5 base pairs) or intact TSO sequences were removed from the 5' end. The error tolerance was set to 0.1 for the TSO trimming to allow for a maximum of 3 errors. For the 3' end homopolymer trimming, a sequence of 10 As was used as a regular 3' adapter to remove potential polyA tail products regardless of its position in the read, also with a minimum overlap of 5 base pairs. The

trimmed data was processed with the SpaceRanger pipeline (10X Genomics), version 1.2.1 (tonsil) and version 1.0.0 (BC) and mapped to the GRCH38 v93 genome assembly.

Target enrichment with hybridization capture

TCR and BCR target enrichment was performed using IDT xGen Hybridization and Wash Kit (#1080584) with one enrichment probe pool (IDT) each for BCR and TCR transcripts (IG and TCR pool, Table 3). Custom blocking oligos (IDT, Table 3) were designed to hybridize to adaptor sequences of the cDNA library and to prevent off-target fragments from binding to BCR/TCR transcripts and contaminating the enriched library. The IG and TCR enrichment probe pools were mixed at ratio 1:3 and 1:12, respectively and each sample was enriched using both settings.

The “xGen hybridization capture of DNA libraries”, version 4 (IDT) protocol was followed with an input of 10 µl Visium cDNA per reaction, corresponding to between about 45-130 ng and the hybridization enrichment reaction was performed overnight.

The enriched and purified libraries were amplified twice with an AMPure bead wash after each PCR reaction, using 25 µl 2xKAPA mix, 7.5 µl cDNA primers (10X Genomics) and 17.5 µl sample in MQ water. The following settings were used for the PCRs: 1. 98°C 3 min; 2. 98°C 15 sec; 3. 63°C 30 sec; 4. 72°C 2 min; 5. Repeat steps 2-5 6x for a total of 7 cycles (1st PCR) and 4x for a total of 5 cycles (2nd PCR); 6. 72°C 1min

Library preparation and sequencing

The resulting product from the hybridization enrichment capture method was used as input into the SMRTbell library preparation protocol (PacBio). The DNA was concentrated by AMPure Bead Purification (0.8x), eluting in 6 µl of Elution Buffer, using 1 µl for Qubit measurements. At least 1 µg of input was used for each library and multiplexed 8 samples in total per sequencing run. PacBio Barcoded Overhand Adapters was used for multiplexing and followed the manufacturer’s instructions for the library preparations. The pooled library had a concentration of 11.4 ng/µl (50 µl total eluted volume). A SMRT Enzyme clean up kit was used to remove linear and single stranded DNA. The final libraries were sequenced at 2.7 million long read sequences (168-422K reads/sample) on a Sequel II at the National Genomics Infrastructure (NGI)/Uppsala Genome Center.

Sequencing data analysis

The input for the analysis was de-multiplexed consensus reads obtained from PacBio sequencing and performed with Python programming language. The fastq files were parsed into a dataframe with readID, sequence and quality columns. Data was searched for the Truseq adapter sequence and the TSO sequence to anchor the ends of each of the reads, and reads that lacked these sequences were discarded. A portion of the Truseq adapter starting in the first seven bases of either the read or its reverse complement was identified. If any of the positions matched the sequence with hamming distance 1 or less they were tagged. The same was performed for a portion of the TSO sequence. The sequences were reverse complemented as needed so that all the reads had the Truseq adapter (SEQ ID NO: 115) at the beginning and the TSO (SEQ ID NO: 114) at the end. The spatial barcode and the UMI were identified. The first 16 bases were obtained following the TruSeq adapter to determine the spatial barcode and subsequent bases determined the unique molecular identifier (UMI). Additionally, following the sequence of the UMI at least 4 bases were identified as all thymines (e.g., the poly(dT) capture domain) and filtered out of the reads that had any other bases within that interval. Any read with a UMI identified as a poly(dT) sequence was removed. The end of poly(dT) region is defined as the first matching position for the pattern '[^T]T{0,2}[^T]T{0,2}[^T]'.

Clonality analysis and visualization

To run MIXCR (version 3.0.3), poly(dT) and TSO sequences were trimmed and the reads were written to a new fastq file. The reads were analyzed with MIXCR and the following command:

```
'mixcr analyze shotgun -s hsa --align -OsaveOriginalReads=true --starting-material  
rna <TrimmedFastq> <SampleName>'
```

The following MIXCR command was performed to report alignments for each read:

```
'mixcr exportAlignments -f -cloneIdWithMappingType -cloneId -readIds -descrsR1  
<SampleName>.cna <ReportFile>'
```

The resulting tabular file was used to assign reads to the clonotypes in MIXCR output. Any reads that did not map to any clone were filtered out (cloneID==-1), then the reads were grouped in a table by the spatial barcode and UMI and counted how many reads were present and how many clones were associated with each UMI. UMIs that were assigned

to more than one clonotype were filtered out, since they are likely due to PCR or sequencing errors.

The resulting clonotype count matrices were subsequently loaded into R (R Core Team, A language and environment for statistical computing, *R Foundation for Statistical Computing*, (2017)). Tissue images, spatial coordinates and total gene expression counts obtained through the Visium platform and SpaceRanger pipeline were also loaded, and one Seurat object (Stuart et al. Comprehensive Integration of Single-Cell Data, *Cell*, 177(7) (2019)) per sample type (tonsil and breast tumor) was created using the STUtility package (Bergensträhle et al., Seamless integration of image and molecular analysis for spatial transcriptomics workflows, *BMC Genomics*, 21(1), (2020)). The clonotype count matrix was extended by adding any missing spatial barcodes that were present in the total gene expression count matrix, and filled with zero counts for all added barcodes. The new, extended matrix was loaded as a new assay into the Seurat object, where genes and clonotypes were visualized on the tissue images using built-in functions of the STUtility package.

Cell Processing for Single-cell RNA Sequencing

Single cell suspensions from five breast tumor regions (Tumor A-E) were prepared by enzymatic tissue dissociation using the human Tumor Dissociation Kit (Miltenyi Biotec, 130-095-929) and gentleMACS dissociator (Miltenyi Biotec). Cell suspensions were stained with the Zombie Aqua Fixable viability dye (Biolegend, 423101) at room temperature for 20 minutes, then washed with Phosphate Buffered Saline (PBS). The cells were incubated with Human TruStain Fc block (Biolegend, 422302) for 10 minutes to limit non-specific antibody binding, then stained for 20 minutes with anti-EPCAM (1:40, Biolegend, 324206) and anti-CD45 (1:40, Biolegend, 304021) in FACS buffer (PBS + 0.5% Bovine Serum Albumin). The cells were subsequently washed and resuspended in FACS buffer. Fluorescence-activated cell sorting (FACS) using an influx flow cytometer (BD Biosciences) was performed to sort live EPCAM+CD45+ single cells into an Eppendorf tube for 10x Genomics Chromium Single Cell gene expression analysis. Single stain controls (e.g., cells and beads) and fluorescence minus one controls (FMO), containing all the fluorochromes in the panel except the one being measured, were used to set voltages and to define the proper gating strategy.

10x Genomics Chromium Single-Cell library preparation and sequencing

Single-cell gene expression and VDJ clonotype libraries were generated from EPCAM-CD45+ cells using the 10x Genomics Chromium Single Cell 5' assay following the manufacturer's instructions. Libraries were profiled and quantified using a Bioanalyzer High Sensitivity DNA kit (Agilent Technologies) and Qubit High sensitivity kit (Thermo Fischer Scientific). Final single-cell gene expression libraries were sequenced (aiming for at least 30,000 reads per cell) on a NovaSeq 6000 SP flowcell (Illumina, 150-8-8-150 read set-up) by the National Genomics Infrastructure, SciLifeLab.

Single-cell gene expression and VDJ data processing

Sequencing outputs were processed by Cell Ranger (version 5.0, 10X Genomics). Gene-barcode count matrices were analyzed with the Seurat package (version 4.0, Satija Lab). Two steps of filtering were introduced here. First, raw gene expression matrices were subset by the barcode list in VDJ output, including T cell subsets and B cell subsets. Based on the UMI count, gene count, and mitochondrial percentage of raw gene expression matrices and their subsets, each threshold was selected to keep the maximum count of high-quality cells and avoid losing T and B cells which have VDJ sequencing outputs. Second, doublets in each sample were detected and filtered out by HTODemux() function in Seurat. All samples were integrated and scaled into one count matrix by Seurat. Dimension reduction, UMAP generation, and clustering, were performed on the merged dataset by Seurat. The merged dataset was clustered by a gradient of the resolution, from 0.2 to 2. The final resolution was determined by the significance of top-listed differentially expressed genes in each cluster. Cell types were annotated by differentially expressed genes and their marker genes expression level. All dimension reduction and annotation results, along with the VDJ output files were imported into Loupe Browser (version 5.0, 10X Genomics) and Loupe VDJ Browser (version 4.0, 10X Genomics) for interactive analysis.

