

CLAIMS

WHAT IS CLAIMED IS:

1. A method of analyzing a sample comprising a nucleic acid molecule, comprising:
 - a. providing:
 - (i) a sample comprising said nucleic acid molecule, wherein said nucleic acid molecule comprises a first target region and a second target region, wherein said first target region and said second target region are disposed on a same strand of said nucleic acid molecule;
 - (ii) a first probe comprising a first probe sequence and a second probe sequence, wherein said first probe sequence of said first probe is complementary to said first target region of said nucleic acid molecule; and
 - (iii) a second probe comprising a third probe sequence, wherein said third probe sequence of said second probe is complementary to said second target region of said nucleic acid molecule;
 - b. subjecting said sample to conditions sufficient to (i) hybridize said first probe sequence of said first probe to said first target region of said nucleic acid molecule, and (ii) hybridize said third probe sequence of said second probe to said second target region of said nucleic acid molecule to yield a probe-associated nucleic acid molecule;
 - c. subjecting said probe-associated nucleic acid molecule to conditions sufficient to yield a probe-linked nucleic acid molecule comprising said first probe linked to said second probe; and
 - d. within a partition, attaching a barcode sequence to said probe-linked nucleic acid molecule.
2. The method of claim 1, wherein said partition is a well among a plurality of wells.
3. The method of claim 1, wherein said partition is a droplet among a plurality of droplets.
4. The method of any one of claims 1-3, wherein (d) comprises (i) providing, in said partition, a nucleic acid barcode molecule comprising a binding sequence and a barcode sequence, wherein said binding sequence is complementary to said second probe sequence of said first probe, and (ii) subjecting said partition to conditions sufficient to hybridize said binding sequence to said second probe sequence. The method of claim 4, further comprising subjecting said partition to conditions sufficient to conduct a nucleic acid extension reaction to generate a barcoded nucleic acid molecule comprising a

- sequence corresponding to said first probe, a sequence corresponding to said second probe, and a sequence corresponding to said barcode sequence.
5. The method of claim 4, further comprising subjecting said partition to conditions sufficient to ligate said probe-linked nucleic acid molecule to said nucleic acid barcode molecule to generate a barcoded nucleic acid molecule comprising a sequence corresponding to said first probe, a sequence corresponding to said second probe, and a sequence corresponding to said barcode sequence.
 6. The method of claim 5 or 6, further comprising subjecting said barcoded nucleic acid molecule to conditions sufficient to conduct an amplification reaction to generate an amplification product, which amplification product comprises nucleic acid molecules comprising said sequence corresponding to said first probe, said sequence corresponding to said second probe, and said sequence corresponding to said barcode sequence.
 7. The method of claim 7, wherein said amplification reaction comprises use of a primer comprising one or more functional sequences and wherein said amplification product comprises nucleic acid molecules further comprising said one or more functional sequences.
 8. The method of any one of claims 7 or 8, wherein said amplification is isothermal amplification.
 9. The method of any one of claims 7-9, wherein said amplification reaction is performed within said partition.
 10. The method of claim 10, further comprising recovering said amplification product from said partition.
 11. The method of any one of claims 7-9, wherein said amplification reaction is performed outside of said partition.
 12. The method of any one of claims 7-12, further comprising sequencing said amplification product or a derivative thereof.
 13. The method of any one of claims 1-3, further comprising (i) providing a splint oligonucleotide comprising a first sequence complementary to said second probe sequence and a second sequence, and (ii) subjecting said partition to conditions sufficient to hybridize said first sequence of said splint oligonucleotide to said second probe sequence.
 14. The method of claim 13, wherein said first sequence of said splint oligonucleotide hybridizes to said second probe sequence prior to (c).
 15. The method of claim 13, wherein said first sequence of said splint oligonucleotide hybridizes to said second probe sequence after (c).

