

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 ~~spatial analysis~~ of genomic DNA ~~present~~ 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 ~~under conditions sufficient to make the genomic DNA in the biological sample accessible to transposon insertion~~; (d) contacting a transposome with providing a transposon sequence and a transposase enzyme to the permeabilized biological sample to insert ~~under conditions wherein the transposon end sequences is inserted~~ into the genomic DNA, thereby generating fragmented genomic DNA; ~~allowing the transposase enzyme to excise the inserted transposon sequence from the genomic DNA, thereby generating fragmented genomic DNA~~; (e) hybridizing ~~contacting the biological sample comprising~~ the fragmented genomic DNA to the capture domain of the capture probe on ~~with the arrayed substrate under conditions where the capture probe interacts with the fragmented genomic DNA~~; 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 ~~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 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

genomic DNA. In some examples, the biological sample is imaged before or after contacting the biological sample with the substrate.

In a another example, the present disclosure generally describes a kit for use in a method of spatially detecting nucleic acids of a biological sample, wherein the kit comprises any two or more of an array on which plurality of capture probes are present; one or more biological sample permeabilization reagents; one or more transposase enzymes; one or more reverse transcriptases; and one or more cleavage enzymes.

In another aspect, the present disclosure refers to a kit for use in a method of spatially detecting ~~genomic DNA from nucleic acids of~~ a biological sample as disclosed herein, wherein the kit comprises any two or more of: (i) an array ~~ed substrate comprising on which~~ a plurality of capture probes ~~are present~~; (ii) one or more ~~biological sample~~ permeabilization reagents; (iii) ~~a plurality of transposomes one or more transposase enzymes~~; (iv) ~~a DNA polymerase or one or more~~ reverse transcriptases; and (v) ~~instructions for performing the methods as disclosed herein one or more cleavage enzymes~~.

In a different example, the present disclosure generally describes a method for spatial analysis of genomic DNA and RNA present in a biological sample wherein an array is provided and the array comprises a plurality of capture probes, wherein a first capture probe of the plurality of capture probes comprises a spatial barcode and a first capture domain, and wherein a second capture probe of the plurality of capture probes comprises the spatial barcode and a second capture domain; permeabilizing the biological sample under conditions sufficient to make the genomic DNA in the biological sample accessible to 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, thereby generating fragmented genomic DNA; contacting the biological sample comprising the fragmented genomic DNA and RNA with the array under conditions where the first capture domain interacts with the fragmented genomic DNA and the second capture domain interacts with the RNA; and correlating the location of the first capture probe on the array to a location in the biological sample and correlating the location of the second capture probe on the array to a location in the biological sample, thereby spatially analyzing the fragmented genomic DNA and RNA at the location in the biological sample.

In another aspect, the present disclosure refers to a method for ~~determining a location~~^{spatial} ~~analysis~~ of genomic DNA and RNA ~~present~~ in a biological sample, the method comprising: (a) providing an array~~ed substrate~~, wherein the array~~ed 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~~^{the} 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 ~~under conditions sufficient to make the genomic DNA in the biological sample accessible to transposon insertion~~; (d) ~~providing a transposon sequence and a transposase enzyme~~^{contacting a plurality of transposomes} to the biological sample under conditions wherein ~~the transposon sequences of the transposome are~~^{is} inserted into the genomic DNA, ~~thereby generating fragmented genomic DNA~~; ~~allowing the transposase enzyme to excise the inserted transposon sequence from the genomic DNA, thereby generating fragmented genomic DNA~~; (e) ~~contacting the biological sample comprising the fragmented genomic DNA and RNA with the array under conditions where the first capture domain interacts with~~^{hybridizing} (i) the fragmented genomic DNA ~~to the first capture domains of the first plurality of capture probes~~ and the (ii) RNA ~~from the biological sample to the second capture domains of the second plurality of capture probes~~^{domain} ~~interacts with the RNA~~; and (f) determining (i) the spatial barcode sequence, or a complement thereof, of the first capture probe comprising the fragmented genomic DNA, or a complement thereof, wherein the determined sequences correlates the location of the fragmented genomic DNA ~~in the biological sample~~; and (ii) the spatial barcode sequence, or a complement thereof, of the second capture probe comprising the RNA, or a complement thereof, wherein the determined sequences correlates the location of the RNA in the biological sample, thereby determining the spatial location of the genomic DNA and RNA in the biological sample~~correlating the location of the first capture probe on the array to a location in the biological sample and correlating the location of the second capture probe on the array to a location in the biological sample, thereby spatially analyzing the fragmented genomic DNA and RNA at the location 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

examples, the biological sample is imaged before or after contacting the biological sample with the substrate.

In another example aspect, the present disclosure refers to a method for determining a location of an analyte in a biological sample, comprising: (a) contacting a biological sample with a substrate comprising a plurality of capture probes, wherein a capture probe of the plurality comprises a spatial barcode and a capture domain that specifically binds to an analyte from the biological sample; and (b) determining (i) all or a portion of the analyte sequence specifically bound to the capture domain, or a complement thereof, and (ii) all or a portion of the spatial barcode, or a complement thereof, and using the determined sequences of (i) or (ii) to determine the location of the analyte in the biological sample, wherein the biological sample is a cell culture, an organism, or an organoid.

In another example aspect, the present disclosure refers to a method for spatial analysis of a nucleic acid in a biological sample, the method comprising: (a) contacting the biological sample to an array comprising a plurality of capture probes immobilized on the array, wherein a capture probe of the plurality of capture probes comprises a spatial barcode and a capture domain; (b) contacting a polymer material to the biological sample, and activating the polymer material to form a hydrogel-embedded biological sample; (c) permeabilizing the hydrogel-embedded biological sample under conditions sufficient to allow the nucleic acid in the biological sample to specifically bind to the capture domain; (d) amplifying the RNA specifically bound to the capture domain to generate an amplicon having a sequence, wherein the amplicon comprises all or a portion of the spatial barcode or a complement thereof, and all or a portion of the nucleic acid sequence or a complement thereof; and (e) determining the sequence of the amplicon by sequential hybridization and detection with a plurality of labelled probes, and using the determined amplicon sequence to identify the location of the nucleic acid in the biological sample.

In another example aspect, the present disclosure refers to a nucleic acid comprising: (a) a first single-stranded oligonucleotide comprising: a spatial barcode; a primer binding site; a unique molecular identifier; and a first hybridization sequence; and (b) a splint oligonucleotide comprising: a second hybridization sequence that specifically binds to the first hybridization sequence; and a capture domain.

In another example aspect, the present disclosure refers to a composition comprising: (a) a nucleic acid disclosed herein; and (b) a single-stranded tagment comprising a first adaptor that comprises a sequence that is capable of specifically binding to the capture domain.