Semi-nested PCR

After hybridization capture and post-capture PCR amplification (14 cycles), semi-nested PCR reactions were performed with the following primers: V primers targeting either the TRAV or TRBV genes, 5' of the CDR3 region (i.e. 'Outer' TRAV or TRBV primers, see Table 3 for sequences) and a primer ('partRead1', see Table 3) targeting the universal partial read 1 sequence present on the transcripts in Visium cDNA libraries. PartRead1 is also compatible with TruSeq indexes to allow multiplexing of samples for sequencing. For the semi-nested PCR experiments, the Visium cDNA was further pre-amplified prior to

hybridization capture to generate more input needed for testing. The Outer V primer PCR input was 1-5ng of hybridization captured cDNA from two breast tumor tissue Visium libraries (replicate, adjacent sections) and the reaction was run with KAPA HiFi HotStart ReadyMix (2X) (KAPA Biosystems). All primers were diluted 40x for a final concentration of 2.5 μ M (Integrated DNA Technologies). The PCR was run for 15 cycles under the following conditions: 1. 98°C 5 min; 2. 98°C 20 sec; 3. 65°C 30 sec; 4. 72°C 1:30 min; 5. Repeat steps 2-5 14x for a total of 15 cycles; and 6. 72°C 7 min.

Quantitative real-time PCR (qPCR) was performed to determine the appropriate number of cycles (to avoid exponential amplification). The Outer V primer PCR product was purified using AMPure beads (0.6x), followed by two 80% EtOH washes. The Outer V primer PCR product was eluted in EB buffer after incubation at 15min at 37°C. The cleaned up PCR product was quantified using Qubit and BioAnalyzer (Agilent). 3-5ng of each PCR product was used as input to the subsequent Inner V primer PCR.

The Inner V primer PCR was performed with the following primers: V primers targeting either the TRAV or the TRBV gene, close/adjacent to the CDR3 region (e.g., 'Inner' V primers) and the same universal partial read 1 primer as described for the Outer V primer PCR ('partRead1'). These Inner V primers have a handle compatible with TruSeq indexing. The primer concentrations and reagents were as described for the OUTER V primer PCR. qPCR was used to determine the optimal number of cycles (7). The following conditions were used for the PCR reaction: 7. 98°C 5 min; 8. 98°C 20 sec; 9. 72°C 30 sec; 10. 72°C 1:30 min; 11. Repeat steps 2-5 14x for a total of 15 cycles; 12. 72°C 7min.

The same AMPure bead-clean up and ethanol washes were performed as described above. The final eluted PCR product was quantified using Qubit and BioAnalyzer (Agilent). The samples were PCR indexed using TruSeq Indexes (5 cycles) and sequenced on a Novaseq sequencing instrument using a short read 1 and a longer read 2 to capture the entire CDR3 region and part of the constant region from the 5' end.

Target enrichment with hybridization capture for TCR and BCR sequences

As discussed above BCR (IGH, IGK, IGL) and TCR (TRA, TRB) clones can be amplified using PCR from poly(dT) captured cDNA libraries, e.g., Visium (10X Genomics). In some instances, the obtained amplicons lacked the spatial barcode. Therefore, to enrich for TCR and BCR sequences while preserving the spatial barcode and the CDR3 clonal information, a target enrichment strategy with hybridization probes (IDT technologies) was tested. Manufacturer's instructions were followed with some minor adaptations according to

the methods described above. Visium cDNA from two tonsil sections (e.g., from the same tonsil, spaced 150 μ M apart) were used as input material. **FIG. 12** shows poly(A) capture with a poly(T) capture domain. A poly(T) capture domain can capture other mRNA analytes from a tissue, including mRNA analytes encoding immune cell receptors, however, immune cell analytes were enriched using a hybridization enrichment probe strategy. BCR hybridization probes (n=174) were designed to span all BCR constant genes (e.g., IGH, IGL, IGK), see Table 3. Similarly, TCR hybridization probes (n=20) were designed to target the TCR constant genes (e.g., TRA, TRB), see Table 3. **FIG. 13** shows an exemplary cDNA library that would include BCR, TCR, other analytes and a pool of hybridization probes specific for BCR and TCR analytes. The Visium cDNA samples were hybridized with the hybridization enrichment capture probes and the hybridization reaction was performed overnight (**FIG. 14**), in the presence of blocking oligos as shown in **FIG. 15** targeting Read 1, Poly(dT)VN, and TSO sequences present on the transcripts in the cDNA library. After a series of washes, a post-capture PCR reaction was performed, which amplifies all, or a portion of, the captured analyte pool. Indexed PacBio libraries were prepared for long read sequencing from the eluted PCR products. To avoid unnecessary PCR cycles, which can introduce artifacts, errors, and chimeric fragments, barcoded overhang adapter ligation was performed to add unique sample indexes to each sample. The enriched libraries were then sequenced, de-multiplexed, and analyzed.

20

Clonotype numbers

cDNA prepared from captured immune cell mRNA analytes were enriched via a hybridization capture approach as described above and combined with PacBio long read sequencing. The resulting data successfully identified spatially barcoded BCR and TCR clones from tonsil Visium libraries (**FIGs. 16A-C**). A clone was defined as a single-chain with a unique combination of VDJ gene segments and a CDR3 region, based on MIXCR analysis (previously described) (Bolotin et al., (2015)). **FIG. 16A** shows the distribution of the clonotype (left) and UMI (right) count for two tonsil sections, spaced 150 μ m apart, from the same tonsil. The number of clonotypes per spot ranged between 0 and 300. For each tonsil sample, approximately 10,000 IGH, IGK and IGL clones (BCR) were identified (**FIG. 16B**). For TCRs, 3,437 TRB clonotypes and 687 TRA clonotypes were captured on average. The approximately five-fold lower capture of TRA clones was likely due to the lower expression of TRAC on a per cell basis consistent with previous results. The data demonstrate more successful capture of BCR clones (relative to TCR clones), which, without

wishing to be bound by theory may be due to several reasons, including a higher receptor expression by B cell lineage cells (particularly plasma cells) and a higher number of cells per B cell clone. Furthermore, all IGH isotypes were found, except for IGHE, which is expressed by very rare IgE positive B cell lineage cells (**FIG. 16C**). A small number of IGH clones were not assigned a constant gene. As expected, IGHG and IGHA-expressing cells dominate, followed by IGHM. The BCR light chains (IGK and IGL) were expressed at comparable numbers.

Collectively, the data demonstrate that target enrichment with hybridization probes from Visium cDNA mRNA libraries successfully enrich BCR and TCR clones from lymphocyte rich tissue.

B and T cell Spatial Segregation in the Tonsil

It was expected that with tonsil and similar tissues, e.g., lymph node, B cell clones would segregate mainly in follicles or germinal centers, in which B cell clonal selection and expansion occurs. In the Visium gene expression data, MS4A1, which encodes CD20 a B cell specific gene, was expressed in a cluster-like pattern that corresponded with increased cell density as visualized by the H&E staining, suggestive of B cell follicles (“B cell follicles”) (**FIG. 17A**, arrows). In contrast, SDC1, which encodes CD138 and is considered a reliable plasma cell enriched gene, was expressed mainly at the borders of the tissue and around B cell follicles, as expected from plasma cells (**FIG. 17A**). This cell type distribution was also supported by the spatial expression of the IGH constant gene (**FIG. 17B**, top), IGHM, which is expressed by B cells prior to class switching into other isotypes and was mostly enriched in the same B cell follicle-like areas as MS4A1. Similarly, IGHD, though more sparsely expressed, was also enriched in the B cell follicular areas. For other IGH isotypes (**FIG. 17A**, top) and the light chain (**FIG. 17B**, bottom), the highest gene expression was mainly outside B cell follicles, suggestive of increased expression by plasma cells. Based on CD3E and TCR constant gene (e.g., TRAC, TRBC1, TRBC2) expression, T cells were likely situated outside or around B cell follicles, which corresponds well with the presence of known, so-called “T cell zones” in lymphoid tissues (**FIG. 17C**).

Clonotype distribution in the tonsil

The data determined whether captured clones spatially segregate in tonsil tissue relative to the observed B and T cell segregation (**FIGs. 17A-C** and **FIGs. 18A-G**). The most abundant clone, IGKC, was highly expressed almost exclusively in a single B cell follicle, as

captured by the two tonsil sections spaced 150 μ M apart (**FIG. 18A**). Similar expression patterns were also observed for many clones; e.g., in **FIG. 18B**, a second representative clone, IGLC, was restricted to another B cell follicle. Without wishing to be bound by theory, these light chains may be expressed by B cells under-going selection and therefore are present in higher concentrations. Large clones, whose expression was not restricted to B cell follicles, were also found (see, e.g., **FIG. 18C**). These results indicate that clones can be captured with distinct spatial segregation within a tissue section. In accordance with IGHM gene expression, IGHM clones were also found in single follicles (see, e.g., **FIG. 18D** for a representative example). In contrast, IGHA-expressing clones, tended to be expressed along the border of the tonsil tissue (**FIG. 18E**), consistent with the spatial IGHA gene expression (**FIG. 18B**). TCR clones tended to locate at the border of B cell follicles (see, e.g., **FIG. 18F** and **FIG. 18G** for representative examples of TRB and TRA clones, respectively). TCR clones also tended to have lower UMI counts per clone on average compared to the BCR clones, again, confirming that TCR transcripts are less abundant in tonsil Visium cDNA libraries and subsequently in the enriched libraries.

