

and a capture domain; permeabilizing the biological sample under conditions sufficient to make the genomic DNA in the biological sample accessible to a transposon insertion; providing a transposon sequence and a transposase enzyme to the biological sample under conditions wherein the transposon sequence is inserted into the genomic DNA; allowing the transposase enzyme to excise the inserted transposon sequence from the genomic DNA thus generating fragmented genomic DNA; contacting the biological sample comprising the fragmented genomic DNA with an array under conditions such that a capture probe interacts with the fragmented genomic DNA; and correlating the location of the capture probe on the array to a location in the biological sample, thereby spatially analyzing the fragmented genomic DNA.

In one aspect, the present disclosure refers to a method for determining a spatial location of genomic DNA in a biological sample, the method comprising: (a) providing an arrayed substrate comprising a plurality of capture probes, wherein a capture probe of the plurality of capture probes comprises a spatial barcode and a capture domain, wherein the spatial barcode is associated with a particular location of the capture probe on the arrayed substrate; (b) contacting the biological sample with the arrayed substrate; (c) permeabilizing the biological sample by contacting the biological sample with an enzyme selected from the group consisting of collagenase, pepsin, and proteinase-K; (d) contacting a transposome with the permeabilized biological sample to insert transposon end sequences into the genomic DNA, thereby generating fragmented genomic DNA, wherein the fragmented genomic DNA includes a sequence complementary to the capture domain; (e) hybridizing the fragmented genomic DNA to the capture domain of the capture probe on the arrayed substrate; and (f) determining the spatial barcode sequence or a complement thereof, and the fragmented genomic DNA sequence or a complement thereof, wherein the determined sequences correlate the location of the genomic DNA in the biological sample, thereby determining the spatial location of the genomic DNA.

In some examples, the array comprising a plurality of capture probes are provided on a substrate. In some examples, the array comprising the plurality of capture probes is provided on a feature. In some examples, the capture probe is directly or indirectly attached. In some examples, the array comprising the plurality of capture probes is provided on the feature on the substrate. In some examples, the substrate comprises a microfluidic channel. In some examples, the capture probe further comprises one or more of a cleavage domain, a functional domain, and a unique identifier, or combinations thereof.

In some examples, a further migration step comprising a step wherein the fragmented genomic DNA is migrated to the substrate. In some examples, the migration step is an active migration step comprising applying an electric field to the fragmented genomic DNA. In some examples, the migration step is a passive migration step comprising diffusion. In some examples, the migration of the fragmented genomic DNA from the biological sample comprises exposing the biological sample and the feature to heat. In some examples, the biological sample is immobilized on the substrate.

In some examples, the transposase enzyme is a dimer comprised of a first monomer complexed with a first adapter comprising a transposon end sequence and a sequence complementary to the capture domain and wherein a second monomer is complexed with a second adapter comprising a transposon end sequence and a second adapter sequence, wherein the transposase enzyme ligates the first adapter and the second adapter to the fragmented genomic DNA. In some examples, the first adapter and the second adapter have a 5' end and a 3' end, wherein the 5' end is phosphorylated in situ. In some examples, prior to fragmenting the DNA, the 5' end of the first adapter complexed with the first monomer and the

In another aspect, the present disclosure refers to a method for determining a spatial location of genomic DNA and RNA in a biological sample, the method comprising: (a) providing an arrayed substrate, wherein the arrayed substrate comprises a plurality of first capture probes and a plurality of second capture probes: (i) wherein a first capture probe of the plurality of first capture probes comprises a spatial barcode and a first capture domain, wherein the spatial barcode of the first capture probe is associated with a particular location of the first capture probe on the arrayed substrate; and (ii) wherein a second capture probe of the plurality of second capture probes comprises a spatial barcode and a second capture domain, wherein the spatial barcode of the second capture probe is associated with a particular location of the second capture probe on the arrayed substrate; (b) contacting the biological sample with the arrayed substrate; (c) permeabilizing the biological sample by contacting the biological sample with an enzyme selected from the group consisting of collagenase, pepsin, and proteinase-K; (d) contacting a transposome comprising a transposase enzyme with the biological sample under conditions wherein transposon end sequences of the transposome are inserted into the genomic DNA, thereby generating fragmented genomic DNA, wherein the fragmented genomic DNA includes a sequence complementary to the first capture domain; (e) hybridizing (i) the fragmented genomic DNA to the first capture domain of the first capture probe and (ii) the RNA from the biological sample to the second capture domain of the second capture probe; and (f) determining (i) the spatial barcode sequence, or a complement thereof, of the first capture probe, (ii) the fragmented genomic DNA sequence, or a complement thereof, (iii) the spatial barcode sequence, or a complement thereof, of the second capture probe, and (iv) the RNA sequence, or a complement thereof, wherein the determined sequences of (i) and (ii) correlate the location of the fragmented genomic DNA in the biological sample, wherein the determined sequences of (iii) and (iv) correlate the location of the RNA in the biological sample, thereby determining the spatial location of the genomic DNA and RNA in the biological sample.

