

CLAIMS

WHAT IS CLAIMED IS:

1. ~~—A method for processing a plurality of nucleic acid molecules derived from a cell free biological sample, comprising (a) bringing said plurality of nucleic acid molecules or derivatives thereof in contact with a plurality of binding agents, to provide a first subset of said plurality of nucleic acid molecules coupled to said plurality of binding agents and a second subset of said plurality of nucleic acid molecules; (b) separating said first subset of said plurality of nucleic acid molecules coupled to said plurality of binding agents from said second subset of said plurality of nucleic acid molecules; (c) subsequent to (b), circularizing a nucleic acid molecule derived from said first subset of said plurality of nucleic acid molecules to obtain a circularized nucleic acid molecule; and (d) identifying said circularized nucleic acid molecule or derivative thereof.~~
2. ~~—The method of claim 1, wherein said plurality of nucleic acid molecules comprise a deoxyribonucleic acid (DNA) molecule or a ribonucleic acid (RNA) molecule.~~
3. ~~—The method of claim 1 or claim 2, wherein circularizing comprises ligating a 5' end and a 3' end of said nucleic acid molecule to one another.~~
4. ~~—The method of any one of claims 1 to 3, wherein circularizing comprises coupling an adapter to a 3' end, a 5' end, or both a 5' end and a 3' end of said nucleic acid molecule.~~
5. ~~—The method of any one of claims 1 to 4, further comprising subjecting said circularized nucleic acid molecule to nucleic acid amplification to generate a plurality of amplification products of said circularized nucleic acid molecule, wherein (d) comprises identifying said plurality of nucleic acid amplification products.~~
6. ~~—The method of claim 5, wherein said nucleic acid amplification is effected by a polymerase having strand displacement activity.~~
7. ~~—The method of claim 5, wherein said nucleic acid amplification is effected by a polymerase that does not have strand displacement activity.~~
8. ~~—The method of claim 5, wherein said nucleic acid amplification comprises contacting said circularized nucleic acid molecule to an amplification reaction mixture comprising random primers.~~
9. ~~—The method of claim 5, wherein said nucleic acid amplification comprises contacting said circularized nucleic acid molecule to an amplification reaction mixture comprising one or more primers, each of which specifically hybridizes to a different target sequence via sequence complementarity.~~
10. ~~—The method of any one of claims 1 to 9, wherein said binding agent comprises an antibody, or fragment thereof.~~
11. ~~—The method of claim 10, wherein said antibody, or fragment thereof specifically binds to a nucleic acid binding protein.~~

- ~~12. — The method of claim 10, wherein said nucleic acid binding protein is a chromatin protein.~~
- ~~13. — The method of claim 10, wherein said nucleic acid binding protein is a histone.~~
- ~~14. — The method of claim 10, wherein said nucleic acid binding protein is a methyl CpG binding protein.~~
- ~~15. — The method of claim 10, wherein said nucleic acid binding protein is a transcription factor.~~
- ~~16. — The method of claim 10, wherein said nucleic acid binding protein is an RNA binding protein.~~
- ~~17. — The method of any one of claims 1 to 9, wherein said antibody, or fragment thereof specifically binds to a nucleic acid sequence.~~
- ~~18. — The method of claim 17, wherein said nucleic acid sequence is methylated.~~
- ~~19. — The method of any one of claims 1 to 9, wherein said binding agent comprises a polypeptide or a nucleic acid.~~
- ~~20. — The method of claim 19, wherein said polypeptide comprises streptavidin.~~
- ~~21. — The method of any one of claims 1 to 18, further comprising determining a size of each cell-free nucleic acid molecule of said plurality of cell-free nucleic acid molecules.~~
- ~~22. — The method of any one of claims 1 to 19, wherein (d) comprises sequencing said circularized nucleic acid molecule or derivative thereof.~~
- ~~23. — The method of claim 22, wherein said sequencing comprises a method selected from one or more of sequencing by synthesis, sequencing by ligation, nanopore sequencing, nanoball sequencing, ion detection, sequencing by hybridization, polymerized colony (POLONY) sequencing, nanogrid-rolling circle sequencing (ROLONY), and ion-torrent sequencing.~~
- ~~24. — The method of any one of claims 1 to 23, wherein said cell-free biological sample comprises less than 75 nanograms of nucleic acids.~~
- ~~25. — The method of any one of claims 1 to 24, wherein said cell-free biological sample comprises a bodily fluid.~~
- ~~26. — The method of claim 25, wherein said bodily fluid is urine, saliva, blood, serum, plasma, tears, sputum, cerebrospinal fluid, synovial fluid, mucus, bile, semen, lymph, amniotic fluid, menstrual fluid, or combinations thereof.~~
- ~~27. — The method of any one of claims 1 to 26, wherein (d) comprises sequencing said circularized nucleic acid molecule or derivative thereof.~~
- ~~28. — The method of claim 27, further comprising processing said sequence against a plurality of reference sequences to identify said sequence as corresponding to at least a subset of said plurality of reference sequences, thereby determining that a subject has or is at risk of having a disease.~~
- ~~29. — The method of claim 28, wherein said disease is cancer.~~