Target enrichment of lymphocyte receptors in breast tumor tissue

Target enrichment strategies as described herein were also tested on breast tumor tissue. Due to the high frequency of tumor cells and stromal cells in breast tumor tissue, it was expected that lymphocyte-associated transcripts would be less abundant, relative to tonsil tissue. Visium libraries were generated from two consecutive sections from breast tumor tissue, isolated from a HER2+ breast tumor patient. **FIG. 19A** shows the distribution of the clonotype (left) and UMI (right) count for two breast tumor sections. The number of clonotypes per spot ranged between 0 and 300. For each tonsil sample, we identified approximately 10,000 IGH, IGK and IGL clones (BCR) (**FIG. 19B**). Using the same approach, approximately 1000 IGH, IGK, and IGL clones and between 20-100 TCR clones from each breast tumor section were captured (**FIGs. 19B** and **19C**). Fewer B and T cell clones were expected in the breast tumor samples relative to tonsil tissue, however, there were far fewer T cell clones relative to the B cell clones. Without wishing to be bound by theory, single-cell gene expression and VDJ libraries from the same tumor were prepared and 10-fold more T cells compared to B cell lineage cells (data not shown) were obtained from the single-cell data. Thus, the spatial methods described herein may be more efficient than single-cell approaches in capturing B cell expression and gene expression indicative of

plasma, whereas single-cell techniques may be superior in capturing T cells, relative to spatial transcriptomics for antigen receptors.

Spatial segregation of IGH clones (e.g., IGHV4-28, IGHD3-3, IGHD3-9, IGHJ4, IGHG1/IGHG3) within the breast tumor tissue was found consistent between two adjacent sections (See **FIG. 20** for a representative example). Furthermore, since the linked single-cell VDJ data from the same tumor sample was available, detection of paired clones in the spatial clonotype data was also performed. While the single-cell data was processed from a much larger tissue section relative to the 10 μ M tissue section used for spatial transcriptomic analysis, it was expected that large clones would be represented in both samples. By comparing the spatial transcriptomics for antigen receptor clonotype lists with the single-cell VDJ data, a total of 6 sets of paired BCR receptors were found in both datasets. The spatial gene expression of three such pairs are shown in **FIGs. 21A-C**. The similar spatial distribution for both chains for each clone and the concordance with the total respective IGH constant gene expression is demonstrated. For TCR clones, only two sets of paired receptors were found, with sparse UMI count (data not shown). The data show detection of paired receptors using spatial transcriptomics for antigen receptors and that these paired receptors are expressed in a spatially concordant manner.

Target enrichment for TCR using semi-nested PCR

As described above, the hybridization probe approach was more efficient at capturing BCR clonotypes, most probably due to higher expression on a per cell basis than TCR clonotypes. In order to improve TCR capture, a second target enrichment step was introduced to increase the T cell clonotype yield and to prepare libraries compatible with Illumina sequencing. After hybridization probe capture and subsequent PCR amplification, TCR analytes were enriched using a semi-nested PCR approach as shown in **FIG. 22**. The PCR is a two-step PCR in which two sets of V (e.g., primer to the variable domain region "V") primers (e.g., "Outer" and "Inner") targeting the TRAV and TRBV genes, respectively, were combined with a universal primer targeting the partial Read1 present on transcripts in the Visium cDNA library. The "Outer" primer can be referred to as a second primer and the "Inner" primer can be referred to as a third primer. The outer primers target gene regions further away from the start of the CDR3 (e.g., between about 200-270 bp from the end of the coding V segment), whereas the inner primers target gene segments closer to the CDR3 (between about 20-25 bp from the end of the coding V segment).

The results show amplification of both TRA and TRB transcripts using the semi-nested PCR approach from breast tumor Visium libraries and that these libraries had the expected sizes (data not shown).

- Collectively, the data demonstrate that spatial transcriptomics for antigen receptors
- 5 can isolate high numbers of BCR and TCR clonotypes from tonsil and breast tumor tissue. These clones segregate in the tissue in characteristic ways concordant with their biology and cell type gene expression patterns.

Embodiments

- 10 Embodiment 1 is a method for determining the presence and/or abundance of an immune cell clonotype at a location in a biological sample, the method comprising: (a) contacting a biological sample with an array comprising a plurality of capture probes, wherein a capture probe of the plurality of capture probes comprises (i) a spatial barcode and (ii) a capture domain that specifically binds to a nucleic acid encoding an immune cell
- 15 receptor of the immune cell clonotype; and (b) determining (i) all or a portion of the sequence of the spatial barcode or a complement thereof, and (ii) all or a portion of the sequence of the nucleic acid encoding the immune cell receptor or a complement thereof, and using the determined sequences of (i) and (ii) to determine the presence and/or abundance of the immune cell clonotype at a location in the biological sample.

- 20 Embodiment 2 is the method of embodiment 1, wherein the immune cell clonotype is a T cell clonotype.

Embodiment 3. The method of embodiment 2, wherein the immune cell receptor is a T cell receptor alpha chain.

- Embodiment 4 is the method of embodiment 3, wherein the capture domain binds
- 25 specifically to a nucleic acid sequence encoding a constant region of the T cell receptor alpha chain.

Embodiment 5 is the method of embodiment 3 or 4, wherein step (b) comprises determining a sequence encoding CDR3 of the T cell receptor alpha chain.

- Embodiment 6 is the method of embodiment 5, wherein step (b) further comprises
- 30 determining a sequence encoding one or both of CDR1 and CDR2 of the T cell receptor alpha chain.

Embodiment 7 is the method of embodiment 5, wherein step (b) further comprises determining a sequence encoding a full-length variable domain of the T cell receptor alpha chain.

Embodiment 8 is the method of embodiment 2, wherein the immune cell receptor is a
5 T cell receptor beta chain.

Embodiment 9 is the method of embodiment 8, wherein the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the T cell receptor beta chain.

Embodiment 10 is the method of embodiment 8 or 9, wherein step (b) comprises
10 determining a sequence encoding CDR3 of the T cell receptor beta chain.

Embodiment 11 is the method of embodiment 10, wherein step (b) further comprises determining a sequence encoding one or both of CDR1 and CDR2 of the T cell receptor beta chain.

Embodiment 12 is the method of embodiment 10, wherein step (b) further comprises
15 determining a full-length variable domain of the T cell receptor beta chain.

Embodiment 13 is the method of embodiment 1, wherein the immune cell clonotype is a B cell clonotype.

Embodiment 14 is the method of embodiment 13, wherein the immune cell receptor is an immunoglobulin kappa light chain.

Embodiment 15 is the method of embodiment 14, wherein the capture domain binds
20 specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin kappa light chain.

Embodiment 16 is the method of embodiment 14 or 15, wherein step (b) comprises determining a sequence encoding CDR3 of the immunoglobulin kappa light chain.

Embodiment 17 is the method of embodiment 16, wherein step (b) further comprises
25 determining a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin kappa light chain.

Embodiment 18 is the method of embodiment 16, wherein step (b) further comprises determining a sequence encoding a full-length variable domain of the immunoglobulin kappa
30 light chain.

Embodiment 19 is the method of embodiment 13, wherein the immune cell receptor is an immunoglobulin lambda light chain.

Embodiment 20 is the method of embodiment 19, wherein the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin lambda light chain.

Embodiment 21 is the method of embodiment 19 or 20, wherein step (b) comprises
5 determining a sequence encoding CDR3 of the immunoglobulin lambda light chain.

Embodiment 22 is the method of embodiment 21, wherein step (b) further comprises determining a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin lambda light chain.

Embodiment 23 is the method of embodiment 21, wherein step (b) further comprises
10 determining a sequence encoding a full-length variable domain of the immunoglobulin lambda light chain.

Embodiment 24 is the method of embodiment 13, wherein the immune cell receptor is an immunoglobulin heavy chain.

Embodiment 25 is the method of embodiment 24, wherein the capture domain binds
15 specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin heavy chain.

Embodiment 26 is the method of embodiment 24 or 25, wherein step (b) comprises determining a sequence encoding CDR3 of the immunoglobulin heavy chain.

Embodiment 27 is the method of embodiment 26, wherein step (b) further comprises
20 determining a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin heavy chain.

Embodiment 28 is the method of embodiment 26, wherein step (b) further comprises determining a sequence encoding a full-length variable domain of the immunoglobulin heavy chain.

Embodiment 29 is the method of any one of embodiments 1-28, wherein step (b)
25 comprises the capture probe using the nucleic acid encoding the immune cell receptor as a template, thereby generating an extended capture probe.

Embodiment 30 is the method of embodiment 29, wherein step (b) comprises extending a 3' end of the capture probe.

Embodiment 31 is the method of embodiment 29 or 30, wherein step (b) further
30 comprises generating a second strand of nucleic acid that comprises (i) a sequence that is complementary to all or a portion of the spatial barcode, and (ii) a sequence that corresponds to all or a portion of the sequence of the nucleic acid encoding the immune cell receptor.

Embodiment 32 is the method of any one of embodiments 1-31, wherein the capture probe further comprises a cleavage domain, a functional domain, a unique molecular identifier, or any combination thereof.

5 Embodiment 33 is the method of any one of embodiments 1-30, wherein the capture probe further comprises a functional domain.

Embodiment 34 is the method of embodiment 33, wherein step (b) further comprises generating a second strand of nucleic acid that comprises (i) a sequence that is complementary to all or a portion of the functional domain, (ii) a sequence that is complementary to all or a portion of the spatial barcode, and (iii) a sequence that corresponds
10 to all or a portion of the sequence of the nucleic acid encoding the immune cell receptor.