16. The method of any one of claims 13-16, wherein (d) comprises (i) providing, in said partition, a nucleic acid barcode molecule comprising a binding sequence and a barcode sequence, wherein said binding sequence is complementary to said second sequence of said splint oligonucleotide, and (ii) subjecting said partition to conditions sufficient to hybridize said binding sequence to said second sequence of said splint oligonucleotide.
17. The method of claim 17, wherein said binding sequence of said nucleic acid barcode molecule comprises one or more ribobases.
18. The method of claim 17 or 18, further comprising subjecting (i) said splint oligonucleotide hybridized to said second probe sequence and (ii) said nucleic acid barcode molecule to conditions sufficient to ligate said probe-linked nucleic acid molecule to said nucleic acid barcode molecule.
19. The method of any one of claims 17-19, further comprising subjecting (i) said splint oligonucleotide hybridized to said second probe sequence and (ii) said nucleic acid barcode molecule to conditions sufficient to conduct a nucleic acid extension reaction to generate a barcoded nucleic acid molecule comprising a sequence corresponding to said first probe, a sequence corresponding to said second probe, and a sequence corresponding to said barcode sequence.
20. The method of any one of claims 19 or 20, further comprising subjecting said barcoded nucleic acid molecule to conditions sufficient to conduct an amplification reaction to generate an amplification product, which amplification product comprises nucleic acid molecules comprising said sequence corresponding to said first probe, said sequence corresponding to said second probe, and said sequence corresponding to said barcode sequence.
21. The method of claim 21, wherein said amplification reaction is a polymerase chain reaction.
22. The method of claim 21 or 22, wherein said amplification reaction is performed within said partition.
23. The method of claim 23, further comprising recovering said amplification product from said partition.
24. The method of claim 21 or 22, wherein said amplification reaction is performed outside of said partition.
25. The method of any one of claims 21-25, wherein said amplification reaction is isothermal amplification.
26. The method of any one of claims 21-26, further comprising sequencing said amplification product or derivative thereof.

27. The method of any one of claims 4-27, wherein said nucleic acid barcode molecule further comprises a unique molecular identifier sequence, a sequencing primer sequence, and/or a partial sequencing primer sequence.
28. The method of any one of claims 4-28, wherein, subsequent to (c), said probe-associated nucleic acid molecule is co-partitioned with said nucleic acid barcode molecule.
29. The method of any one of claims 4-28, wherein, subsequent to (a), said nucleic acid molecule is co-partitioned with said first probe, said second probe, and said nucleic acid barcode molecule.
30. The method of claim 30, wherein (c) is performed within said partition.
31. The method of claim 31, wherein (b) and (c) are performed within said partition.
32. The method of any one of claims 4-32, wherein said second probe comprises a fourth probe sequence, and wherein said method further comprises providing a nucleic acid binding molecule in said partition, wherein said nucleic acid binding molecule comprises a second binding sequence that is complementary to said fourth probe sequence of said second probe.
33. The method of claim 33, further comprising hybridizing said second binding sequence to said fourth probe sequence of said second probe within said partition.
34. The method of any one of claims 4-34, wherein said nucleic acid barcode molecule is coupled to a bead.
35. The method of claim 35, wherein said bead is a gel bead.
36. The method of claim 35 or 36, wherein said nucleic acid barcode molecule is coupled to said bead via a labile moiety.
37. The method of any one of claims 35-37, wherein said bead comprises a plurality of nucleic acid barcode molecules coupled thereto, wherein said plurality of nucleic acid barcode molecules comprise said nucleic acid barcode molecule.
38. The method of claim 38, wherein said bead comprises at least 100,000 nucleic acid barcode molecules coupled thereto.
39. The method of claim 38 or 39, wherein said plurality of nucleic acid barcode molecules are releasably coupled to said bead.
40. The method of claim 40, wherein said plurality of nucleic acid barcode molecules are releasable from said bead upon application of a stimulus.
41. The method of claim 41, wherein said stimulus is selected from the group consisting of a thermal stimulus, a photo stimulus, a biological stimulus, and a chemical stimulus.
42. The method of claim 42, wherein said stimulus is a reducing agent.