In some examples, the RNA is a mRNA. In some examples, the first capture domain and the second capture domain are identical. In some examples, the first capture domain and the second capture domain comprise a homopolymeric poly (T) sequence. In some examples, the first capture domain and the second capture domain are different. In some examples, the first capture domain comprises a random sequence and the second capture domain comprises a poly (T) sequence. In some examples, the array comprising the plurality of capture probes is provided on a substrate. In some examples, the array comprising the

WHAT IS CLAIMED IS:

1. A method for determining a spatial location of genomic DNA in a biological sample, the method comprising:
 - (a) providing an arrayed substrate comprising a plurality of capture probes, wherein a capture probe of the plurality of capture probes comprises a spatial barcode and a capture domain, wherein the spatial barcode is associated with a particular location of the capture probe on the arrayed substrate;
 - (b) contacting the biological sample with the arrayed substrate;
 - (c) permeabilizing the biological sample by contacting the biological sample with an enzyme selected from the group consisting of collagenase, pepsin, and proteinase-K;
 - (d) contacting a transposome with the permeabilized biological sample to insert transposon end sequences into the genomic DNA, thereby generating fragmented genomic DNA, wherein the fragmented genomic DNA includes a sequence complementary to the capture domain;
 - (e) hybridizing the fragmented genomic DNA to the capture domain of the capture probe on the arrayed substrate; and
 - (f) determining the spatial barcode sequence or a complement thereof, and the fragmented genomic DNA sequence or a complement thereof, wherein the determined sequences correlate the location of the genomic DNA in the biological sample, thereby determining the spatial location of the genomic DNA.
2. The method of claim 1, wherein the plurality of capture probes are provided on a plurality of features on the arrayed substrate.
3. The method of claim 1 or 2, wherein the plurality of capture probes are directly or indirectly affixed on the arrayed substrate.
4. The method of any one of claims 1 to 3, wherein the arrayed substrate comprises a microfluidic channel.

5. The method of any one of claims 1 to 4, wherein the capture probe of the plurality of capture probes further comprises one or more of a cleavage domain, a functional domain, a unique identifier, and combinations thereof.
6. The method of any one of claims 2 to 5, further comprising a migration step wherein the fragmented genomic DNA is migrated from the biological sample to the arrayed substrate.
7. The method of claim 6, wherein the migration step is an active migration step comprising applying an electric field to the biological sample.
8. The method of claim 6, wherein the migration step is a passive migration step comprising diffusion.
9. The method of any one of claims 6 to 8, wherein the migration of the fragmented genomic DNA from the biological sample comprises exposing the biological sample to heat.
10. The method of any one of claims 2 to 9, wherein the biological sample is immobilized on the arrayed substrate.
11. The method of any one of claims 1 to 10, wherein the transposome comprises a transposase enzyme as a dimer comprising a first monomer complexed with a first adapter comprising a transposon end sequence and a sequence complementary to the capture domain and wherein a second monomer is complexed with a second adapter

comprising a transposon end sequence and a second adapter sequence, wherein the transposase enzyme ligates the first adapter and the second adapter to the genomic DNA, thereby generating fragmented genomic DNA.

12. The method of claim 11, wherein the first adapter and the second adapter have a 5' end and a 3' end, wherein the 5' end is phosphorylated.
13. The method of claim 11 or 12, wherein prior to fragmenting the genomic DNA, the method further comprises phosphorylating the 5' end of the first adapter complexed with the first monomer and the 5' end of the second adapter complexed with the second monomer.
14. The method of claim 12 or 13, wherein phosphorylating the first adapter complexed with the first monomer and the second adapter complexed with the second monomer comprises contacting a first monomer: first adapter complex and a second monomer: second adapter complex with a polynucleotide kinase in the presence of ATP.
15. The method of any one of claims 11 to 15, wherein the capture domain of the capture probe comprises a sequence that hybridizes to the first adapter.
16. The method of any one of claims 11 to 15, wherein the capture domain of the capture probe is complementary to a portion of a splint oligonucleotide and wherein the splint oligonucleotide comprises a sequence complementary to a portion of the first adapter of the fragmented genomic DNA.