Embodiment 35 is the method of embodiment 34, wherein step (b) further comprises amplifying the second strand of nucleic acid using (i) a first primer comprising all or a portion of the functional domain, wherein the functional domain is 5' to the spatial barcode in the second strand of nucleic acid, and (ii) a second primer comprising a sequence that is
15 substantially complementary to a portion of a sequence encoding a variable region of the immune cell receptor.

Embodiment 36 is the method of any one of embodiments 1-35, wherein the biological sample comprises a tissue sample.

Embodiment 37 is the method of embodiment 36, wherein the tissue sample is a tissue
20 section.

Embodiment 38 is the method of embodiment 37, wherein the tissue section is a fixed tissue section.

Embodiment 39 is the method of embodiment 38, wherein the fixed tissue section is a
25 formalin-fixed paraffin-embedded tissue section.

Embodiment 40 is the method of any one of embodiments 37-39, wherein the tissue section comprises a tumor region.

Embodiment 41 is the method of any one of embodiments 1-40, wherein the nucleic acid encoding the immune cell receptor comprises RNA.

30 Embodiment 42 is the method of embodiment 41, wherein the RNA is mRNA.

Embodiment 43 is the method of any one of embodiments 1-40, wherein the nucleic acid encoding the immune cell receptor comprises DNA.

Embodiment 44 is the method of embodiment 43, wherein the DNA is genomic DNA.

Embodiment 45 is the method of any one of embodiments 1-44, wherein the method further comprises, prior to step (b), contacting the biological sample with ribosomal RNA depletion probes and mitochondrial RNA depletion probes.

5 Embodiment 46 is the method of any one of embodiments 1-45, wherein the method further comprises imaging the biological sample.

Embodiment 47 is the method of any one of embodiments 1-46, wherein the determining in step (b) comprises sequencing (i) all or a portion of the sequence of the spatial barcode or a complement thereof, and (ii) all or a portion of the sequence of the nucleic acid encoding the immune cell receptor or a complement thereof.

10 Embodiment 48 is the method of any one of embodiments 1-47, wherein step (b) comprises determining the presence of the immune cell clonotype at a location in the biological sample.

Embodiment 49 is the method of any one of embodiments 1-47, wherein step (b) comprises determining the abundance of the immune cell clonotype at a location in the
15 biological sample.

Embodiment 50 is the method of any one of embodiments 1-47, wherein step (b) comprises determining the presence and abundance of the immune cell clonotype at a location in the biological sample.

Embodiment 51 is the method of any one of embodiments 1-47, wherein step (b)
20 comprises determining the presence of two or more immune cell clonotypes at a location in the biological sample.

Embodiment 52 is the method of any one of embodiments 1-47, wherein step (b) comprises determining the abundance of two or more immune cell clonotypes at a location in the biological sample.

25 Embodiment 53 is the method of any one of embodiments 1-47, wherein step (b) comprises determining the presence and abundance of two or more immune cell clonotypes at a location in the biological sample.

Embodiment 54 is the method of any one of embodiments 51-53, wherein the method further comprises comparing the two or more immune cell clonotypes.

30 Embodiment 55 is the method of any one of embodiments 51-54, wherein the two or more immune cell clonotypes are each a B cell clonotype.

Embodiment 56 is the method of any one of embodiments 51-54, wherein the two or more immune cell clonotypes are each a T cell clonotype.

Embodiment 57 is the method of any one of embodiments 51-54, wherein the two or more immune cell clonotypes comprise at least one T cell clonotype and at least one B cell clonotype.

Embodiment 58 is a method for determining the presence and/or abundance of an immune cell receptor at a location in a biological sample, the method comprising: (a) contacting a biological sample with an array comprising a plurality of capture probes, wherein a capture probe of the plurality of capture probes comprises (i) a spatial barcode and (ii) a capture domain that specifically binds to a nucleic acid encoding an immune cell receptor; and (b) determining (i) all or a portion of the sequence of the spatial barcode or a complement thereof, and (ii) all or a portion of the sequence of the nucleic acid encoding the immune cell receptor or a complement thereof, and using the determined sequences of (i) and (ii) to determine the presence and/or abundance of the immune cell receptor at a location in the biological sample.

Embodiment 59 is the method of embodiment 58, wherein the immune cell receptor is a T cell receptor alpha chain.

Embodiment 60 is the method of embodiment 59, wherein the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the T cell receptor alpha chain.

Embodiment 61 is the method of embodiment 59 or 60, wherein step (b) comprises determining a sequence encoding CDR3 of the T cell receptor alpha chain.

Embodiment 62 is the method of embodiment 61, wherein step (b) further comprises determining a sequence encoding one or both of CDR1 and CDR2 of the T cell receptor alpha chain.

Embodiment 63 is the method of embodiment 61, wherein step (b) further comprises determining a sequence encoding a full-length variable domain of the T cell receptor alpha chain.

Embodiment 64 is the method of embodiment 58, wherein the immune cell receptor is a T cell receptor beta chain.

Embodiment 65 is the method of embodiment 64, wherein the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the T cell receptor beta chain.

Embodiment 66 is the method of embodiment 64 or 65, wherein step (b) comprises determining a sequence encoding CDR3 of the T cell receptor beta chain.

Embodiment 67 is the method of embodiment 66, wherein step (b) further comprises determining a sequence encoding one or both of CDR1 and CDR2 of the T cell receptor beta chain.

Embodiment 68 is the method of embodiment 66, wherein step (b) further comprises
5 determining a full-length variable domain of the T cell receptor beta chain.

Embodiment 69 is the method of embodiment 58, wherein the immune cell receptor is an immunoglobulin kappa light chain.

Embodiment 70 is the method of embodiment 69, wherein the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin
10 kappa light chain.

Embodiment 71 is the method of embodiment 69 or 70, wherein step (b) comprises determining a sequence encoding CDR3 of the immunoglobulin kappa light chain.

Embodiment 72 is the method of embodiment 71, wherein step (b) further comprises determining a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin
15 kappa light chain.

Embodiment 73 is the method of embodiment 71, wherein step (b) further comprises determining a sequence encoding a full-length variable domain of the immunoglobulin kappa light chain.

Embodiment 74 is the method of embodiment 58, wherein the immune cell receptor is
20 an immunoglobulin lambda light chain.

Embodiment 75 is the method of embodiment 74, wherein the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin lambda light chain.

Embodiment 76 is the method of embodiment 74 or 75, wherein step (b) comprises
25 determining a sequence encoding CDR3 of the immunoglobulin lambda light chain.

Embodiment 77 is the method of embodiment 76, wherein step (b) further comprises determining a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin lambda light chain.

Embodiment 78 is the method of embodiment 76, wherein step (b) further comprises
30 determining a sequence encoding a full-length variable domain of the immunoglobulin lambda light chain.

Embodiment 79 is the method of embodiment 58, wherein the immune cell receptor is an immunoglobulin heavy chain.

Embodiment 80 is the method of embodiment 79, wherein the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin heavy chain.

Embodiment 81 is the method of embodiment 79 or 80, wherein step (b) comprises
5 determining a sequence encoding CDR3 of the immunoglobulin heavy chain.

Embodiment 82 is the method of embodiment 81, wherein step (b) further comprises determining a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin heavy chain.

Embodiment 83 is the method of embodiment 81, wherein step (b) further comprises
10 determining a sequence encoding a full-length variable domain of the immunoglobulin heavy chain.

Embodiment 84 is the method of any one of embodiments 58-83, wherein step (b) comprises extending an end of the capture probe using the nucleic acid encoding the immune cell receptor as a template, thereby generating an extended capture probe.

Embodiment 85 is the method of embodiment 84, wherein step (b) comprises
15 extending a 3' end of the capture probe.

Embodiment 86 is the method of embodiment 84 or 85, wherein step (b) further comprises generating a second strand of nucleic acid that comprises (i) a sequence that is complementary to all or a portion of the spatial barcode, and (ii) a sequence that corresponds
20 to all or a portion of the sequence of the nucleic acid encoding the immune cell receptor.

Embodiment 87 is the method of any one of embodiments 58-86, where the capture probe further comprises a cleavage domain, a functional domain, a unique molecular identifier, or any combination thereof.

Embodiment 88 is the method of any one of embodiments 58-85, wherein the capture
25 probe further comprises a functional domain.

Embodiment 89 is the method of embodiment 88, wherein step (b) further comprises generating a second strand of nucleic acid that comprises (i) a sequence that is complementary to all or a portion of the functional domain, (ii) a sequence that is complementary to all or a portion of the spatial barcode, and (iii) a sequence that corresponds
30 to all or a portion of the sequence of the nucleic acid encoding the immune cell receptor.

Embodiment 90 is the method of embodiment 89, wherein step (b) further comprises amplifying the second strand of nucleic acid using (i) a first primer comprising all or a portion of the functional domain, wherein the functional domain is 5' to the spatial barcode in the second strand of nucleic acid, and (ii) a second primer comprising a sequence that is

substantially complementary to a portion of a sequence encoding a variable region of the immune cell receptor.

Embodiment 91 is the method of any one of embodiments 58-90, wherein the biological sample comprises a tissue sample.

5 Embodiment 92 is the method of embodiment 91, wherein the tissue sample is a tissue section.

Embodiment 93 is the method of embodiment 92, wherein the tissue section is a fixed tissue section.

