poly(A) mRNA capture, targeted RNA capture as described herein is less affected by RNA degradation associated with FFPE fixation compared to methods dependent on oligo-dT capture and reverse transcription of mRNA. Further targeted RNA capture as described herein allows for sensitive measurement of specific genes of interest that otherwise might be missed with a whole transcriptomic approach. Targeted RNA capture can be used to capture a defined set of RNA molecules of interest, or it can be used at a whole transcriptome level, or anything in between. When combined with the spatial methods disclosed herein, the location and abundance of the RNA targets can be determined.

In one example, this disclosure features a method for determining a location of an analyte in a biological sample including: (a) providing the biological sample on an array including a plurality of capture probes, where a capture probe of the plurality includes: (i) a spatial barcode and (ii) a capture domain; (b) contacting a first probe and a second probe with the biological sample, where the first probe and the second probe each include one or more sequences that are substantially complementary to sequences of the analyte, and where the second probe includes a capture probe capture domain; (c) hybridizing the first probe and the second probe to the analyte; (d) generating a ligation product by ligating the first probe and the second probe; (e) releasing the ligated product from the analyte; (f) hybridizing the ligation product to the capture domain; and (g) determining (i) all or a part of the sequence of the ligation product bound to the capture domain, or a complement thereof, and (ii) all or a part of the sequence of the spatial barcode, or a complement thereof, and using the determined sequence of (i) and (ii) to identify the location of the analyte in the biological sample. In some instances, the methods include determining the abundance and location of the analyte in the biological sample. In some instances, the methods include determining the abundance of an analyte at a location in the biological sample.

In one aspect, the present disclosure refers to a method for determining a location of an analyte in a biological sample comprising: (a) providing the biological sample on 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; (b) contacting a first probe and a second probe with the biological sample, wherein the first probe and the second probe each comprise sequences that are substantially complementary to sequences of the analyte, and wherein the second probe comprises a capture probe capture domain that is complementary to all or a portion of the capture domain; (c) hybridizing the first probe and the second probe to the analyte; (d) generating a ligation product by ligating the first probe and the second probe; (e) releasing the ligation product from the analyte; (f) hybridizing the ligation product to the capture domain; and (g) determining (i) all or part of the sequence of the ligation product bound to the capture domain, or a complement thereof, and (ii) the spatial barcode, or a complement thereof, and using the determined sequences of (i) and (ii) to determine the location of the analyte in the biological sample.

In some examples, the first probe and the second probe are substantially complementary to adjacent sequences of the analyte. In some examples, the first probe and the second probe hybridize to sequences that are not adjacent to each other on the analyte.

In some examples, the method further includes hybridizing a third probe to the first probe and the second probe, where the third probe includes a first sequence that is substantially complementary to a portion of the first probe and a second sequence that is substantially complementary to a portion of the second probe. In some examples, hybridizing the first probe and the second probe to the analyte is performed at a first temperature. In some examples, hybridizing the third probe to the first probe and the

WHAT IS CLAIMED IS:

1. A composition comprising:

(a) a spatial array comprising a plurality of capture probes, wherein a capture probe of the plurality of capture probes comprises a spatial barcode and a capture domain;

(b) a biological sample on the spatial array wherein the biological sample comprises a plurality of analytes; and

(c) a first probe and a second probe that are ligated together, wherein the first probe and the second probe each comprise one or more sequences that are substantially complementary to sequences of the analyte and wherein one of the first probe or the second probe comprises a capture probe capture domain that is hybridized to the capture domain of the capture probe on the spatial array.

2. The composition of claim 1, wherein the first probe or the second probe that does not comprise the capture probe capture domain comprises a functional domain.

3. The composition of claim 1 or 2, wherein the second probe comprises the capture probe capture domain.

4. The composition of any one of claims 1-3, wherein the first probe and the second probe are substantially complementary to adjacent sequences of the analyte.

5. The composition of any one of claims 1-4, wherein the first probe and the second probe are substantially complementary to sequences that are not adjacent to each other on the analyte, wherein one of the first probe or the second probe is extended with a DNA polymerase.

6. The composition of any one of claims 1-5, wherein the first probe and/or the second probe is a DNA probe.

7. The composition of any one of claims 1-6, further comprising 5000 or more ligated probe pairs, wherein a ligated probe pair of the 5000 or more ligated probe pairs comprises the first probe and the second probe that are ligated together, and wherein at least one of the ligated probe pairs is hybridized to the capture domain of a capture probe on the spatial array.

8. The composition of any one of claims 1-7, wherein prior to ligation to the second probe the first probe comprised a pre-adenylated phosphate group at its 5' end.

9. The composition of any one of claims 1-8, wherein the capture probe capture domain comprises a poly-adenylated sequence or a complement thereof.

10. The composition of any one of claims 1-9, further comprising a DNA polymerase.

11. The composition of any one of claims 1-10, further comprising an RNase H enzyme, wherein the RNase H enzyme comprises RNase H1, RNase H2, or both.