17. The method of claim 16, wherein the splint oligonucleotide hybridizes to the first adapter, or a portion thereof, and to the capture domain of the capture probe, or a portion thereof.
18. The method of claim 17, wherein the first adapter and the capture domain of the capture probe hybridized to the splint oligonucleotide are ligated together.
19. The method of claim 15, wherein the fragmented genomic DNA hybridized to the capture domain of the capture probe is used as a template to generate an extended capture probe comprising the spatial barcode and a sequence complementary to the fragmented genomic DNA.
20. The method of claim 19, wherein the extended capture probe is generated using a DNA polymerase.
21. The method of claim 20, wherein the DNA polymerase has strand displacement activity.
22. The method of any one of claims 1 to 21, further comprising gap repair of single stranded breaks in the fragmented genomic DNA.
23. The method of any one of claims 11 to 16, wherein the sequence complementary to the capture domain is a unique sequence.
24. The method of claim 18, wherein the capture domain of the capture probe is ligated to the fragmented genomic DNA by a DNA ligase enzyme.

25. The method of claim 11, wherein the transposase enzyme of the transposome is a Tn5 transposase enzyme, or a functional derivative thereof, and comprises a sequence having at least 80% identity to SEQ ID NO: 1.
26. The method of claim 11, wherein the transposase enzyme of the transposome is a Mu transposase enzyme, or a functional derivative thereof, and comprises a sequence having at least 80% identity to SEQ ID NO: 2.
27. The method of claim 1, wherein the transposon end sequence comprises a sequence having at least 80% identity to SEQ ID NO: 8.
28. The method of claim 1, wherein the transposon end sequences comprise a sequence having at least 80% identity to any one of SEQ ID NO: 9 to 14.
29. The method of claim 1, wherein at step (c), the enzyme is pepsin comprising a sequence having at least 80% identity to SEQ ID NO: 3 or 4.
30. The method of claim 1, wherein at step (c), the enzyme is collagenase comprising a sequence having at least 80% identity to SEQ ID NO: 5 or 6.
31. The method of claim 1, wherein at step (c), the enzyme is proteinase K comprising a sequence having at least 80% identity to SEQ ID NO: 7.
32. The method of claim 1, wherein the determining step comprises sequencing.

33. The method of claim 32, wherein sequencing comprises sequencing the spatial barcode sequence or a complement thereof, and the fragmented genomic DNA sequence or a complement thereof.

34. The method of any one of claims 1 to 33, further comprising a step of imaging the biological sample before or after contacting the biological sample with the arrayed substrate.

35. A method for determining a spatial location of genomic DNA and RNA in a biological sample, the method comprising:

(a) providing an arrayed substrate, wherein the arrayed substrate comprises a plurality of first capture probes and a plurality of second capture probes:

(i) wherein a first capture probe of the plurality of first capture probes comprises a spatial barcode and a first capture domain, wherein the spatial barcode of the first capture probe is associated with a particular location of the first capture probe on the arrayed substrate; and

(ii) wherein a second capture probe of the plurality of second capture probes comprises a spatial barcode and a second capture domain, wherein the spatial barcode of the second capture probe is associated with a particular location of the second capture probe on the arrayed substrate;

(b) contacting the biological sample with the arrayed substrate;

(c) permeabilizing the biological sample by contacting the biological sample with an enzyme selected from the group consisting of collagenase, pepsin, and proteinase-K;

(d) contacting a transposome comprising a transposase enzyme with the biological sample under conditions wherein transposon end sequences of the transposome are inserted into the genomic DNA, thereby generating fragmented genomic DNA, wherein the fragmented genomic DNA includes a sequence complementary to the first capture domain;

(e) hybridizing (i) the fragmented genomic DNA to the first capture domain of the first capture probe and (ii) the RNA from the biological sample to the second capture domain of the second capture probe; and

(f) determining: (i) the spatial barcode sequence, or a complement thereof, of the first capture probe, (ii) the fragmented genomic DNA sequence, or a complement thereof, (iii) the spatial barcode sequence, or a complement thereof, of the second capture probe, and (iv) the RNA sequence, or a complement thereof, wherein the determined sequences of (i) and (ii) correlate the location of the fragmented genomic DNA in the biological sample, wherein the determined sequences of (iii) and (iv) correlate the location of the RNA in the biological sample, thereby determining the spatial location of the genomic DNA and RNA in the biological sample.