5 In another example aspect, the present disclosure refers to a composition comprising: (a) a nucleic acid disclosed herein; (b) DNA from a biological sample; and (c) a transposase dimer comprising: (i) a first transposase monomer complexed with a first adaptor comprising a first transposon end sequence and a sequence that is capable of specifically binding to the capture domain; (ii) a second transposase monomer complexed with a second adaptor comprising a
10 second transposon end sequence and a primer binding site or a complement thereof.

In another example aspect, the present disclosure refers to a composition comprising: (a) a nucleic acid disclosed herein; (b) DNA from a biological sample; and (c) a first transposase monomer complexed with a first adaptor comprising a first transposon end sequence and a sequence that is capable of specifically binding to the capture domain; and (d) a second
15 transposase monomer complexed with a second adaptor comprising a second transposon end sequence and a primer binding site or a complement thereof.

All publications, patents, patent applications, and information available on the internet and 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
20 and individually indicated to be 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
25 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.

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,
30 unless expressly stated otherwise, or unless the context of the usage clearly indicates otherwise.

WHAT IS ~~ITEMIZED~~CLAIMED IS:

1. A method for spatial analysis of genomic DNA present in a biological sample, the method comprising:
5 providing an 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;
permeabilizing the biological sample under conditions sufficient to make the genomic DNA in the biological sample accessible to transposon insertion;
providing a transposon sequence and a transposase enzyme to the biological sample
10 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, thereby generating fragmented genomic DNA;
contacting the biological sample comprising the fragmented genomic DNA with the array under conditions where the capture probe interacts with the fragmented genomic DNA; and
15 correlating the location of the capture probe on the array to a location in the biological sample, thereby spatially analyzing the fragmented genomic DNA.

2. The method of ~~claimitem~~ 1, wherein the array comprising the plurality of capture probes is provided on a substrate.

3. The method of ~~claimitem~~ 1, wherein the array comprising the plurality of capture probes is provided on a feature.

4. The method of any one of ~~claimitems~~ 1 to 3, wherein the capture probe is directly or indirectly attached.

5. The method of any one of ~~claimitems~~ 1 to 4, wherein the array comprising the plurality of capture probes is provided on the feature on the substrate.

6. The method of any one of ~~claimitems~~ 1 to 5, wherein the substrate comprises a microfluidic channel.

7. The method of any one of [claimitem](#)s 1 to 6, wherein the capture probe further comprises one or more of a cleavage domain, a functional domain, and a unique identifier, or combinations thereof.

5 8. The method of any one of [claimitem](#)s 1 to 7, further comprising a migration step wherein the fragmented genomic DNA is migrated to the substrate.

9. The method of [claimitem](#) 8, wherein the migration step is an active migration step comprising applying an electric field to the fragmented genomic DNA.

10 10. The method of [claimitem](#) 8, wherein the migration step is a passive migration step comprising diffusion.

15 11. The method of any one of [claimitem](#)s 8 to 10, wherein the migration of the fragmented genomic DNA from the biological sample comprises exposing the biological sample and the feature to heat.

12. The method of any one of [claimitem](#)s 1 to 11, wherein the biological sample is immobilized on the substrate.

20 13. The method of any one of [claimitem](#)s 1 to 12, wherein 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
25 the second adapter to the fragmented genomic DNA.

14. The method of [claimitem](#) 13, wherein the first adapter and the second adapter have a 5' end and a 3' end, wherein the 5' end is phosphorylated in situ.

15. The method of [claimitem](#) 13 or 14, wherein prior to fragmenting the DNA, the 5' end of the first adapter complexed with the first monomer and the second adapter complexed with the second monomer are phosphorylated.

16. The method of [claimitem](#) 14 or 15, wherein the step of phosphorylating the 5' end of 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.

17. The method of any one of [claimitems](#) 1 to 16, wherein the capture domain of the capture probe comprises a sequence that hybridizes to the sequence complementary to the capture domain of the first adapter.

18. The method of any one of [claimitems](#) 13 to 17, wherein the capture probe is a partially double stranded molecule comprising a first strand comprising the capture domain hybridized to a second strand, and wherein the first strand templates the ligation of the first adapter to the second strand.

19. The method of any one of [claimitems](#) 13 to 18, wherein the first adapter sequence complementary to the capture domain, or portion thereof, hybridized to the capture probe templates the ligation and ligating the 5' end of the first adapter to the 3' end of the capture probe.

20. The method of any one of [claimitem](#)s 1 to 19, wherein the capture probe comprises a surface probe and a splint oligonucleotide and wherein the splint oligonucleotide comprises a sequence complementary to a hybridization domain of the surface probe, or portion thereof.

21. The method of [claimitem](#) 20, wherein the splint oligonucleotide comprises the capture domain with a sequence complementary to the first adapter, or portion thereof.

22. The method of [claimitem](#) 20 or 21, wherein the splint oligonucleotide hybridizes to the first adapter, or portion thereof, and to the hybridization domain of the surface probe, or portion thereof.

23. The method of any one of [claimitem](#)s 20 to 22, wherein ligation is performed in the presence of the splint oligonucleotide, thereby ligating the surface probe of the capture probe and the first adapter.

24. The method of any one of [claimitem](#)s 13 to 23, wherein the fragmented genomic DNA hybridized to the capture probe by the first adapter is an extension template used to produce an extended capture probe that comprises the sequences of the spatial barcode and a sequence complementary to the fragmented genomic DNA.

25. The method of [claimitem](#) 24, wherein the capture probe hybridized to the fragmented genomic DNA is extended with a DNA polymerase.

26. The method of [claimitem](#) 25, wherein the DNA polymerase has strand displacement activity.

27. The method of any one of claimitems 1 to 26, further comprising gap repair of single stranded breaks in the fragmented genomic DNA.

5 28. The method of any one of claimitems 13 to 26, wherein the sequence complementary to the capture domain is a unique sequence.

29. The method of any one of claimitems 1-28, wherein the capture probe is ligated to the fragmented genomic DNA by a DNA ligase enzyme.

10 30. The method of any one of claimitems 1 to 29, wherein the transposase enzyme is a Tn5 transposase enzyme, or a functional derivative thereof.

15 31. The method of claimitem 30, wherein the Tn5 transposase enzyme comprises a sequence having at least 80% identity to SEQ ID NO: 1.

32. The method of any one of claimitems 1 to 29, wherein the transposase enzyme is a Mu transposase enzyme, or the functional derivative thereof.

20 33. The method of claimitem 32, wherein the Mu transposase enzyme comprises a sequence having at least 80% identity to SEQ ID NO: 2.

34. The method of claimitem 13, wherein the transposon end sequence comprises a sequence having at least 80% identity to SEQ ID NO: 8.

25 35. The method of claimitem 13, wherein the transposon end sequence comprises a sequence having at least 80% identity to any one of SEQ ID NO: 9 to 14.

36. The method of any one of claimitems 1 to 35, wherein permeabilizing the biological sample is performed under a chemical permeabilization condition, an enzymatic permeabilization condition, or both.