12. The composition of any one of claims 1-11, further comprising a permeabilization agent, wherein the permeabilization agent is proteinase K or pepsin.

13. The composition of any one of claims 1-12, further comprising a ligase, wherein the ligase is one or more of a T4 RNA ligase (Rnl2), a splintR ligase, a single stranded DNA ligase, or a T4 DNA ligase.

14. The composition of any one of claims 1-13, wherein the tissue sample is a formalin-fixed, paraffin-embedded (FFPE) tissue sample, a fresh tissue sample, or a frozen tissue sample.

15. A kit comprising:

(a) a spatial array comprising a plurality of capture probes, wherein a capture probe of the plurality of capture probes comprises a spatial barcode and a capture domain;

(b) a first probe and a second probe, wherein the first probe and the second probe each comprise one or more sequences that are substantially complementary to sequences of the analyte and wherein one of the first probe or the second probe comprises a capture probe capture domain that can hybridize to the capture domain of the capture probe on the spatial array;

(c) one or more reagents comprising a permeabilization agent; and

(d) one or more enzymes selects from a DNA polymerase, an endonuclease, a ligase, and any combination thereof.

16. The kit of claim 15, wherein the permeabilization agent is proteinase K or pepsin.

17. The kit of claim 15 or 16, wherein the endonuclease is RNAse H.

18. The kit of any one of claims 15-17, wherein the ligase is one or more of a T4 RNA ligase (Rnl2), a splintR ligase, a single stranded DNA ligase, or a T4 DNA ligase.

19. A method for determining a location of an analyte in a biological sample comprising:

(a) providing the biological sample on 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;

(b) contacting a first probe and a second probe with the biological sample, wherein the first probe and the second probe each comprise sequences that are substantially complementary to sequences of the analyte, and wherein the second probe comprises a capture probe capture domain that is complementary to all or a portion of the capture domain;

(c) hybridizing the first probe and the second probe to the analyte;

(d) generating a ligation product by ligating the first probe and the second probe;

(e) releasing the ligation product from the analyte;

(f) hybridizing the ligation product to the capture domain; and

(g) determining (i) all or part of the sequence of the ligation product bound to the capture domain, or a complement thereof, and (ii) the spatial barcode, or a complement thereof, and using the determined sequences of (i) and (ii) to determine the location of the analyte in the biological sample.

20. The method of claim 19, wherein the first probe and the second probe are substantially complementary to adjacent sequences of the analyte.

21. The method of claim 19, where the first probe and the second probe hybridize to sequences that are not adjacent to each other on the analyte, and wherein the first probe is extended with a DNA polymerase, thereby (i) filling in a gap between the first probe and the second probe and (ii) generating an extended first probe.

22. The method of any one of claims 19-21, further comprising (i) hybridizing the first probe and the second probe to the analyte at a temperature from about 50°C to about 75°C,

and (ii) hybridizing the first probe and the second probe to a third probe at a temperature from about 15°C to about 35°C.

23. The method of any one of claims 19-22, wherein the contacting the first probe and the second probe with the biological sample comprises contacting the biological sample with 5000 or more probe pairs, wherein a probe pair of the 5000 or more probe pairs comprises the first probe and the second probe.

24. The method of any one of claims 19-23, wherein the generating the ligation product comprises ligating the first probe to the second probe using enzymatic ligation or chemical ligation, wherein the enzymatic ligation utilizes a ligase,
wherein the ligase is one or more of a T4 RNA ligase (Rnl2), a splintR ligase, a single stranded DNA ligase, or a T4 DNA ligase.

25. The method of any one of claims 19-24, wherein the first probe further comprises a primer sequence.

26. The method of any one of claims 19-25, wherein the first probe and/or the second probe is a DNA probe.

27. The method of any one of claims 19-26, wherein the releasing the ligation product from the analyte utilizes an endonuclease.

28. The method of claim 27, wherein the endonuclease is an RNase H enzyme.

29. The method of any one of claims 19-28, wherein the biological sample is a formalin-fixed, paraffin-embedded (FFPE) tissue sample, a fresh tissue sample, or a frozen tissue sample.

30. The method of any one of claims 19-29, wherein the biological sample was previously stained using immunofluorescence, immunohistochemistry, or hematoxylin and eosin.

31. The method of any one of claims 19-30, further comprising contacting the biological sample with a permeabilization agent, wherein the permeabilization agent is selected from an organic solvent, a detergent, an enzyme, or a combination thereof.

32. The method of claim 31, wherein the permeabilization agent comprises proteinase K or pepsin.

33. The method of any one of claims 19-32, wherein the analyte comprises RNA, optionally wherein the RNA is an mRNA.

34. The method of any one of claims 19-33, wherein the determining step (g) comprises sequencing (i) all or a part of the sequence of the ligation product, or a complement thereof, and (ii) the spatial barcode, or a complement thereof.