36. The method of claim 35, wherein the RNA is mRNA.

37. The method of claim 35 or 36, wherein the first capture domain and the second capture domain are identical.

38. The method of claim 37, wherein the first capture domain and the second capture domain comprise a homopolymeric poly(T) sequence.

39. The method of claim 35 or 36, wherein the first capture domain and the second capture domain are different.

40. The method of claim 39, wherein the first capture domain comprises a non-homopolymer sequence and the second capture domain comprises a poly(T) sequence.

41. The method of any one of claims 35 to 40, wherein the arrayed substrate comprises a plurality of features.

42. The method of claim 41, wherein a feature of the plurality of features comprises the first capture probe, the second capture probe, or both.

43. The method of claim 42, wherein the first capture probe, the second capture probe, or both, are directly or indirectly affixed to the feature of the plurality of features.

44. The method of claim 35, wherein the arrayed substrate comprises a microfluidic channel.

45. The method of any one of claims 35 to 44, wherein the plurality of first capture probes and the plurality of second capture probes comprise one or more of a cleavage domain, a functional domain, a unique molecular identifier, and combinations thereof.

46. The method of any one of claims 35 to 45, comprising a migration step wherein the fragmented genomic DNA and the RNA are migrated from the biological sample to the arrayed substrate.

47. The method of claim 46, wherein the migration step is an active migration step comprising applying an electric field to the biological sample.

48. The method of claim 46, wherein the migration step is a passive migration step comprising diffusion.

49. The method of any one of claims 46 to 48, wherein the migration of the fragmented genomic DNA and the RNA from the biological sample comprises exposing the biological sample to heat.

50. The method of claim 35, wherein the biological sample is immobilized on the arrayed substrate.

51. The method of claim 35, further comprising gap repair of single stranded breaks in the fragmented genomic DNA.

52. The method of any one of claims 35 to 51, wherein the sequence complementary to the first capture domain of the first capture probe is inserted into the fragmented genomic DNA and hybridizes to the first capture domain of the first capture probe.

53. The method of any one of claims 35 to 52, wherein the second capture domain of the second capture probe is a poly(T) sequence and hybridizes to a complementary sequence in the RNA.

54. The method of any one of claims 35 to 53, further comprising extending the first capture probe using the hybridized fragmented genomic DNA as a template, and extending the second capture probe using the hybridized RNA as a template.

55. The method of claim 54, wherein extending the first capture probe is performed with a DNA polymerase and extending the second capture probe is performed with a reverse transcriptase.

56. The method of claim 35, wherein the transposase enzyme of the transposome is a Tn5 transposase enzyme, or a functional derivative thereof and comprises a sequence having at least 80% identity to SEQ ID NO: 1.

57. The method of claim 35, wherein the transposase enzyme of a transposome is a Mu transposase enzyme, or a functional derivative thereof and comprises a sequence having at least 80% identity to SEQ ID NO: 2.

58. The method of claim 56, wherein the transposon end sequence of a transposome comprises a sequence having at least 80% identity to SEQ ID NO: 8, and further comprises an adapter sequence.

59. The method of claim 57, wherein the transposon end sequence of a transposome comprises a sequence having at least 80% identity to any one of SEQ ID NO: 9 to 14, and further comprises an adapter sequence.

60. The method of claim 35, wherein at step (c), the enzyme is pepsin comprising a sequence having at least 80% identity to SEQ ID NO: 3 or 4.

61. The method of claim 35, wherein at step (c), the enzyme is collagenase comprising a sequence having at least 80% identity to SEQ ID NO: 5 or 6.

62. The method of claim 35, wherein at step (c), the enzyme is proteinase K comprising a sequence having at least 80% identity to SEQ ID NO: 7.

63. The method of claim 35, wherein the determining step comprises sequencing.

64. The method of claim 63, wherein the sequencing comprises sequencing: (i) the spatial barcode sequence, or a complement thereof, of the first capture probe, (ii) the fragmented genomic DNA sequence, or a complement thereof, (iii) the spatial barcode sequence, or a complement thereof, of the second capture probe, and (iv) the RNA sequence, or a complement thereof.

65. The method of any one of claims 35 to 64, further comprising imaging the biological sample before or after contacting the biological sample with the arrayed substrate.