37. The method of claimitem 36, wherein the chemical permeabilization condition comprises contacting the biological sample with an alkaline solution.

38. The method of claimitem 36 or 37, wherein the enzymatic permeabilization condition comprises contacting the biological sample with an acidic solution comprising a protease enzyme.

39. The method of claimitem 38, wherein the protease enzyme is an aspartyl protease, preferably a pepsin enzyme, a pepsin-like enzyme, or the functional equivalent thereof.

40. The method of claimitem 39, wherein the pepsin enzyme, the pepsin-like enzyme, or the functional equivalent thereof, comprises a sequence having at least 80% identity to SEQ ID NO: 3 or 4.

41. The method of any one of claimitems 36-40, wherein the enzymatic permeabilization condition comprises contacting the biological sample with:

(i) a zinc endopeptidase, a collagenase enzyme, a collagenase-like enzyme, or a functional equivalent thereof;

(ii) a serine protease, a proteinase K enzyme, a proteinase K-like enzyme, or a functional equivalent thereof; or

(iii) both.

42. The method of [claimitem](#) 41, wherein the collagenase enzyme, the collagenase-like enzyme, or the functional equivalent thereof comprises a sequence having at least 80% identity to SEQ ID NO: 5 or 6.

43. The method of [claimitem](#) 41 or 42, wherein the proteinase K enzyme, the proteinase K-like enzyme, or the functional equivalent thereof comprises a sequence having at least 80% identity to SEQ ID NO: 7.

44. The method of [claimitem](#) 25 or 26, wherein using the fragmented genomic DNA hybridized to the capture probe as the extension template generates a DNA molecule.

45. The method of [claimitem](#) 18 or 19, where using the fragmented genomic DNA hybridized to the capture probe as a ligation template generates a DNA molecule.

46. The method of [claimitem](#) 44 or 45, comprising a step of analyzing the DNA molecule.

47. The method of [claimitem](#) 46, wherein the step of analyzing the DNA molecule includes sequencing.

48. The method of [claimitem](#) any one of [claimitems](#) 1 to 47, wherein correlating the spatial barcode of the capture probe with the fragmented genomic DNA associated with the capture probe spatially analyzes the fragmented genomic DNA.

49. The method of any one of [claimitems](#) 1 to 48, further comprising a step wherein the biological sample is imaged before or after contacting the biological sample with the substrate.

50. A kit for use in a method of spatially detecting nucleic acids of a biological sample as defined in any one of [claimitems](#) 1 to 49, wherein the kit comprises any two or more of:
(i) an array on which a plurality of capture probes are present;
(ii) one or more biological sample permeabilization reagents;
(iii) one or more transposase enzymes;

- (iv) one or more reverse transcriptases; and
- (v) one or more cleavage enzymes.

51. A method for spatial analysis of genomic DNA and RNA present in a biological sample, the method comprising:

providing an array, wherein the array comprises a plurality of capture probes, wherein a first capture probe of the plurality of capture probes comprises a spatial barcode and a first capture domain, and wherein a second capture probe of the plurality of capture probes comprises the spatial barcode and a second capture domain;

permeabilizing the biological sample under conditions sufficient to make the genomic DNA in the biological sample accessible to 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, thereby generating fragmented genomic DNA;

contacting the biological sample comprising the fragmented genomic DNA and RNA with the array under conditions where the first capture domain interacts with the fragmented genomic DNA and the second capture domain interacts with the RNA; and

correlating the location of the first capture probe on the array to a location in the biological sample and correlating the location of the second capture probe on the array to a location in the biological sample, thereby spatially analyzing the fragmented genomic DNA and RNA at the location in the biological sample.

52. The method of [claimitem](#) 51, wherein the RNA is a mRNA.

53. The method of [claimitem](#) 51 or 52, wherein the first capture domain and the second capture domain are identical.

54. The method of [claimitem](#) 53, wherein the first capture domain and the second capture domain comprise a homopolymeric poly(T) sequence.

55. The method of any one of [claimitem](#)s 51 to 54, wherein the first capture domain and the second capture domain are different.

56. The method of [claimitem](#) 55, wherein the first capture domain comprises a random sequence and the second capture domain comprises a poly(T) sequence.

57. The method of any one of [claimitem](#)s 51 to 56, wherein the array comprising the plurality of capture probes is provided on a substrate.

58. The method of any one of [claimitem](#)s 51 to 56, wherein the array comprising the plurality of capture probes is provided on a feature.

59. The method of [claimitem](#) 58, wherein the feature comprises the first capture probe, the second capture probe, or both.

60. The method of any one of [claimitem](#)s 51 to 59, wherein the first capture probe, the second capture probe, or both, are directly or indirectly attached.

61. The method of any one of [claimitem](#)s 51 to 60, wherein the array comprising the plurality of capture probes is provided on the feature on the substrate.

62. The method of any one of [claimitem](#)s 51 to 61, wherein the substrate comprises a microfluidic channel.

63. The method of any one of [claimitem](#)s 51 to 62, wherein the first capture probe, the second capture probe, or both, comprise one or more of a cleavage domain, a functional domain, and a unique identifier, or combinations thereof.

64. The method of any one of [claimitem](#)s 51 to 63, comprising a migration step wherein the fragmented genomic DNA and the RNA are migrated to the substrate.

65. The method of [claimitem](#) 64, wherein the migration step is an active migration step.

66. The method of [claimitem](#) 64, wherein the migration step is a passive migration step.

5 67. The method of any one of [claimitems](#) 64 to 66, wherein the migration of the fragmented genomic DNA and the RNA from the biological sample comprises exposing the biological sample to heat.

10 68. The method of any one of [claimitems](#) 51 to 67, wherein the biological sample is immobilized on the substrate.

69. The method of any one of [claimitems](#) 51 to 68, wherein the fragmented genomic DNA is repaired by ligating breaks with a ligase enzyme.

15 70. The method of any one of [claimitems](#) 51 to 69, further comprising gap repair of single stranded breaks in the fragmented genomic DNA.

20 71. The method of any one of [claimitems](#) 51 to 70, wherein a sequence complementary to the first capture domain of the first capture probe is introduced to the fragmented genomic DNA.

72. The method of any one of [claimitems](#) 51 to 71, wherein the first capture domain of the first capture probe hybridizes to the sequence complementary to the capture domain introduced to the fragmented genomic DNA.

25 73. The method of [claimitem](#) 57, wherein the random sequence of the first capture domain hybridizes the fragmented genomic DNA.

74. The method of any one of [claimitems](#) 51 to 73, wherein the second capture domain of the second capture probe hybridizes to a complementary sequence in the mRNA.

75. The method of any one of [claimitem](#)s 71 or 74, wherein the sequence complementary to the first capture domain and the complementary sequence in the mRNA is a homopolymeric sequence.

5 76. The method of [claimitem](#) 75, wherein the homopolymeric sequence is a poly(A) sequence.

