

In some examples, the spatial array further includes a template oligonucleotide including a reverse complement of a unique molecular identifier (UMI), and where the capture probe includes the UMI.

In some examples of spatial arrays provided herein, the first primer is affixed to the substrate at a 5' end of the first primer. In some examples of spatial arrays provided herein, the first primer includes a sequence that is complementary to the template oligonucleotide. In some examples of spatial arrays provided herein, the first primer includes poly (A) domain. In some examples of spatial arrays provided herein, the first primer includes a cleavage domain.

In some examples of spatial arrays provided herein, the second primer is affixed to the substrate at a 5' end of the second primer. In some examples of spatial arrays provided herein, the second primer includes a cleavage domain.

In some examples of spatial arrays provided herein, the template oligonucleotide is attached to a bead. In some examples of spatial arrays provided herein, the bead is a gel bead. In some examples of spatial arrays provided herein, the bead is 2 microns or less in diameter. In some examples of spatial arrays provided herein, the bead is 1 micron or less in diameter.

In some examples of spatial arrays provided herein, the substrate includes a glass slide. In some examples of spatial arrays provided herein, the glass slide is a treated glass slide.

In some examples of spatial arrays provided herein, the first primer and the second primer are at a ratio of between 1:1.5 and 1.5:1. In some examples of spatial arrays provided herein, the first primer and the second primer are less than 5 μm apart.

In one aspect, the present disclosure refers to a method of generating a spatial array comprising:

(a) providing a substrate comprising a plurality of acceptor oligonucleotides, wherein an acceptor oligonucleotide of the plurality of acceptor oligonucleotides comprises a spatial barcode and a first ligation handle, and wherein the acceptor oligonucleotide comprises a 5' end that is attached to the substrate and a 3' end comprising a photocleavable protective group;

(b) providing a single-stranded splint oligonucleotide comprising a sequence substantially complementary to the first ligation handle and a sequence substantially complementary to a second ligation handle;

(c) releasing the photocleavable protective group from the acceptor oligonucleotide;

(d) providing a plurality of donor oligonucleotides, wherein a donor oligonucleotide of the plurality of donor oligonucleotides comprises the second ligation handle and a capture domain;

(e) hybridizing the single-stranded splint oligonucleotide to the first ligation handle and the second ligation handle; and

(f) ligating the acceptor oligonucleotide to the donor oligonucleotide to generate a capture probe, thereby generating a spatial array.

Provided herein are methods of generating a spatial array including (a) providing a substrate including an arrayed plurality of acceptor oligonucleotides, where an acceptor oligonucleotide of the plurality of acceptor oligonucleotides includes a spatial barcode and a first ligation handle, and where the 5' end of the acceptor oligonucleotide is attached to the substrate, (b) providing a splint oligonucleotide including a sequence complementary to the first ligation handle and a sequence complementary to a donor oligonucleotide, and (c) ligating a donor oligonucleotide including a capture domain to the 3' end the acceptor oligonucleotide to generate a capture probe, thereby generating a spatial array.

WHAT IS CLAIMED IS:

1. A method of generating a spatial array comprising:
 - (a) providing a substrate comprising a plurality of acceptor oligonucleotides,
5 wherein an acceptor oligonucleotide of the plurality of acceptor oligonucleotides comprises a spatial barcode and a first ligation handle, and wherein the acceptor oligonucleotide comprises a 5' end that is attached to the substrate and a 3' end comprising a photocleavable protective group;
 - (b) providing a single-stranded splint oligonucleotide comprising a sequence
10 substantially complementary to the first ligation handle and a sequence substantially complementary to a second ligation handle;
 - (c) releasing the photocleavable protective group from the acceptor oligonucleotide;
 - (~~d~~) providing a plurality of donor oligonucleotides, wherein a donor
15 oligonucleotide of the plurality of donor oligonucleotides comprises the second ligation handle and a capture domain;
 - (~~e~~) hybridizing the single-stranded splint oligonucleotide to the first ligation handle and the second ligation handle; and
 - (~~f~~) ligating the acceptor oligonucleotide to the donor oligonucleotide to generate a capture probe, thereby generating a spatial array.
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2. The method of claim 1, wherein the donor oligonucleotide further comprises a functional sequence, a donor spatial barcode, a unique molecular identifier, or a combination thereof.
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3. The method of claim 1 or 2, wherein the acceptor oligonucleotide further comprises a cleavage domain, a unique molecular identifier, a functional sequence, or a combination thereof.
- 30 4. The method of any one of claims 1 to 3, wherein the capture domain comprises a poly(T) sequence.