43. The method of any one of claims 41-43, wherein the application of said stimulus results in one or more of (i) cleavage of a linkage between nucleic acid barcode molecules of said plurality of nucleic acid barcode molecules and said bead, and (ii) degradation of said bead to release nucleic acid barcode molecules of said plurality of nucleic acid barcode molecules from said bead.
44. The method of any one of claims 35-44, wherein said bead is provided in said partition, and wherein said nucleic acid barcode molecule is released from said bead within said partition.
45. The method of any one of claims 1-45, wherein (c) is performed before (d).
46. The method of any one of claims 1-45, wherein (d) is performed before (c).
47. The method of any one of claims 1-47, wherein said first probe or said second probe further comprises a barcode sequence or unique molecular identifier.
48. The method of any one of claims 1-48, wherein said second probe comprises a fourth probe sequence, which fourth probe sequence hybridizes to a third target region of said nucleic acid molecule.
49. The method of claim 49, wherein said second target region is not adjacent to said third target region, and wherein said third probe sequence and said fourth probe sequence of said second probe are separated by a linker sequence.
50. The method of any one of claims 1-50, wherein said first probe sequence of said first probe comprises a first reactive moiety and said third probe sequence of said second probe comprises a second reactive moiety, wherein, subsequent to (b), said first reactive moiety is adjacent to said second reactive moiety.
51. The method of claim 51, wherein (c) comprises subjecting said first reactive moiety and said second reactive moiety to conditions sufficient to link said first probe sequence to said third probe sequence.
52. The method of claim 51 or 52, wherein said first reactive moiety of said first probe or said second reactive moiety of said second probe comprises an azide moiety, an alkyne moiety, a phosphorothioate moiety, an iodide moiety, an amine moiety, or a phosphate moiety.
53. The method of any one of claims 51-53, wherein said first probe is linked to said second probe in said probe-linked nucleic acid molecule via a linker, wherein said linker comprises a triazole moiety, a phosphorothioate bond, or a phosphoroamidatephosphoramidate bond.
54. The method of any one of claims 1-50, wherein (c) comprises performing an enzymatic ligation reaction and/or an extension reaction.

55. The method of claim 55, wherein said enzymatic ligation reaction and/or said extension reaction comprises use of an enzyme selected from the group consisting of T4 RNL2, SplintR, T4 DNA ligase, PBCV1, DNA polymerase, and Mu polymerase, or a derivative thereof.
56. The method of any one of claims 1-56, wherein, prior to (a), said first probe is linked to said second probe via one or more linking sequences.
57. The method of claim 57, wherein said one or more linking sequences comprise one or more of a spacer sequence, a sequencing primer or complement thereof, a capture sequence, a restriction site, a transposition site, and a unique molecular identifier sequence.
58. The method of claim 57 or 58, wherein said one or more linking sequences comprise a thermolabile, photocleavable, or enzymatically cleavable site.
59. The method of any one of claims 1-59, wherein said first target region is adjacent to said second target region.
60. The method of any one of claims 1-59, wherein said first target region and said second target region are separated by a gap region disposed between said first target region and said second target region.
61. The method of claim 61, wherein said gap region is at least one nucleotide long.
62. The method of claim 62, wherein said gap region is at least 10 nucleotides long.
63. The method of claim 63, wherein said gap region is at least 100 nucleotides long.
64. The method of any one of claims 1-64, further comprising digesting one or more nucleic acid molecules or portions thereof using an exonuclease.
65. The method of any one of claims 1-65, wherein said first probe or said second probe comprises a known sequence or a degenerate sequence.
66. The method of any one of claims 1-66, wherein said first probe or said second probe comprises a Phi-29 based rolling circle amplification sequence.
67. The method of any one of claims 1-67, wherein said first probe or said second probe comprises a cleavable site, wherein said cleavable site is cleavable using a thermal, photo-, chemical, or biological stimulus.
68. The method of any one of claims 1-68, further comprising contacting said first probe or said second probe with a transposase.
69. The method of any one of claims 1-69, wherein said sample comprises a cell, and wherein said nucleic acid molecule is contained within said cell.
70. The method of claim 70, further comprising, subsequent to (a), lysing or permeabilizing said cell, thereby providing access to said nucleic acid molecule.