77. The method of any one of [claimitem](#)s 51-76, comprising extending the first capture probe using the fragmented genomic DNA as an extension template, and extending the second
10 capture probe using the RNA as an extension template.

78. The method of [claimitem](#) 77, wherein extending the first capture probe is performed with a DNA polymerase.

15 79. The method of [claimitem](#) 78, wherein extending the second capture probe is performed with reverse transcriptase.

80. The method of any one of [claimitem](#)s 51 to 79, wherein the transposase enzyme is a Tn5 transposase enzyme, or a functional derivative thereof.

20 81. The method of [claimitem](#) 80, wherein the Tn5 transposase enzyme comprises a sequence having at least 80% identity to SEQ ID NO: 1.

82. The method of any one of [claimitem](#)s 51 to 79, wherein the transposase enzyme is a
25 Mu transposase enzyme, or a functional derivative thereof.

83. The method of [claimitem](#) 82, wherein the Mu transposase comprises a sequence having at least 80% identity to SEQ ID NO: 2.

30 84. The method of [claimitem](#) any one of [claimitem](#)s 51 to 83, wherein the transposase enzyme is complexed with an adapter comprising a transposon end sequence.

85. The method of ~~claim~~item 84, wherein the transposon end sequence comprises a sequence having at least 80% identity to SEQ ID NO: 8.

5 86. The method of ~~claim~~item 84, wherein the transposon end sequence comprises a sequence having at least 80% identity to any one of SEQ ID NO: 9 to 14.

87. The method of any one of ~~claim~~items 51 to 86, wherein permeabilizing the biological sample is performed under a chemical permeabilization condition, an enzymatic
10 permeabilization condition, or both.

88. The method of ~~claim~~item 87, wherein the chemical permeabilization condition comprises contacting the biological sample with an alkaline solution.

15 89. The method of ~~claim~~item 86 or 87, wherein the enzymatic permeabilization condition comprises contacting the biological sample with an acidic solution comprising a protease enzyme.

90. The method of ~~claim~~item 89, wherein the protease enzyme is an aspartyl protease,
20 preferably a pepsin enzyme, a pepsin-like enzyme, or a functional equivalent thereof.

91. The method of ~~claim~~item 90, wherein the pepsin enzyme, the pepsin-like enzyme, or functional equivalent thereof, comprises a sequence having at least 80% identity to SEQ ID NO:
3 or 4.

25 92. The method of any one of ~~claim~~items 86 to 91, wherein the enzymatic permeabilization condition comprises contacting the biological sample with:

(i) a zinc endopeptidase, a collagenase enzyme, a collagenase-like enzyme, or a functional equivalent thereof;

30 (ii) a serine protease, a proteinase K enzyme, a proteinase K-like enzyme, or a functional equivalent thereof; or

(iii) both.

93. The method of [claimitem](#) 92, wherein the collagenase enzyme, the collagenase-like enzyme, or the functional equivalent thereof comprises a sequence having at least 80% identity to SEQ ID NO: 5 or 6.

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94. The method of [claimitem](#) 92 or 93, wherein the proteinase K enzyme, the proteinase K-like enzyme, or the functional equivalent thereof comprises a sequence having at least 80% identity to SEQ ID NO: 7.

10 95. The method of [claimitem](#) any one [claimitem](#)s 51 to 94, wherein the step of analyzing the DNA molecule includes sequencing.

15 96. The method of any one of [claimitem](#)s 51 to 95, wherein correlating the spatial barcode of the first capture probe with the fragmented genomic DNA associated with the first capture probe spatially analyzes the fragmented genomic DNA.

97. The method of any one of [claimitem](#)s 51 to 96, wherein correlating the spatial barcode of the second capture probe with the mRNA associated with the second capture probe spatially analyzes the mRNA.

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98. The method of any one of [claimitem](#)s 51 to 97, further comprising a step wherein the biological sample is imaged before or after contacting the biological sample with the substrate.

WHAT IS CLAIMED IS:

1. A method for ~~determining a spatial location~~spatial analysis of genomic DNA ~~present~~ in a biological sample, the method comprising:
 - (a) providing an ~~array~~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 ~~under conditions sufficient to make the genomic DNA in the biological sample accessible to transposon insertion~~;
 - (d) contacting a transposome with providing a transposon sequence and a transposase enzyme to the permeabilized biological sample to insert~~under conditions wherein the transposon end sequences is inserted~~ into the genomic DNA, thereby generating fragmented genomic DNA;
~~allowing the transposase enzyme to excise the inserted transposon sequence from the genomic DNA, thereby generating fragmented genomic DNA~~;
 - (e) ~~hybridizing~~contacting the biological sample comprising the fragmented genomic DNA ~~to the capture domain of the capture probe on with the arrayed substrate under conditions where the capture probe interacts with the fragmented genomic DNA~~; 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.
~~correlating the location of the capture probe on the array to a location in the biological sample, thereby spatially analyzing the fragmented genomic DNA.~~
2. ~~The method of claim 1, wherein the array comprising the plurality of capture probes is provided on a substrate.~~

~~3.2.~~ The method of claim 1, wherein ~~the array comprising~~ the plurality of capture probes ~~is~~are provided on a plurality of features on the arrayed substrate.

~~4.3.~~ The method of claim 1 or 2~~any one of claims 1 to 3~~, wherein the plurality of capture probes ~~are~~is directly or indirectly ~~attached~~affixed on the arrayed substrate.

~~5. The method of any one of claims 1 to 4, wherein the array comprising the plurality of capture probes is provided on the feature on the substrate.~~

~~6.4.~~ The method of any one of claims 1 to ~~5~~3, wherein the arrayed substrate comprises a microfluidic channel.

~~7.5.~~ The method of any one of claims 1 to ~~6~~4, wherein the capture probe of the plurality of capture probes further comprises one or more of a cleavage domain, a functional domain, ~~and~~ a unique identifier, ~~and/or~~ combinations thereof.

~~8.6.~~ The method of any one of claims ~~2~~1 to ~~7~~5, further comprising a migration step wherein the fragmented genomic DNA is migrated from the biological sample to the arrayed substrate.

~~9.7.~~ The method of claim ~~8~~6, wherein the migration step is an active migration step comprising applying an electric field to the biological sample~~fragmented genomic DNA~~.

~~10.8.~~ The method of claim ~~8~~6, wherein the migration step is a passive migration step comprising diffusion.

~~11.9.~~ The method of any one of claims ~~68~~ to ~~810~~, wherein the migration of the fragmented genomic DNA from the biological sample comprises exposing the biological sample ~~and the feature~~ to heat.

~~12.10.~~ The method of any one of claims ~~21~~ to ~~119~~, wherein the biological sample is immobilized on the arrayed substrate.

~~13.11.~~ The method of any one of claims 1 to ~~1210~~, wherein the transposome comprises a transposase enzyme ~~as is~~ a dimer ~~comprising~~~~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 inserts the transposon end sequences and ligates the first adapter and the second adapter into the genomic DNA, thereby generating fragmented genomic DNA.