5. The method of any one of claims 1 to 4, wherein the capture domain is a non-homopolymeric sequence.
6. The method of any one of claims 1 to 5, wherein the method further comprises
5 contacting a biological sample to the generated spatial array.
7. The method of claim 6, further comprising permeabilizing the biological sample after contacting with the generated spatial array to allow an analyte in the biological sample to
10 ~~bind to~~~~interact with~~ the capture domain.
8. The method of claim 7, wherein the ~~binding~~~~interacting~~ comprises hybridization to the capture domain.
9. The method of claim 7, wherein the ~~binding~~~~interacting~~ comprises ligation to the
15 capture domain.
10. The method of claim 7, wherein the ~~binding~~~~interacting~~ comprises hybridization and ligation to the capture domain.
- 20 11. The method of any one of claims 7 to 10, wherein the method further comprises migrating the analyte in the biological sample to the capture probe.
12. The method of claim 11, wherein the migrating comprises passive migration.
- 25 13. The method of claim 11, wherein the migrating comprises active migration.
14. The method of any one of claims 6 to 13, further comprising imaging the biological sample.
- 30 15. The method of any one of claims 6 to 14, further comprising staining the biological sample.
16. The method of claim 15, wherein the staining comprises hematoxylin and eosin (H&E) staining.

17. The method of any one of claims 1 to 16, wherein the ligation comprises enzymatic ligation or chemical ligation.

5 18. The method of claim 17, wherein the enzymatic ligation comprises a ligase selected from SplintR ligase, T4 DNA ligase, App ligase, and RNA ligase.

19. The method of any one of claims 1 to 18, wherein the single-stranded splint oligonucleotide comprises DNA, RNA, or a combination thereof.

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~~20. The method of any one of claims 1 to 19, wherein the acceptor oligonucleotide comprises a 3' end comprising a protective group which is released prior to ligation to the donor oligonucleotide.~~

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~~21. The method of claim 20, wherein the protective group is a photocleavable protective group.~~

~~22~~20. The method of any one of claims 1 to 19~~21~~, wherein the photocleavable protective group is released by exposing the acceptor oligonucleotide to light.

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~~23~~21. The method of claim ~~20~~21 ~~or 22~~, wherein, prior to releasing, the photocleavable protective group is protected by applying a photomask.

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~~24~~22. The method of any one of claims 1 to ~~21~~23, wherein the substrate comprises two or more partitions and wherein a partition of the two or more partitions comprises a plurality of features.

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~~25~~23. The method of claim ~~22~~24, wherein the plurality of features comprises a plurality of immobilized acceptor oligonucleotides.

~~26~~24. The method of claim ~~25~~23, wherein an immobilized acceptor oligonucleotide of the plurality of immobilized acceptor oligonucleotides within the partition comprises a spatial barcode that is different from immobilized acceptor oligonucleotides in other partitions.

5 2725. The method of claim 2624, wherein the plurality of donor oligonucleotides is delivered to the partition, wherein the donor oligonucleotide of the plurality of donor oligonucleotides further comprises a donor spatial barcode, wherein the donor spatial barcode comprises a different sequence compared to the spatial barcode of the immobilized acceptor oligonucleotides within the partition.

2826. The method of claim 2624 or 2725, wherein the two or more partitions are separated by physical barriers.

10 2927. The method of claim 2826, wherein the physical barriers comprise walls or hydrophobic regions on the substrate.

3028. The method of any one of claims 2527 to 2729, wherein the plurality of donor oligonucleotides is delivered using a microfluidic device.

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