~~30. — The method of claim 29, wherein said cancer is selected from the group consisting of colon cancer, non-small-cell lung cancer, small-cell lung cancer, breast cancer, hepatocellular carcinoma, liver cancer, skin cancer, malignant melanoma, endometrial cancer, esophageal cancer, gastric cancer, ovarian cancer, pancreatic cancer, brain cancer, leukemia, lymphoma, and myeloma.~~

~~31. — The method of claim 28, further comprising using said sequence identified to output an electronic report indicating that said subject has or is at risk of having a disease.~~

~~32. — The method of claim 28, further comprising using said sequence identified to provide a therapeutic intervention to said subject for a disease.~~

~~33. — The method of claim 28, further comprising using said sequence identified to treat said subject for said disease.~~

~~34. — The method of claim 32 or claim 33, wherein said subject is treated by administering a chemotherapy or immunotherapy to said subject.~~

~~35. — The method of claim 28, further comprising using said sequence identified to monitor said subject for a progression or regression of said subject.~~

~~36. — The method of claim 1, wherein in (c) said nucleic acid molecule is coupled to a binding agent of said plurality of binding agents.~~

37.1. A method for processing a plurality of nucleic acid molecules derived from a cell-free biological sample, comprising (a) determining a methylation state for a nucleic acid molecule of said plurality of nucleic acid molecules; (b) determining a size for said nucleic acid molecule of said plurality of nucleic acid molecules; and (c) processing (i) said methylation state for said nucleic acid molecule of said plurality of nucleic acid molecules against a first database, and (ii) said size for said nucleic acid molecule of said plurality of nucleic acid molecules against a second database, to identify an association of said methylation state and of said size with at least a disease.

38.2. The method of claim 37.1, wherein determining said methylation state comprises sequencing said nucleic acid molecule of said plurality of nucleic acid molecules.

~~39. — The method of claim 38, wherein said sequencing comprises a method selected from one or more of sequencing by synthesis, sequencing by ligation, nanopore sequencing, nanoball sequencing, ion detection, sequencing by hybridization, polymerized colony (POLONY) sequencing, nanogrid-rolling circle sequencing (ROLONY), and ion-torrent sequencing.~~

40.3. The method of claim 37.1, wherein determining said methylation state comprises contacting said nucleic acid molecule to a binding agent that binds specifically to methylated nucleic acids or a derivative thereof.

41.4. The method of any one of claims 37.1 to 40.3, wherein determining said methylation state comprises (a) bringing said plurality of nucleic acid molecules in contact with a plurality of binding

agents, to provide a first subset of said plurality of nucleic acid molecules coupled to said plurality of binding agents and a second subset of said plurality of nucleic acid molecules; (b) separating said first subset of said plurality of nucleic acid molecules coupled to said plurality of binding agents from said second subset of said plurality of nucleic acid molecules; (c) subsequent to (b), circularizing a nucleic acid molecule derived from said first subset of said plurality of nucleic acid molecules to obtain a circularized nucleic acid molecule; and (d) identifying said circularized nucleic acid molecule or derivative thereof.

~~42.5.~~ The method of any one of claims ~~37-1~~ to ~~414~~, wherein said binding agent comprises an antibody, or fragment thereof.

~~43.6.~~ The method of any one of claims ~~37-1~~ to ~~414~~, wherein said binding agent comprises a polypeptide or a nucleic acid.

~~44.7.~~ The method of claim ~~436~~, wherein said polypeptide comprises streptavidin.

~~45.8.~~ The method of any one of claims ~~37-1~~ to ~~447~~, wherein said plurality of nucleic acid molecules comprise a deoxyribonucleic acid (DNA) molecule or a ribonucleic acid (RNA) molecule.

~~46.9.~~ The method of claim ~~41-4~~ or claim ~~458~~, wherein circularizing comprises ligating a 5' end and a 3' end of said nucleic acid molecule to one another.

~~47.10.~~ The method of any one of claims ~~41-4~~ to ~~469~~, wherein circularizing comprises coupling an adapter to a 3' end, a 5' end, or both a 5' end and a 3' end of said nucleic acid molecule.

~~48.11.~~ The method of any one of claims ~~41-4~~ to ~~4710~~, further comprising subjecting said circularized nucleic acid molecule to nucleic acid amplification to generate a plurality of amplification products of said circularized nucleic acid molecule, wherein (d) comprises identifying said plurality of nucleic acid amplification products.

~~49. The method of claim 48, wherein said nucleic acid amplification is effected by a polymerase having strand displacement activity.~~

~~50. The method of claim 48, wherein said nucleic acid amplification is effected by a polymerase that does not have strand displacement activity.~~

~~51.12.~~ The method of claim ~~4811~~, wherein said nucleic acid amplification comprises contacting said circularized nucleic acid molecule to an amplification reaction mixture comprising random primers.