~~14.12.~~ The method of claim ~~1113~~, wherein the first adapter and the second adapter have a 5' end and a 3' end, wherein the 5' end is phosphorylated ~~in situ~~.

~~15.13.~~ The method of claim ~~1113~~ or ~~1214~~, wherein prior to fragmenting the genomic DNA, the 5' end of the first adapter complexed with the first monomer and the second adapter complexed with the second monomer are phosphorylated.

~~16.14.~~ The method of claim ~~1214~~ or ~~1315~~, wherein ~~the step of~~ phosphorylating the ~~5' end of 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.

~~17.~~15. ____ The method of any one of claims ~~1 to 16~~11 to 15, wherein the capture domain of the capture probe comprises a sequence that hybridizes to ~~the sequence complementary to the capture domain of~~ the first adapter.

~~18. The method of any one of claims 13 to 17, wherein the capture probe is a partially double stranded molecule comprising a first strand comprising the capture domain hybridized to a second strand, and wherein the first strand templates the ligation of the first adapter to the second strand.~~

~~19. The method of any one of claims 13 to 18, wherein the first adapter sequence complementary to the capture domain, or portion thereof, hybridized to the capture probe templates the ligation and ligating the 5' end of the first adapter to the 3' end of the capture probe.~~

~~20.~~16. ____ The method of any one of claims ~~11 to 19~~11 to 15, wherein the capture domain of the capture probe ~~comprises a surface probe and is complementary to a portion of~~ a splint oligonucleotide and wherein the splint oligonucleotide comprises a sequence complementary to a ~~hybridization domain of the surface probe, or portion thereof~~portion of the first adapter of the fragmented genomic DNA.

~~21. The method of claim 20, wherein the splint oligonucleotide comprises the capture domain with a sequence complementary to the first adapter, or portion thereof.~~

~~22.17. The method of claim 20 or 2116, wherein the splint oligonucleotide hybridizes to the first adapter, or a portion thereof, and to the capture hybridization domain of the capture surface probe, or a portion thereof.~~

~~23.18. The method of claim 17any one of claims 20 to 22, wherein the first adapter and the capture domain of the capture probe hybridized to the splint oligonucleotide are ligated togetherligation is performed in the presence of the splint oligonucleotide, thereby ligating the surface probe of the capture probe and the first adapter.~~

~~24.19. The method of claim 15any one of claims 13 to 23, wherein the fragmented genomic DNA hybridized to the capture domain of the capture probe is by the first adapter is an extension template used to produceused as a template to generate an extended capture probe comprisingthat comprises the sequences of the spatial barcode and a sequence complementary to the fragmented genomic DNA.~~

~~25.20. The method of claim 1924, wherein the capture probe hybridized to the fragmented genomic DNA is extended capture probe is generated usingwith a DNA polymerase.~~

~~26.21. The method of claim 2025, wherein the DNA polymerase has strand displacement activity.~~

~~27.22.~~ The method of any one of claims 1 to ~~21~~~~26~~, further comprising gap repair of single stranded breaks in the fragmented genomic DNA.

~~28.23.~~ The method of any one of claims ~~11~~~~13~~ to ~~16~~~~26~~, wherein the sequence complementary to the capture domain is a unique sequence.

~~29.24.~~ The method of ~~claim 18~~~~any one of claims 1-28~~, wherein the capture ~~domain of the capture~~ probe is ligated to the fragmented genomic DNA by a DNA ligase enzyme.

~~30.25.~~ The method of ~~claim 11~~~~any one of claims 1 to 29~~, 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.~~

~~31.~~ The method of claim 30, wherein the Tn5 transposase enzyme comprises a sequence having at least 80% identity to SEQ ID NO: 1.

~~32.26.~~ The method of ~~claim 11~~~~any one of claims 1 to 29~~, wherein the transposase enzyme ~~of the transposome~~ is a Mu transposase enzyme, or the functional derivative thereof, ~~and comprises a sequence having at least 80% identity to SEQ ID NO: 2.~~

~~33.~~ The method of claim 32, wherein the Mu transposase enzyme comprises a sequence having at least 80% identity to SEQ ID NO: 2.

~~34.27.~~ The method of claim ~~13~~, wherein the transposon end sequence comprises a sequence having at least 80% identity to SEQ ID NO: 8.

~~35~~28. The method of claim ~~13~~, wherein the transposon end sequence comprises a sequence having at least 80% identity to any one of SEQ ID NO: 9 to 14.

~~36~~29. The method of any one of claims 1 to ~~35~~28, wherein permeabilizing the biological sample is performed under a chemical permeabilization condition, an enzymatic permeabilization condition, or both.

~~37~~30. The method of claim ~~36~~29, wherein the chemical permeabilization condition comprises contacting the biological sample with an alkaline solution.

~~38~~31. The method of claim ~~29~~36 ~~or 37~~, wherein the enzymatic permeabilization condition comprises contacting the biological sample with ~~an acidic solution comprising~~ a protease enzyme.

~~39~~32. The method of claim ~~38~~31, wherein the protease enzyme is an aspartyl protease, ~~preferably~~ a pepsin enzyme, a pepsin-like enzyme, or the functional equivalent thereof.

~~40~~33. The method of claim ~~32~~39, wherein the pepsin enzyme, the pepsin-like enzyme, or the functional equivalent thereof, comprises a sequence having at least 80% identity to SEQ ID NO: 3 or 4.

~~41~~34. The method of any one of claims ~~31-33~~36-40, wherein the enzymatic permeabilization condition comprises contacting the biological sample with:

- (i) a zinc endopeptidase, a collagenase enzyme, a collagenase-like enzyme, or a functional equivalent thereof;
- (ii) a serine protease, a proteinase K enzyme, a proteinase K-like enzyme, or a functional equivalent thereof; or
- (iii) both a combination thereof.

~~42.35.~~ The method of claim ~~41~~34, wherein the collagenase enzyme, the collagenase-like enzyme, or the functional equivalent thereof comprises a sequence having at least 80% identity to SEQ ID NO: 5 or 6.

~~43.36.~~ The method of claim ~~34~~41 or 42, wherein the proteinase K enzyme, the proteinase K-like enzyme, or the functional equivalent thereof comprises a sequence having at least 80% identity to SEQ ID NO: 7.

~~44. The method of claim 25 or 26, wherein using the fragmented genomic DNA hybridized to the capture probe as the extension template generates a DNA molecule.~~

~~45. The method of claim 18 or 19, where using the fragmented genomic DNA hybridized to the capture probe as a ligation template generates a DNA molecule.~~

~~46. The method of claim 44 or 45, comprising a step of analyzing the DNA molecule.~~

~~47.37.~~ The method of claim 146, wherein the determining step comprises step of ~~analyzing the DNA molecule includes~~ sequencing.

38. The method of claim 37, wherein sequencing comprises sequencing the spatial barcode sequence or a complement thereof, and the fragmented genomic DNA sequence or a complement thereof.