10 Embodiment 94 is the method of embodiment 93, wherein the fixed tissue section is a formalin-fixed paraffin-embedded tissue section.

Embodiment 95 is the method of any one of embodiments 92-94, wherein the tissue section comprises a tumor region.

Embodiment 96 is the method of any one of embodiments 58-95, wherein the nucleic acid encoding the immune cell receptor comprises RNA.

15 Embodiment 97 is the method of embodiment 96, wherein the RNA is mRNA.

Embodiment 98 is the method of any one of embodiments 58-95, wherein the nucleic acid encoding the immune cell receptor comprises DNA.

Embodiment 99 is the method of embodiment 98, wherein the DNA is genomic DNA.

20 Embodiment 100 is the method of any one of embodiments 58-99, wherein the method further comprises, prior to step (b), contacting the biological sample with ribosomal RNA depletion probes and mitochondrial RNA depletion probes.

Embodiment 101 is the method of any one of embodiments 58-100, wherein the method further comprises imaging the biological sample.

25 Embodiment 102 is the method of any one of embodiments 58-101, wherein the determining in step (b) comprises sequencing (i) all or a portion of the sequence of the spatial barcode or a complement thereof, and (ii) all or a portion of the sequence of the nucleic acid encoding the immune cell receptor or a complement thereof.

30 Embodiment 103 is the method of any one of embodiments 58-102, wherein step (b) comprises determining the presence of the immune cell receptor at a location in the biological sample.

Embodiment 104 is the method of any one of embodiments 58-102, wherein step (b) comprises determining the abundance of the immune cell receptor at a location in the biological sample.

Embodiment 105 is the method of any one of embodiments 58-102, wherein step (b) comprises determining the presence and abundance of the immune cell receptor at a location in the biological sample.

Embodiment 106 is the method of any one of embodiments 58-102, wherein step (b)
5 comprises determining the presence of two or more immune cell receptors at a location in the biological sample.

Embodiment 107 is the method of any one of embodiments 58-102, wherein step (b) comprises determining the abundance of two or more immune cell receptors at a location in the biological sample.

10 Embodiment 108 is the method of any one of embodiments 58-102, wherein step (b) comprises determining the presence and abundance of two or more immune cell receptors at a location in the biological sample.

Embodiment 109 is the method of any one of embodiments 106-108, wherein the method further comprises comparing the two or more immune cell receptors.

15 Embodiment 110 is the method of any one of embodiments 106-109, wherein the two or more immune cell clonotypes are each an immune cell receptor of a B cell.

Embodiment 111 is the method of any one of embodiments 106-109, wherein the two or more immune cell clonotypes are each an immune cell receptor of a T cell.

Embodiment 112 is the method of any one of embodiments 106-109, wherein the two
20 or more immune cell clonotypes comprise at least one immune cell receptor of a T cell and at least one immune cell receptor from a B cell.

Embodiment 113 is an array comprising a plurality of capture probes, wherein a capture probe of the plurality of capture probes comprises (i) a spatial barcode and (ii) a capture domain that specifically binds to a nucleic acid encoding an immune cell receptor of
25 an immune cell clonotype.

Embodiment 114 is the array of embodiment 113, wherein the immune cell clonotype is a T cell clonotype.

Embodiment 115 is the array of embodiment 114, wherein the immune cell receptor is a T cell receptor alpha chain.

30 Embodiment 116 is the array of embodiment 115, wherein the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the T cell receptor alpha chain.

Embodiment 117 is the array of embodiment 114, wherein the immune cell receptor is a T cell receptor beta chain.

Embodiment 118 is the array of embodiment 117, wherein the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the T cell receptor beta chain.

Embodiment 119 is the array of embodiment 113, wherein the immune cell clonotype
5 is a B cell clonotype.

Embodiment 120 is the array of embodiment 119, wherein the immune cell receptor is an immunoglobulin kappa light chain.

Embodiment 121 is the array of embodiment 120, wherein the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin
10 kappa light chain.

Embodiment 122 is the array of embodiment 119, wherein the immune cell receptor is an immunoglobulin lambda light chain.

Embodiment 123 is the array of embodiment 122, wherein the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin
15 lambda light chain.

Embodiment 124 is the array of embodiment 119, wherein the immune cell receptor is an immunoglobulin heavy chain.

Embodiment 125 is the array of embodiment 124, wherein the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin
20 heavy chain.

Embodiment 126 is the array of any one of embodiments 113-125, where the capture probe further comprises a cleavage domain, a functional domain, a unique molecular identifier, or any combination thereof.

Embodiment 127. A kit comprising: an array of any one of embodiments 113-126;
25 and one or both of ribosomal RNA depletion probes and mitochondrial RNA depletion probes.

Embodiment 128 is a method for determining the presence and/or abundance of an immune cell clonotype at a location in a biological sample, the method comprising: (a) contacting a biological sample with an array comprising a plurality of capture probes,
30 wherein a capture probe of the plurality of capture probes comprises (i) a spatial barcode and (ii) a capture domain that binds to a nucleic acid encoding an immune cell receptor of the immune cell clonotype; (b) determining (i) all or a portion of the sequence of the spatial barcode or a complement thereof, and (ii) all or a portion of the sequence of the nucleic acid encoding the immune cell receptor or a complement thereof, and using the determined

sequences of (i) and (ii) to determine the presence and/or abundance of the immune cell clonotype at a location in the biological sample.

Embodiment 129 is the method of embodiment 1, wherein step (b) comprises extending the capture probe using the nucleic acid encoding the immune cell receptor as a
5 template, thereby generating an extended capture probe.

Embodiment 130 is the method of embodiment 129, wherein step (b) comprises extending a 3' end of the capture probe.

Embodiment 131 is the method of embodiment 129 or 130, wherein step (b) further comprises generating a second strand of nucleic acid that comprises (i) a sequence that is
10 complementary to all or a portion of the spatial barcode, and (ii) a sequence that corresponds to all or a portion of the sequence of the nucleic acid encoding the immune cell receptor.

Embodiment 132 is the method of any one of embodiments 128-131, wherein the capture probe further comprises a cleavage domain, a functional domain, a unique molecular identifier, or any combination thereof.

15 Embodiment 133 is the method of any one of embodiments 128-132, wherein the capture domain comprises a poly(T) sequence.

Embodiment 134 is the method of any one of embodiments 128-133, wherein the capture probe further comprises a functional domain.

Embodiment 135 is the method of embodiment 134, wherein step (b) further
20 comprises generating a second strand of nucleic acid that comprises (i) a sequence that is complementary to all or a portion of the functional domain, (ii) a sequence that is complementary to all or a portion of the spatial barcode, and (iii) a sequence that corresponds to all or a portion of the sequence of the nucleic acid encoding the immune cell receptor.

Embodiment 136 is the method of embodiment 135, wherein step (b) further
25 comprises amplifying the second strand of nucleic acid using (i) a first primer comprising all or a portion of the functional domain, wherein the functional domain is 5' to the spatial barcode in the second strand of nucleic acid, and (ii) a second primer comprising a sequence that is substantially complementary to a portion of a sequence encoding a variable region of the immune cell receptor.

30 Embodiment 137 is the method of any one of embodiments 128-136, wherein the immune cell clonotype is a T cell clonotype.

Embodiment 138 is the method of embodiment 137, wherein the immune cell receptor is a T cell receptor alpha chain.

Embodiment 139 is the method of embodiment 138, wherein step (b) comprises determining a sequence encoding CDR3 of the T cell receptor alpha chain.

Embodiment 140 is the method of embodiment 139, wherein step (b) further comprises determining a sequence encoding one or both of CDR1 and CDR2 of the T cell
5 receptor alpha chain.

Embodiment 141 is the method of embodiment 139, wherein step (b) further comprises determining a sequence encoding a full-length variable domain of the T cell receptor alpha chain.

Embodiment 142 is the method of embodiment 137, wherein the immune cell receptor
10 is a T cell receptor beta chain.

Embodiment 143 is the method of embodiment 142, wherein step (b) comprises determining a sequence encoding CDR3 of the T cell receptor beta chain.

Embodiment 144 is the method of embodiment 143, wherein step (b) further comprises determining a sequence encoding one or both of CDR1 and CDR2 of the T cell
15 receptor beta chain.

Embodiment 145 is the method of embodiment 143, wherein step (b) further comprises determining a full-length variable domain of the T cell receptor beta chain.

Embodiment 146 is the method of any one of embodiments 128-136, wherein the immune cell clonotype is a B cell clonotype.

Embodiment 147 is the method of embodiment 146, wherein the immune cell receptor
20 is an immunoglobulin kappa light chain.

Embodiment 148 is the method of embodiment 147, wherein step (b) comprises determining a sequence encoding CDR3 of the immunoglobulin kappa light chain.

Embodiment 149 is the method of embodiment 148, wherein step (b) further
25 comprises determining a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin kappa light chain.

Embodiment 150 is the method of embodiment 148, wherein step (b) further comprises determining a sequence encoding a full-length variable domain of the immunoglobulin kappa light chain.

Embodiment 151 is the method of embodiment 146, wherein the immune cell receptor
30 is an immunoglobulin lambda light chain.