71. The method of claim 70 or 71, wherein said cell is a prokaryotic cell.
72. The method of claim 70 or 71, wherein said cell is a eukaryotic cell.
73. The method of claim 70 or 71, wherein said cell is a human cell.
74. The method of any one of claims 70-74, wherein said cell is a fixed suspension cell or a formalin-fixed paraffin-embedded cell.
75. The method of any one of claims 70-75, wherein said cell is provided within said partition.
76. The method of claim 76, wherein said cell is a single cell.
77. The method of any one of claims 1-77, wherein said nucleic acid molecule is a ribonucleic acid (RNA) molecule.
78. The method of claim 78, wherein said nucleic acid molecule is a messenger RNA (mRNA) molecule.
79. The method of claim 78 or 79, wherein said nucleic acid molecule comprises a poly-A sequence at a terminus of said nucleic acid molecule.
80. The method of any one of claims 1-77, wherein said nucleic acid molecule is a deoxyribonucleic acid (DNA) molecule.
81. The method of any one of claims 1-81, wherein said partition further comprises one or more reagents selected from the group consisting of fluorophores, oligonucleotides, primers, nucleic acid barcode molecules, barcodes, buffers, deoxynucleotide triphosphates, DNA splints, detergents, reducing agents, chelating agents, oxidizing agents, nanoparticles, antibodies, temperature-sensitive enzymes, pH-sensitive enzymes, light-sensitive enzymes, proteases, ligases, polymerases, reverse transcriptases, restriction enzymes, nucleases, protease inhibitors, and nuclease inhibitors.
82. The method of claim 82, wherein said polymerase is a polymerase selected from the group of DNA polymerase, RNA polymerase, Hot Start polymerase, and Warm start polymerase
83. The method of any one of claims 1-83, wherein said sample comprises a cell bead, and wherein said nucleic acid molecule is contained within said cell bead.
84. The method of any one of claims 1-84, wherein (a)-(c) are performed without reverse transcription.
85. A method of analyzing a sample comprising a nucleic acid molecule, comprising:
 - a. providing:
 - (i) a sample comprising said nucleic acid molecule, wherein said nucleic acid molecule comprises a target region;

- (ii) a probe comprising a probe sequence and a binding sequence, wherein said probe sequence is complementary to said target region; and
 - (iii) an adapter comprising a first sequence and a second sequence, wherein said first sequence of said adapter is complementary to said binding sequence of said probe;
 - b. subjecting said sample to conditions sufficient to hybridize (i) said probe sequence of said probe to said target region, and (ii) said binding sequence of said probe to said first sequence of said adapter, to yield an adapter-bound probe; and
 - c. within a partition, barcoding said adapter-bound probe to provide a barcoded nucleic acid molecule.
- 86. A method of analyzing a sample comprising a nucleic acid molecule, comprising:
 - a. providing:
 - (i) a sample comprising said nucleic acid molecule, wherein said nucleic acid molecule comprises a target region;
 - (ii) a probe comprising a probe sequence and a first reactive moiety, wherein said probe sequence is complementary to said target region; and
 - (iii) a nucleic acid barcode molecule comprising a second reactive moiety and a barcode sequence;
 - b. subjecting said sample to conditions sufficient to hybridize said probe sequence of said probe to said target region to provide a probe-associated nucleic acid molecule; and
 - c. within a partition, subjecting said first reactive moiety of said probe-associated nucleic acid molecule and said second reactive moiety of said nucleic acid barcode molecule to conditions sufficient to link said probe-associated nucleic acid molecule and said nucleic acid barcode molecule to provide a barcoded nucleic acid product.