~~48. The method of claim any one of claims 1 to 47, wherein correlating the spatial barcode of the capture probe with the fragmented genomic DNA associated with the capture probe spatially analyzes the fragmented genomic DNA.~~

~~4939.~~ The method of any one of claims 1 to ~~3848~~, further comprising a step of imaging~~wherein~~ the biological sample ~~is imaged~~ before or after contacting the biological sample with the arrayed substrate.

~~5040.~~ A kit for use in a method of spatially detecting genomic DNA from~~nucleic acids of~~ a biological sample as defined in any one of claims 1 to ~~3949~~, wherein the kit comprises any two or more of:

- ~~(i) an array~~ed substrate comprising~~on which~~ a plurality of capture probes ~~are present~~;
- ~~(ii) one or more~~ biological sample permeabilization reagents;
- ~~(iii) a plurality of transposomes~~one or more transposase enzymes;
- ~~(iv) a DNA polymerase or a reverse transcriptase~~one or more reverse transcriptases; and
- ~~(v) instructions for performing the methods of any one of claims 1-39~~one or more cleavage enzymes.

~~5141.~~ A method for determining a location~~spatial analysis~~ of genomic DNA and RNA ~~present~~ 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~~the~~ 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~~under conditions sufficient to make the genomic DNA in the biological sample accessible to transposon insertion;~~

(d) providing a transposon sequence and a transposase enzyme~~contacting a plurality of transposomes~~ to the biological sample under conditions wherein~~the~~ transposon sequences of the transposome are~~is~~ inserted into the genomic DNA, thereby generating fragmented genomic DNA;

~~allowing the transposase enzyme to excise the inserted transposon sequence from the genomic DNA, thereby generating fragmented genomic DNA;~~

(e) contacting the biological sample comprising the fragmented genomic DNA and RNA~~with the array under conditions where the first capture domain interacts with~~ hybridizing (i) the fragmented genomic DNA to the first capture domains of the first plurality of capture probes and the (ii) RNA from the biological sample to the second capture domains of the second plurality of capture probes~~domain interacts with the RNA;~~ and

(f) determining (i) the spatial barcode sequence, or a complement thereof, of the first capture probe comprising the fragmented genomic DNA, or a complement thereof, wherein the determined sequences correlates the location of the fragmented genomic DNA in the biological sample; and

(ii) the spatial barcode sequence, or a complement thereof, of the second capture probe comprising the RNA, or a complement thereof, wherein the determined sequences correlates the location of the RNA in the biological sample, thereby determining the spatial location of the genomic DNA and RNA in the biological sample.

~~correlating the location of the first capture probe on the array to a location in the biological sample and correlating the location of the second capture probe on the array to a location in the biological sample, thereby spatially analyzing the fragmented genomic DNA and RNA at the location in the biological sample.~~

5242. The method of claim 4151, wherein the RNA is a-mRNA.

~~5343~~. The method of claim ~~4151~~ or ~~4252~~, wherein the first capture domain and the second capture domain are identical.

~~5444~~. The method of claim ~~5343~~, wherein the first capture domain and the second capture domain comprise a homopolymeric poly(T) sequence.

~~5545~~. The method of ~~claim 41 or 42~~~~any one of claims 51 to 54~~, wherein the first capture domain and the second capture domain are different.

~~5646~~. The method of claim ~~4555~~, wherein the first capture domain comprises a ~~non-homopolymer~~~~random~~ sequence and the second capture domain comprises a poly(T) sequence.

~~57. The method of any one of claims 51 to 56, wherein the array comprising the plurality of capture probes is provided on a substrate.~~

~~5847~~. The method of any one of claims ~~4151~~ to ~~4656~~, wherein ~~the array comprising the plurality of capture probes is provided on a~~the arrayed substrate comprises a plurality of features.

~~5948~~. The method of claim ~~4758~~, wherein ~~a~~the feature of the plurality of features comprises the first capture probe, the second capture probe, or both.

~~6049~~. The method of ~~claim 48~~~~any one of claims 51 to 59~~, wherein the first capture probe, the second capture probe, or both, are directly or indirectly affixed to a feature of the plurality of features~~attached~~.

~~61. The method of any one of claims 51 to 60, wherein the array comprising the plurality of capture probes is provided on the feature on the substrate.~~

~~62~~50. The method of claim 41~~any one of claims 51 to 61~~, wherein the arrayed substrate comprises a microfluidic channel.

~~63~~51. The method of any one of claims ~~41~~51 to ~~50~~62, wherein the plurality of first capture probes, and the plurality of second capture probes, ~~or both~~, comprise one or more of a cleavage domain, a functional domain, ~~and~~ a unique molecular identifier, ~~and/or~~ combinations thereof.

~~64~~52. The method of any one of claims ~~41~~51 to ~~51~~63, comprising a migration step wherein the fragmented genomic DNA and the RNA are migrated from the biological sample to the arrayed substrate.

~~65~~53. The method of claim ~~52~~64, wherein the migration step is an active migration step comprising applying an electric field to the biological sample.

~~66~~54. The method of claim ~~52~~64, wherein the migration step is a passive migration step comprising diffusion.

~~67~~55. The method of any one of claims ~~52~~64 to ~~54~~66, wherein the migration of the fragmented genomic DNA and the RNA from the biological sample comprises exposing the biological sample to heat.

~~68~~56. The method of claim 41~~any one of claims 51 to 67~~, wherein the biological sample is immobilized on the arrayed substrate.

~~69~~. ~~The method of any one of claims 51 to 68, wherein the fragmented genomic DNA is repaired by ligating breaks with a ligase enzyme.~~

~~70~~57. The method of claim 41~~any one of claims 51 to 69~~, further comprising gap repair of single stranded breaks in the fragmented genomic DNA.

~~7158.~~ The method of any one of claims ~~4151~~ to ~~5770~~, wherein a sequence complementary to the first capture domain of the first capture probe is introduced to the fragmented genomic DNA and hybridizes to the first capture domain of the first capture probe.

~~72.~~ ~~The method of any one of claims 51 to 71, wherein the first capture domain of the first capture probe hybridizes to the sequence complementary to the capture domain introduced to the fragmented genomic DNA.~~

~~73.~~ ~~The method of claim 57, wherein the random sequence of the first capture domain hybridizes the fragmented genomic DNA.~~

~~7459.~~ The method of any one of claims ~~4151~~ to ~~5873~~, wherein the second capture domain of the second capture probe is a poly(T) sequence and hybridizes to a complementary sequence in the RNAmRNA.

~~75.~~ ~~The method of any one of claims 71 or 74, wherein the sequence complementary to the first capture domain and the complementary sequence in the mRNA is a homopolymeric sequence.~~

~~76.~~ ~~The method of claim 75, wherein the homopolymeric sequence is a poly(A) sequence.~~

~~7760.~~ The method of any one of claims ~~41~~ to ~~5951-76~~, further comprising extending the first capture probe using the hybridized fragmented genomic DNA as a an-extension template, and extending the second capture probe using the hybridized RNA as a an-extension template.