Embodiment 152 is the method of embodiment 151, wherein step (b) comprises determining a sequence encoding CDR3 of the immunoglobulin lambda light chain.

Embodiment 153 is the method of embodiment 152, wherein step (b) further comprises determining a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin lambda light chain.

Embodiment 154 is the method of embodiment 152, wherein step (b) further
5 comprises determining a sequence encoding a full-length variable domain of the immunoglobulin lambda light chain.

Embodiment 155 is the method of embodiment 146, wherein the immune cell receptor is an immunoglobulin heavy chain.

Embodiment 156 is the method of embodiment 155, wherein step (b) comprises
10 determining a sequence encoding CDR3 of the immunoglobulin heavy chain.

Embodiment 157 is the method of embodiment 156, wherein step (b) further comprises determining a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin heavy chain.

Embodiment 158 is the method of embodiment 156, wherein step (b) further
15 comprises determining a sequence encoding a full-length variable domain of the immunoglobulin heavy chain.

Embodiment 159 is the method of any one of embodiments 128-158, wherein the biological sample comprises a tissue sample.

Embodiment 160 is the method of embodiment 159, wherein the tissue sample is a
20 tissue section.

Embodiment 161 is the method of embodiment 160, wherein the tissue section is a fixed tissue section.

Embodiment 162 is the method of embodiment 161, wherein the fixed tissue section is a formalin-fixed paraffin-embedded tissue section.

Embodiment 163 is the method of any one of embodiments 160-162, wherein the
25 tissue section comprises a tumor region.

Embodiment 164 is the method of any one of embodiments 128-163, wherein the nucleic acid encoding the immune cell receptor comprises RNA.

Embodiment 165 is the method of embodiment 164, wherein the RNA is mRNA.

Embodiment 166 is the method of any one of embodiments 128-163, wherein the
30 nucleic acid encoding the immune cell receptor comprises DNA.

Embodiment 167 is the method of embodiment 166, wherein the DNA is genomic DNA.

Embodiment 168 is the method of any one of embodiments 128-167, wherein the method further comprises, prior to step (b), contacting the biological sample with ribosomal RNA depletion probes and mitochondrial RNA depletion probes.

Embodiment 169 is the method of any one of embodiments 128-168, wherein the
5 method further comprises imaging the biological sample.

Embodiment 170 is the method of any one of embodiments 128-169, wherein the determining in step (b) comprises sequencing (i) all or a portion of the sequence of the spatial barcode or a complement thereof, and (ii) all or a portion of the sequence of the nucleic acid encoding the immune cell receptor or a complement thereof.

10 Embodiment 171 is the method of any one of embodiments 128-170, wherein step (b) comprises determining the presence of the immune cell clonotype at a location in the biological sample.

Embodiment 172 is the method of any one of embodiments 128-171, wherein step (b) comprises determining the abundance of the immune cell clonotype at a location in the
15 biological sample.

Embodiment 173 is the method of any one of embodiments 128-172, wherein step (b) comprises determining the presence and abundance of the immune cell clonotype at a location in the biological sample.

Embodiment 174 is the method of any one of embodiments 128-173, wherein step (b)
20 comprises determining the presence of two or more immune cell clonotypes at a location in the biological sample.

Embodiment 175 is the method of any one of embodiments 128-174, wherein step (b) comprises determining the abundance of two or more immune cell clonotypes at a location in the biological sample.

25 Embodiment 176 is the method of any one of embodiments 128-174, wherein step (b) comprises determining the presence and abundance of two or more immune cell clonotypes at a location in the biological sample.

Embodiment 177 is the method of any one of embodiments 174-176, wherein the method further comprises comparing the two or more immune cell clonotypes.

30 Embodiment 178 is the method of any one of embodiments 174-177, wherein the two or more immune cell clonotypes are each a B cell clonotype.

Embodiment 179 is the method of any one of embodiments 174-177, wherein the two or more immune cell clonotypes are each a T cell clonotype.

Embodiment 180 is the method of any one of embodiments 174-177, wherein the two or more immune cell clonotypes comprise at least one T cell clonotype and at least one B cell clonotype.

5

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

10

All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition section headings, the materials, methods, and examples are illustrative only and not intended to be limiting.

WHAT IS CLAIMED IS:

1. A method for determining the presence and/or abundance of an immune cell clonotype at a location in a biological sample, the method comprising:
 - 5 (a) contacting a biological sample with an array comprising a plurality of capture probes, wherein a capture probe of the plurality of capture probes comprises (i) a spatial barcode and (ii) a capture domain that binds to a nucleic acid encoding an immune cell receptor of the immune cell clonotype; and
 - (b) determining (i) all or a portion of the sequence of the spatial barcode or a
10 complement thereof, and (ii) all or a portion of the sequence of the nucleic acid encoding the immune cell receptor or a complement thereof, and using the determined sequences of (i) and (ii) to determine the presence and/or abundance of the immune cell clonotype at a location in the biological sample.
- 15 2. The method of claim 1, wherein the immune cell clonotype is a T cell clonotype.
3. The method of claim 2, wherein the T cell clonotype is a T cell receptor alpha chain.
- 20 4. The method of claim 3, wherein the capture domain binds to a nucleic acid sequence encoding a constant region of the T cell receptor alpha chain.
5. The method of claim 3 or 4, wherein step (b) comprises determining a sequence encoding CDR3 of the T cell receptor alpha chain.
- 25 6. The method of claim 5, wherein step (b) further comprises determining a sequence encoding one or both of CDR1 and CDR2 of the T cell receptor alpha chain.
7. The method of claim 5, wherein step (b) further comprises determining a sequence
30 encoding a full-length variable domain of the T cell receptor alpha chain.
8. The method of claim 2, wherein the immune cell receptor is a T cell receptor beta chain.

9. The method of claim 8, wherein the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the T cell receptor beta chain.

10. The method of claim 8 or 9, wherein step (b) comprises determining a sequence
5 encoding CDR3 of the T cell receptor beta chain.

11. The method of claim 10, wherein step (b) further comprises determining a sequence encoding one or both of CDR1 and CDR2 of the T cell receptor beta chain.

12. The method of claim 10, wherein step (b) further comprises determining a
10 sequence encoding a full-length variable domain of the T cell receptor beta chain.

13. The method of claim 1, wherein the immune cell clonotype is a B cell clonotype.

14. The method of claim 13, wherein the B cell clonotype is an immunoglobulin
15 kappa light chain.

15. The method of claim 14, wherein the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin kappa light chain.

20 16. The method of claim 14 or 15, wherein step (b) comprises determining a sequence encoding CDR3 of the immunoglobulin kappa light chain.

17. The method of claim 16, wherein step (b) further comprises determining a
25 sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin kappa light chain.

18. The method of claim 16, wherein step (b) further comprises determining a sequence encoding a full-length variable domain of the immunoglobulin kappa light chain.

30 19. The method of claim 13, wherein the B cell clonotype is an immunoglobulin lambda light chain.

20. The method of claim 19, wherein the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin lambda light chain.

21. The method of claim 19 or 20, wherein step (b) comprises determining a sequence
5 encoding CDR3 of the immunoglobulin lambda light chain.

22. The method of claim 21, wherein step (b) further comprises determining a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin lambda light chain.
10

23. The method of claim 21, wherein step (b) further comprises determining a sequence encoding a full-length variable domain of the immunoglobulin lambda light chain.

24. The method of claim 13, wherein the B cell clonotype is an immunoglobulin
15 heavy chain.

25. The method of claim 24, wherein the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin heavy chain.

26. The method of claim 24 or 25, wherein step (b) comprises determining a sequence
20 encoding CDR3 of the immunoglobulin heavy chain.

27. The method of claim 26, wherein step (b) further comprises determining a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin heavy chain.
25

28. The method of claim 26, wherein step (b) further comprises determining a sequence encoding a full-length variable domain of the immunoglobulin heavy chain.

29. The method of claim 3 or 13, wherein the capture domain binds a poly(A)
30 sequence of a nucleic acid encoding an immune cell clonotype.

30. The method of claim 29, wherein the capture domain binds to a nucleic acid sequence encoding a T cell clonotype.

31. The method of claim 30, wherein the T cell clonotype is a T cell receptor alpha chain, a T cell receptor beta chain, and combinations thereof.

32. The method of claim 29 or 30, wherein step (b) comprises determining: a
5 sequence encoding CDR3 of the T cell receptor alpha chain, a sequence encoding CDR3 of
the T cell receptor beta chain, and combinations thereof.

33. The method of claim 32, wherein step (b) further comprises determining: a
sequence encoding one or both of CDR1 and CDR2 of the T cell receptor alpha chain, a
10 sequence encoding one or both of CDR1 and CDR2 of the T cell receptor beta chain, and
combinations thereof.

34. The method of claim 32, wherein step (b) further comprises determining: a
sequence encoding a full-length variable domain of the T cell receptor alpha chain, a
15 sequence encoding a full-length variable domain of the T cell receptor beta chain, and
combinations thereof.

35. The method of claim 29, wherein the capture domain binds to a nucleic acid
encoding a B cell clonotype.
20

36. The method of claim 35, wherein the B cell clonotype is an immunoglobulin
kappa light chain, an immunoglobulin lambda light chain, an immunoglobulin heavy chain,
and combinations thereof.