~~52.13.~~ The method of claim ~~4811~~, wherein said nucleic acid amplification comprises contacting said circularized nucleic acid molecule to an amplification reaction mixture comprising one or more primers, each of which specifically hybridizes to a different target sequence via sequence complementarity.

~~53. The method of any one of claims 41 to 52, wherein (d) comprises sequencing said circularized nucleic acid molecule or derivative thereof.~~

~~54. — The method of claim 53, wherein said sequencing comprises a method selected from one or more of sequencing by synthesis, sequencing by ligation, nanopore sequencing, nanoball sequencing, ion detection, sequencing by hybridization, polymerized colony (POLONY) sequencing, nanogrid-rolling circle sequencing (ROLONY), and ion-torrent sequencing.~~

~~55. — The method of any one of claims 37 to 54, wherein said cell-free biological sample comprises less than 75 nanograms of nucleic acids.~~

~~56.14.~~ The method of any one of claims ~~37.1~~ to ~~55.13~~, wherein said cell-free biological sample comprises a bodily fluid.

~~57. — The method of claim 56, wherein said bodily fluid is urine, saliva, blood, serum, plasma, tears, sputum, cerebrospinal fluid, synovial fluid, mucus, bile, semen, lymph, amniotic fluid, menstrual fluid, or combinations thereof.~~

~~58.15.~~ The method of any one of claims ~~37.1~~ to ~~57.14~~, further comprising processing said methylation state and against a plurality of reference methylation states and processing said size against a plurality of reference sizes to identify said methylation state as corresponding to at least a subset of said plurality of reference methylation states and said size as corresponding to at least a subset of said reference sizes, thereby determining that a subject has or is at risk of having a disease.

~~59.16.~~ The method of claim ~~58.15~~, wherein said disease is cancer.

~~60. — The method of claim 59, wherein said cancer is selected from the group consisting of colon cancer, non-small-cell lung cancer, small-cell lung cancer, breast cancer, hepatocellular carcinoma, liver cancer, skin cancer, malignant melanoma, endometrial cancer, esophageal cancer, gastric cancer, ovarian cancer, pancreatic cancer, brain cancer, leukemia, lymphoma, and myeloma.~~

~~61.17.~~ The method of claim ~~58.15~~, further comprising using said methylation state and said size identified to output an electronic report indicating that said subject has or is at risk of having a disease.

~~62.18.~~ The method of claim ~~58.15~~, further comprising using said methylation state and said size identified to provide a therapeutic intervention to said subject for a disease.

~~63. — The method of claim 58, further comprising using said methylation state and said size identified to treat said subject for said disease.~~

~~64. — The method of claim 62 or claim 63, wherein said subject is treated by administering a chemotherapy or immunotherapy to said subject.~~

~~65. — The method of claim 58, further comprising using said methylation state and said size identified to monitor said subject for a progression or regression of said subject.~~

~~66.19.~~ A method for processing a plurality of nucleic acid molecules derived from a cell-free biological sample of a subject, comprising (a) bringing said plurality of nucleic acid molecules or derivatives thereof in contact with a plurality of binding agents, to provide a first subset of said plurality of nucleic acid

molecules coupled to said plurality of binding agents and a second subset of said plurality of nucleic acid molecules; (b) separating said first subset of said plurality of nucleic acid molecules coupled to said plurality of binding agents from said second subset of said plurality of nucleic acid molecules; (c) subsequent to (b) circularizing nucleic acid molecules derived from said first subset of said plurality of nucleic acid molecules to obtain a first subset of circularized nucleic acid molecules; (d) subsequent to (b) circularizing nucleic acid molecules derived from said second subset of said plurality of nucleic acid molecules to obtain a second subset of circularized nucleic acid molecules; (e) sequencing said first subset of circularized nucleic acid molecules or derivatives thereof to obtain a first size and said second subset of circularized nucleic acid molecules or derivatives thereof to obtain a second size; (f) compare said first size with said second size to determine a disease level of said subject.

67.20. A method for processing a plurality of nucleic acid molecules derived from a cell-free biological sample of a subject, comprising (a) bringing said plurality of nucleic acid molecules or derivatives thereof in contact with a plurality of binding agents, to provide a first subset of said plurality of nucleic acid molecules coupled to said plurality of binding agents and a second subset of said plurality of nucleic acid molecules; (b) separating said first subset of said plurality of nucleic acid molecules coupled to said plurality of binding agents from said second subset of said plurality of nucleic acid molecules; (c) subsequent to (b) circularizing nucleic acid molecules derived from said first subset of said plurality of nucleic acid molecules to obtain a first subset of circularized nucleic acid molecules; (d) subsequent to (b) circularizing nucleic acid molecules derived from said second subset of said plurality of nucleic acid molecules to obtain a second subset of circularized nucleic acid molecules; (e) sequencing said first subset of circularized nucleic acid molecules or derivatives thereof to obtain a first end sequence and said second subset of circularized nucleic acid molecules or derivatives thereof to obtain a second end sequence; (f) compare said first end sequence with said second end sequence to determine a disease level of said subject.