~~7861.~~ The method of claim ~~6077~~, wherein extending the first capture probe is performed with a DNA polymerase and extending the second capture probe is performed with a reverse transcriptase.

~~79. The method of claim 78, wherein extending the second capture probe is performed with reverse transcriptase.~~

~~8062. The method of claim 41~~any one of claims 51 to 79~~, 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.~~

~~81. The method of claim 80, wherein the Tn5 transposase enzyme comprises a sequence having at least 80% identity to SEQ ID NO: 1.~~

~~8263. The method of claim 41~~any one of claims 51 to 79~~, 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.~~

~~83. The method of claim 82, wherein the Mu transposase comprises a sequence having at least 80% identity to SEQ ID NO: 2.~~

~~84. The method of claim ~~any one of claims 51 to 83~~, wherein the transposase enzyme is complexed with an adapter comprising a transposon end sequence.~~

~~8564. The method claim 6284, wherein the transposon end sequence of a transposome comprises a sequence having at least 80% identity to SEQ ID NO: 8, and further comprising an adapter sequence.~~

~~8665. The method of claim 6384, 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 comprising an adapter sequence.~~

~~8766~~. The method of ~~claim 41~~~~any one of claims 51 to 86~~, wherein permeabilizing the biological sample is performed under a chemical permeabilization condition, an enzymatic permeabilization condition, or both.

~~8867~~. The method of claim ~~6687~~, wherein the chemical permeabilization condition comprises contacting the biological sample with an alkaline solution.

~~8968~~. The method of claim ~~6686 or 87~~, wherein the enzymatic permeabilization condition comprises contacting the biological sample with ~~an acidic solution comprising~~ a protease enzyme.

~~9069~~. The method of claim ~~6889~~, wherein the protease enzyme is an aspartyl protease, ~~preferably~~ a pepsin enzyme, a pepsin-like enzyme, or a functional equivalent thereof.

~~9170~~. The method of claim ~~6990~~, wherein the pepsin enzyme, the pepsin-like enzyme, or ~~the~~ functional equivalent thereof, comprises a sequence having at least 80% identity to SEQ ID NO: 3 or 4.

~~9271~~. The method of ~~claim 66~~~~any one of claims 86 to 91~~, wherein the enzymatic permeabilization condition comprises contacting the biological sample with:

- (i) a zinc endopeptidase, a collagenase enzyme, a collagenase-like enzyme, or a functional equivalent thereof;
- (ii) a serine protease, a proteinase K enzyme, a proteinase K-like enzyme, or a functional equivalent thereof; or
- (iii) ~~a combination thereof~~~~both~~.

~~9372~~. The method of claim ~~7192~~, wherein the collagenase enzyme, the collagenase-like enzyme, or the functional equivalent thereof comprises a sequence having at least 80% identity to SEQ ID NO: 5 or 6.

~~94~~73. The method of claim ~~71~~92 ~~or 93~~, wherein the proteinase K enzyme, the proteinase K-like enzyme, or the functional equivalent thereof comprises a sequence having at least 80% identity to SEQ ID NO: 7.

~~95~~74. The method of claim ~~41~~any one claims 51 to 94, wherein the determining step comprises~~step of analyzing the DNA molecule includes~~ sequencing.

75. The method of claim 74, wherein the sequencing comprises sequencing: (i) the spatial barcode sequence, or a complement thereof, of the first capture probe comprising the fragmented genomic DNA, or a complement thereof, and (ii) the spatial barcode sequence, or a complement thereof, of the second capture probe comprising RNA, or a complement thereof.

~~96. The method of any one of claims 51 to 95, wherein correlating the spatial barcode of the first capture probe with the fragmented genomic DNA associated with the first capture probe spatially analyzes the fragmented genomic DNA.~~

~~97. The method of any one of claims 51 to 96, wherein correlating the spatial barcode of the second capture probe with the mRNA associated with the second capture probe spatially analyzes the mRNA.~~

~~98~~76. The method of any one of claims ~~41~~51 to ~~75~~97, further comprising imaging a step ~~wherein~~ the biological sample ~~is imaged~~ before or after contacting the biological sample with the arrayed substrate.

~~99. A method for determining a location of an analyte in a biological sample, comprising:~~
~~(a) contacting a biological sample with a substrate comprising a plurality of capture probes, wherein a capture probe of the plurality comprises a spatial barcode and a capture domain that specifically binds to an analyte from the biological sample; and~~
~~(b) determining (i) all or a portion of the analyte sequence specifically bound to the capture domain, or a complement thereof, and (ii) all or a portion of the spatial barcode, or a~~

~~complement thereof, and using the determined sequences of (i) or (ii) to determine the location of the analyte in the biological sample, wherein the biological sample is a cell culture, an organism, or an organoid.~~

~~100. The method of claim 99, wherein the biological sample is permeabilized prior to step (b).~~

~~101. The method of claim 99, further comprising permeabilizing the biological sample prior to step (b).~~

~~102. The method of claim 101, further comprising fixing the biological sample, prior to the step of permeabilizing the biological sample.~~

~~103. The method of any one of claims 99-102, wherein the organism is a nematode, a plant, a fungi, an insect, an arachnid, a fish, an amphibian, or a reptile.~~

~~104. The method of any one of claims 99-102, wherein the cell culture comprises a plurality of cells.~~

~~105. The method of claim 104, wherein the plurality of cells are non-adherent cells.~~

~~106. The method of claim 104 or 105, wherein the plurality of cells is from disassociated tissue or a tissue section.~~

~~107. The method of any one of claims 104-106, wherein a cell of the plurality of cells is immobilized on the substrate after the cell is distributed onto the substrate.~~

~~108. The method of claim 107, wherein the cell of the plurality of cells is immobilized on the substrate by a polymer coating.~~

~~109. The method of claim 107, wherein the cell of the plurality of cells is immobilized on the substrate by a lectin, poly lysine, an antibody, or a polysaccharide.~~

~~110. The method of claim 104, wherein the plurality of cells are adherent cells.~~

~~111. The method of claim 110, wherein the adherent cells are from a cell line selected from the group consisting of: BT549, HS 578T, MCF7, MDA MB 231, MDAMB 468, T 47D, SF268, SF295, SF539, SNB 19, SNB 75, U251, Colo205, HCC 2998, HCT 116, HCT 15, HT29, KM12, SW620, 786 O, A498, ACHN, CAKI, RXF 393, SN12C, TK 10, UO 31, A549, EKVX, HOP 62, HOP 92, NCI H226, NCI H23, NCI H460, NCI H522, LOX IMVI, M14, MALME 3M, MDA MB 435, SK , EL 2, SK MEL 28, SK MEL 5, UACC 257, UACC 62, IGROV1, OVCAR 3, OVCAR 4, OVCAR 5, OVCAR 8, SK OV 3, NCI ADR RES, DU145, PC 3, DU145, H295R, HeLa, KBM 7, LNCaP, MCF 7, MDA MB 468, PC3, SaOS 2, SH SY5Y, T 47D, THP 1, U87, vero, MC3T3, GH3, PC12, dog MDCK kidney epithelial, Xenopus A6 kidney epithelial, zebrafish AB9, and Sf9 insect epithelial cell lines.~~