37. The method of claim 36, wherein step (b) comprises determining: a sequence
25 encoding CDR3 of the immunoglobulin kappa light chain, a sequence encoding CDR3 of
immunoglobulin lambda light chain, a sequence encoding CDR3 of the immunoglobulin
heavy chain, and combinations thereof.

38. The method of claim 37, wherein step (b) further comprises determining: a
30 sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin kappa light
chain, a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin lambda
light chain, a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin
heavy chain, and combinations thereof.

39. The method of claim 37, wherein step (b) further comprises determining: a sequence encoding a full-length variable domain of the immunoglobulin kappa light chain, a sequence encoding a full-length variable domain of the immunoglobulin lambda light chain, a sequence encoding a full-length variable domain of the immunoglobulin heavy chain, and combinations thereof.

40. The method of any one of claims 1-39, wherein step (b) comprises the capture probe using the nucleic acid encoding the immune cell receptor as a template, thereby generating an extended capture probe.

41. The method of claim 40, wherein step (b) comprises extending a 3' end of the capture probe.

42. The method of claim 40 or 41, wherein step (b) further comprises generating a second strand of nucleic acid that comprises (i) a sequence that is complementary to all or a portion of the spatial barcode, and (ii) a sequence that corresponds to all or a portion of the sequence of the nucleic acid encoding the immune cell receptor.

43. The method of any one of claims 1-42, wherein the capture probe further comprises a cleavage domain, a functional domain, a unique molecular identifier, or any combination thereof.

44. The method of any one of claims 1-43, wherein the capture probe further comprises a functional domain.

45. The method of claim 44, wherein step (b) further comprises generating a second strand of nucleic acid that comprises (i) a sequence that is complementary to all or a portion of the functional domain, (ii) a sequence that is complementary to all or a portion of the spatial barcode, and (iii) a sequence that corresponds to all or a portion of the sequence of the nucleic acid encoding the immune cell receptor.

46. The method of any one of claims 1-45, wherein the method further comprises enriching the nucleic acid encoding the immune cell receptor of the immune cell clonotype.

47. The method of claim 46, wherein enriching comprises hybridizing a plurality of hybridization probes to the nucleic acid encoding the immune cell receptor of the immune cell clonotype, wherein a hybridization probe comprises (i) a sequence complementary to a portion of the nucleic acid encoding the immune cell receptor and (ii) a binding moiety that interacts with a capture moiety.

48. The method of claim 47, wherein the binding moiety comprises biotin and the capture moiety comprises streptavidin.

49. The method of any one of claims 46-48, wherein enriching the nucleic acid encoding the immune cell receptor of the immune cell clonotype further comprises one or more blocking probes.

50. The method of claim 49, wherein the one or more blocking probes comprises a sequence having at least 80% identity to SEQ ID NO: 639.

51. The method of claim 49, wherein the one or more blocking probes comprises a sequence having at least 80% identity to SEQ ID NO: 640.

52. The method of any one of claims 1-51, wherein the method further comprises amplifying the nucleic acid encoding the immune cell receptor of the immune cell clonotype, or a complement thereof, using (i) a first primer comprising all or a portion of the functional domain, wherein the functional domain is 5' to the spatial barcode, and (ii) a second primer comprising a sequence that is substantially complementary to a portion of a sequence encoding a variable region of the immune cell receptor.

53. The method of claim 52, further comprising amplifying the nucleic acid encoding the immune cell receptor of the immune cell clonotype, or a complement thereof, using (i) the first primer comprising all or a portion of the functional domain, wherein the functional domain is 5' to the spatial barcode, and (ii) a third primer comprising a sequence that is substantially complementary to a portion of the nucleic acid sequence encoding a variable region of the immune cell receptor, wherein the third primer is 5' to the second primer.

54. The method of any one of claims 1-53, wherein the biological sample comprises a tissue sample.

5

55. The method of claim 54, wherein the tissue sample is a tissue section.

56. The method of claim 55, wherein the tissue section is a fixed tissue section.

10

57. The method of claim 56, wherein the fixed tissue section is a formalin-fixed paraffin-embedded tissue section.

58. The method of any one of claims 55-57, wherein the tissue section comprises a tumor region.

15

59. The method of any one of claims 1-58, wherein the nucleic acid encoding the immune cell receptor comprises RNA.

60. The method of claim 59, wherein the RNA is mRNA.

20

61. The method of any one of claims 1-60, wherein the nucleic acid encoding the immune cell receptor comprises DNA.

62. The method of claim 61, wherein the DNA is genomic DNA.

25

63. The method of any one of claims 1-62, wherein the method further comprises imaging the biological sample.

64. The method of any one of claims 1-63, wherein the determining in step (b) comprises sequencing (i) all or a portion of the sequence of the spatial barcode or a complement thereof, and (ii) all or a portion of the sequence of the nucleic acid encoding the immune cell receptor or a complement thereof.

30

65. The method of any one of claims 1-64, wherein step (b) comprises determining the presence of the immune cell clonotype at a location in the biological sample.

5 66. The method of any one of claims 1-64, wherein step (b) comprises determining the abundance of the immune cell clonotype at a location in the biological sample.

67. The method of any one of claims 1-64, wherein step (b) comprises determining the presence and abundance of the immune cell clonotype at a location in the biological sample.
10

68. The method of any one of claims 1-64, wherein step (b) comprises determining the presence of two or more immune cell clonotypes at a location in the biological sample.

69. The method of any one of claims 1-64, wherein step (b) comprises determining
15 the abundance of two or more immune cell clonotypes at a location in the biological sample.

70. The method of any one of claims 1-64, wherein step (b) comprises determining the presence and abundance of two or more immune cell clonotypes at a location in the biological sample.
20

71. The method of any one of claims 68-70, wherein the method further comprises comparing the two or more immune cell clonotypes.

72. The method of any one of claims 68-71, wherein the two or more immune cell
25 clonotypes are each a B cell clonotype.

73. The method of any one of claims 68-71, wherein the two or more immune cell clonotypes are each a T cell clonotype.

30 74. The method of any one of claims 68-71, wherein the two or more immune cell clonotypes comprise at least one T cell clonotype and at least one B cell clonotype.

75. A method for determining the presence and/or abundance of an immune cell receptor at a location in a biological sample, the method comprising:

(a) contacting a biological sample with an array comprising a plurality of capture probes, wherein a capture probe of the plurality of capture probes comprises (i) a spatial barcode and (ii) a capture domain that specifically binds to a nucleic acid encoding an immune cell receptor; and

5 (b) determining (i) all or a portion of the sequence of the spatial barcode or a complement thereof, and (ii) all or a portion of the sequence of the nucleic acid encoding the immune cell receptor or a complement thereof, and using the determined sequences of (i) and (ii) to determine the presence and/or abundance of the immune cell receptor at a location in the biological sample.

10

76. The method of claim 75, wherein the immune cell receptor is a T cell receptor alpha chain.

15 77. The method of claim 76, wherein the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the T cell receptor alpha chain.

78. The method of claim 76 or 77, wherein step (b) comprises determining a sequence encoding CDR3 of the T cell receptor alpha chain.

20 79. The method of claim 78, wherein step (b) further comprises determining a sequence encoding one or both of CDR1 and CDR2 of the T cell receptor alpha chain.

80. The method of claim 78, wherein step (b) further comprises determining a sequence encoding a full-length variable domain of the T cell receptor alpha chain.

25

81. The method of claim 75, wherein the immune cell receptor is a T cell receptor beta chain.

30 82. The method of claim 81, wherein the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the T cell receptor beta chain.

83. The method of claim 81 or 82, wherein step (b) comprises determining a sequence encoding CDR3 of the T cell receptor beta chain.

84. The method of claim 83, wherein step (b) further comprises determining a sequence encoding one or both of CDR1 and CDR2 of the T cell receptor beta chain.

85. The method of claim 83, wherein step (b) further comprises determining a full-length variable domain of the T cell receptor beta chain.

86. The method of claim 75, wherein the immune cell receptor is an immunoglobulin kappa light chain.

87. The method of claim 86, wherein the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin kappa light chain.

88. The method of claim 86 or 87, wherein step (b) comprises determining a sequence encoding CDR3 of the immunoglobulin kappa light chain.

89. The method of claim 88, wherein step (b) further comprises determining a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin kappa light chain.

90. The method of claim 88, wherein step (b) further comprises determining a sequence encoding a full-length variable domain of the immunoglobulin kappa light chain.

91. The method of claim 75, wherein the immune cell receptor is an immunoglobulin lambda light chain.

92. The method of claim 91, wherein the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin lambda light chain.

93. The method of claim 91 or 92, wherein step (b) comprises determining a sequence encoding CDR3 of the immunoglobulin lambda light chain.

94. The method of claim 93, wherein step (b) further comprises determining a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin lambda light chain.

95. The method of claim 93, wherein step (b) further comprises determining a sequence encoding a full-length variable domain of the immunoglobulin lambda light chain.

5 96. The method of claim 75, wherein the immune cell receptor is an immunoglobulin heavy chain.

97. The method of claim 96, wherein the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin heavy chain.

10

98. The method of claim 96 or 97, wherein step (b) comprises determining a sequence encoding CDR3 of the immunoglobulin heavy chain.

99. The method of claim 98, wherein step (b) further comprises determining a
15 sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin heavy chain.

100. The method of claim 98, wherein step (b) further comprises determining a sequence encoding a full-length variable domain of the immunoglobulin heavy chain.