~~112. The method of any one of claims 99 111, further comprising imaging the biological sample.~~

~~113. The method of claim 112, wherein the imaging is used to determine a region of interest in the biological sample.~~

~~114. The method of claim 112, wherein the imaging is used to determine morphology of the biological sample or a region of the biological sample.~~

~~115. The method of claim 114, further comprising correlating the morphology of the biological sample or the region of the biological sample to the location of the analyte in the biological sample.~~

~~116. The method of any one of claims 99 115, wherein the analyte is DNA.~~

- ~~117. The method of any one of claims 99-115, wherein the analyte is RNA.~~
- ~~118. The method of claim 117, wherein the capture domain comprises a poly-dT sequence.~~
- ~~119. The method of any one of claims 99-118, wherein the capture probe further comprises a unique molecular identifier (UMI).~~
- ~~120. The method of any one of claims 99-119, wherein the capture probe further comprises a functional domain.~~
- ~~121. The method of any one of claims 99-120, wherein the capture probe further comprises a cleavage domain.~~
- ~~122. A method for spatial analysis of a nucleic acid in a biological sample, the method comprising:~~
- ~~(a) contacting the biological sample to an array comprising a plurality of capture probes immobilized on the array, wherein a capture probe of the plurality of capture probes comprises a spatial barcode and a capture domain;~~
 - ~~(b) contacting a polymer material to the biological sample, and activating the polymer material to form a hydrogel-embedded biological sample;~~
 - ~~(c) permeabilizing the hydrogel-embedded biological sample under conditions sufficient to allow the nucleic acid in the biological sample to specifically bind to the capture domain;~~
 - ~~(d) amplifying the RNA specifically bound to the capture domain to generate an amplicon having a sequence, wherein the amplicon comprises all or a portion of the spatial barcode or a complement thereof, and all or a portion of the nucleic acid sequence or a complement thereof; and~~

~~(e) determining the sequence of the amplicon by sequential hybridization and detection with a plurality of labelled probes, and using the determined amplicon sequence to identify the location of the nucleic acid in the biological sample.~~

~~123. The method of claim 122, wherein step (d) comprises amplifying the nucleic acid specifically bound to the capture domain *in situ*.~~

~~124. The method of claim 122 or 123, wherein step (e) comprises sequencing the amplicon.~~

~~125. The method of any one of claims 122-124, wherein the plurality of labelled probes is a plurality of fluorescently-labelled probes.~~

~~126. The method of any one of claims 122-125, wherein the permeabilizing in step (c) is performed using chemical permeabilization conditions, enzymatic permeabilization conditions, or both.~~

~~127. The method of any one of claims 122-126, wherein the biological sample comprises a tissue section.~~

~~128. The method of any one of claims 122-127, wherein the nucleic acid is RNA.~~

~~129. The method of any one of claims 122-127, wherein the nucleic acid is DNA.~~

~~130. A nucleic acid comprising:~~

~~(a) a first single-stranded oligonucleotide comprising:~~

~~a spatial barcode;~~

~~a primer binding site;~~

~~a unique molecular identifier; and~~

~~a first hybridization sequence; and~~

~~(b) a splint oligonucleotide comprising:~~

~~a second hybridization sequence that specifically binds to the first hybridization sequence; and
a capture domain.~~

~~131. The nucleic acid of claim 130, wherein the first single-stranded oligonucleotide comprises, in a 5' to a 3' direction:~~

~~the spatial barcode;
the primer binding site;
the unique molecular identifier; and
the first hybridization sequence.~~

~~132. The nucleic acid of claim 131, wherein a 5' end of the first single-stranded oligonucleotide is attached to a substrate.~~

~~133. The nucleic acid of any one of claims 130-132, wherein the splint oligonucleotide comprises, in a 5' to 3' direction the capture domain and the second hybridization sequence.~~

~~134. The nucleic acid of claim 133, wherein the capture domain comprises a sequence that is capable of specifically binding to a sequence present in a single-stranded tagment.~~

~~135. The nucleic acid of claim 134, wherein the sequence present in the single-stranded tagment is a sequence present in a first adaptor.~~

~~136. A composition comprising:~~

~~(a) a nucleic acid of any one of claims 130-133; and
(b) a single-stranded tagment comprising a first adaptor that comprises a sequence that is capable of specifically binding to the capture domain.~~

~~137. The composition of claim 136, wherein the single-stranded tagment comprises the first adaptor sequence at its 5' end.~~

~~138. The composition of claim 136 or 137, wherein the first adaptor sequence further comprises a first adaptor transposon end sequence.~~

~~139. The composition of claim 138, wherein the first adaptor sequence comprises, in a 5' to 3' direction, the sequence that is capable of specifically binding to the capture domain and the first adaptor transposon end sequence.~~

~~140. The composition of any one of claims 136-139, wherein the single-stranded tagment further comprises a second adaptor.~~

~~141. The method of any one of claims 136-138, wherein the single-stranded tagment comprises the second adaptor at its 3' end.~~

~~142. The method of claim 141, wherein the second adaptor sequence comprises a second adaptor transposon end sequence and a primer binding site or a complement thereof.~~

~~143. A composition comprising:~~

~~(a) a nucleic acid of any one of claims 130-133;~~

~~(b) DNA from a biological sample; and~~

~~(c) a transposase dimer comprising:~~

~~(i) — a first transposase monomer complexed with a first adaptor comprising a first transposon end sequence and a sequence that is capable of specifically binding to the capture domain;~~

~~(ii) — a second transposase monomer complexed with a second adaptor comprising a second transposon end sequence and a primer binding site or a complement thereof.~~

~~144. The composition of claim 143, wherein a 5' end of the first adaptor is phosphorylated.~~

~~145. The composition of claim 143, wherein a 5' end of the second adaptor is phosphorylated.~~

~~146. A composition comprising:~~

- ~~(a) a nucleic acid of any one of claims 130-133;~~
- ~~(b) DNA from a biological sample; and~~
- ~~(c) a first transposase monomer complexed with a first adaptor comprising a first transposon end sequence and a sequence that is capable of specifically binding to the capture domain; and~~
- ~~(d) a second transposase monomer complexed with a second adaptor comprising a second transposon end sequence and a primer binding site or a complement thereof.~~

~~147. The composition of claim 146, wherein a 5' end of the first adaptor is phosphorylated.~~

~~148. The composition of claim 146, wherein a 5' end of the second adaptor is phosphorylated.~~