20 101. The method of claim 75, wherein the capture domain binds a poly(A) sequence of a nucleic acid encoding an immune cell receptor.

102. The method of claim 101, wherein the immune cell receptor is a T cell receptor alpha chain, a T cell receptor beta chain, and combinations thereof.

25 103. The method of claim 101, wherein step (b) comprises determining: a sequence encoding CDR3 of the T cell receptor alpha chain, a sequence encoding CDR3 of the T cell receptor beta chain, and combinations thereof.

104. The method of claim 103, wherein step (b) further comprises determining: a
30 sequence encoding one or both of CDR1 and CDR2 of the T cell receptor alpha chain, a sequence encoding one or both of CDR1 and CDR2 of the T cell receptor beta chain, and combinations thereof.

105. The method of claim 103, wherein step (b) further comprises determining: a sequence encoding a full-length variable domain of the T cell receptor alpha chain, a sequence encoding a full-length variable domain of the T cell receptor beta chain, and combinations thereof.

5

106. The method of claim 101, wherein the immune cell receptor is a B cell receptor an immunoglobulin kappa light chain, an immunoglobulin lambda light chain, an immunoglobulin heavy chain, and combinations thereof.

10

107. The method of claim 106, wherein step (b) comprises determining: a sequence encoding CDR3 of the immunoglobulin kappa light chain, a sequence encoding CDR3 of immunoglobulin lambda light chain, a sequence encoding CDR3 of the immunoglobulin heavy chain, and combinations thereof.

15

108. The method of claim 107, wherein step (b) further comprises determining: a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin kappa light chain, a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin lambda light chain, a sequence encoding one or both of CDR1 and CDR2 of the immunoglobulin heavy chain, and combinations thereof.

20

109. The method of claim 107, wherein step (b) further comprises determining: a sequence encoding a full-length variable domain of the immunoglobulin kappa light chain, a sequence encoding a full-length variable domain of the immunoglobulin lambda light chain, a sequence encoding a full-length variable domain of the immunoglobulin heavy chain, and combinations thereof.

25

110. The method of any one of claims 75-109, wherein step (b) comprises extending an end of the capture probe using the nucleic acid encoding the immune cell receptor as a template, thereby generating an extended capture probe.

30

111. The method of claim 110, wherein step (b) comprises extending a 3' end of the capture probe.

112. The method of claim 110 or 111, wherein step (b) further comprises generating a second strand of nucleic acid that comprises (i) a sequence that is complementary to all or a portion of the spatial barcode, and (ii) a sequence that corresponds to all or a portion of the sequence of the nucleic acid encoding the immune cell receptor.

5

113. The method of any one of claims 75-112, where the capture probe further comprises a cleavage domain, a functional domain, a unique molecular identifier, or any combination thereof.

10

114. The method of any one of claims 75-87, wherein the capture probe further comprises a functional domain.

115. The method of claim 114, wherein step (b) further comprises generating a second strand of nucleic acid that comprises (i) a sequence that is complementary to all or a portion of the functional domain, (ii) a sequence that is complementary to all or a portion of the spatial barcode, and (iii) a sequence that corresponds to all or a portion of the sequence of the nucleic acid encoding the immune cell receptor.

15

116. The method of any one of claims 75-115, wherein the method further comprises enriching the nucleic acid encoding the immune cell receptor.

20

117. The method of claim 116, wherein enriching comprises hybridizing a plurality of hybridization probes to the nucleic acid encoding the immune cell receptor, wherein a hybridization probe comprises (i) a sequence complementary to a portion of the nucleic acid encoding the immune cell receptor and (ii) a binding moiety that interacts with a capture moiety.

25

118. The method of claim 117, wherein the binding moiety comprises biotin and the capture moiety comprises streptavidin.

30

119. The method of any one of claims 116-118, wherein enriching the nucleic acid encoding the immune cell receptor of the immune cell receptor further comprises one or more blocking probes.

120. The method of claim 119, wherein the one or more blocking probes comprises a sequence having at least 80% identity to SEQ ID NO: 639.

121. The method of claim 119, wherein the one or more blocking probes comprises a
5 sequence having at least 80% identity to SEQ ID NO: 640.

122. The method of any one of claims 75-121, wherein the method further comprises amplifying the nucleic acid encoding an immune cell receptor, or a complement thereof, using (i) a first primer comprising all or a portion of the functional domain, wherein the
10 functional domain is 5' to the spatial barcode in the second strand of nucleic acid, and (ii) a second primer comprising a sequence that is substantially complementary to a portion of a sequence encoding a variable region of the immune cell receptor.

123. The method of claim 122, further comprising amplifying the nucleic acid
15 encoding the immune cell receptor, or a complement thereof, using (i) the first primer comprising all or a portion of the functional domain, wherein the functional domain is 5' to the spatial barcode, and (ii) a third primer comprising a sequence that is substantially complementary to a portion of the nucleic acid sequence encoding a variable region of the immune cell receptor, wherein the third primer is 5' to the second primer.

20

124. The method of any one of claims 75-123, wherein the biological sample comprises a tissue sample.

125. The method of claim 124, wherein the tissue sample is a tissue section.

25

126. The method of claim 125, wherein the tissue section is a fixed tissue section.

127. The method of claim 126, wherein the fixed tissue section is a formalin-fixed paraffin-embedded tissue section.

30

128. The method of any one of claims 125-127, wherein the tissue section comprises a tumor region.

129. The method of any one of claims 75-128, wherein the nucleic acid encoding the immune cell receptor comprises RNA.

130. The method of claim 129, wherein the RNA is mRNA.

5

131. The method of any one of claims 75-130, wherein the nucleic acid encoding the immune cell receptor comprises DNA.

132. The method of claim 131, wherein the DNA is genomic DNA.

10

133. The method of any one of claims 75-132, wherein the method further comprises, prior to step (b), contacting the biological sample with ribosomal RNA depletion probes and mitochondrial RNA depletion probes.

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134. The method of any one of claims 75-133, wherein the method further comprises imaging the biological sample.

135. The method of any one of claims 75-134, wherein the determining in step (b) comprises sequencing (i) all or a portion of the sequence of the spatial barcode or a complement thereof, and (ii) all or a portion of the sequence of the nucleic acid encoding the immune cell receptor or a complement thereof.

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136. The method of any one of claims 75-135, wherein step (b) comprises determining the presence of the immune cell receptor at a location in the biological sample.

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137. The method of any one of claims 75-135, wherein step (b) comprises determining the abundance of the immune cell receptor at a location in the biological sample.

138. The method of any one of claims 75-135, wherein step (b) comprises determining the presence and abundance of the immune cell receptor at a location in the biological sample.

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139. The method of any one of claims 75-135, wherein step (b) comprises determining the presence of two or more immune cell receptors at a location in the biological sample.

5 140. The method of any one of claims 75-135, wherein step (b) comprises determining the abundance of two or more immune cell receptors at a location in the biological sample.

10 141. The method of any one of claims 75-135, wherein step (b) comprises determining the presence and abundance of two or more immune cell receptors at a location in the biological sample.

142. The method of any one of claims 139-141, wherein the method further comprises comparing the two or more immune cell receptors.

15 143. The method of any one of claims 139-142, wherein the two or more immune cell clonotypes are each an immune cell receptor of a B cell.

20 144. The method of any one of claims 139-142, wherein the two or more immune cell clonotypes are each an immune cell receptor of a T cell.

25 145. The method of any one of claims 139-142, wherein the two or more immune cell clonotypes comprise at least one immune cell receptor of a T cell and at least one immune cell receptor from a B cell.

 146. An array comprising a plurality of capture probes, wherein a capture probe of the plurality of capture probes comprises (i) a spatial barcode and (ii) a capture domain that binds to a nucleic acid encoding an immune cell receptor of an immune cell clonotype.

30 147. The array of claim 146, wherein the immune cell clonotype is a T cell clonotype.

 148. The array of claim 147, wherein the immune cell receptor is a T cell receptor alpha chain.

149. The array of claim 148, wherein the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the T cell receptor alpha chain.

5 150. The array of claim 147, wherein the immune cell receptor is a T cell receptor beta chain.

151. The array of claim 150, wherein the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the T cell receptor beta chain.

10 152. The array of claim 146, wherein the immune cell clonotype is a B cell clonotype.

153. The array of claim 152, wherein the immune cell receptor is an immunoglobulin kappa light chain.

15 154. The array of claim 153, wherein the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin kappa light chain.

155. The array of claim 152, wherein the immune cell receptor is an immunoglobulin lambda light chain.

20 156. The array of claim 155, wherein the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin lambda light chain.

25 157. The array of claim 152, wherein the immune cell receptor is an immunoglobulin heavy chain.

158. The array of claim 157, wherein the capture domain binds specifically to a nucleic acid sequence encoding a constant region of the immunoglobulin heavy chain.

30 159. The array of any one of claims 146-158, where the capture probe further comprises a cleavage domain, a functional domain, a unique molecular identifier, or any combination thereof.

160. A kit comprising:

- an array of any one of claims 146-159;
- one or more hybridization probes, wherein a hybridization probe comprises (i) a sequence substantially complementary to a nucleic acid encoding an immune cell receptor and (ii) a binding moiety that interacts with a capturing moiety; and
- 5 one or more blocking probes.