CLAIMS

1. A method of analyzing a biological sample, comprising:

binding a plurality of detection probes directly or indirectly to a DNA concatemer in the biological sample, wherein a detection probe of the plurality of detection probes comprises a sequence of nucleobases on a synthetic backbone, wherein the detection probe is a morpholino and wherein the detection probe directly or indirectly binds to a target sequence in the DNA concatemer~~,~~; and

detecting signals from said plurality of bound detection probes, where the signal generated is greater than a plurality of reference detection probes having the same sequence of nucleobases on a sugar-phosphate backbone.

2. The method of claim 1, wherein the detection probe is less electrostatically negative than the reference detection probe.

3. The method of claim 1 or 2, wherein the detection probe is directly or indirectly coupled to a detectable label, optionally a fluorescent label.

4. The method of any one of claims 1-3, wherein the detection probe directly hybridizes to the target sequence.

~~5. The method of any one of claims 1-3, wherein the detection probe hybridizes to an intermediate probe which directly or indirectly binds to the target sequence.~~

~~6~~5. The method of ~~claim 5~~any one of claims1-3, wherein the detection probe directly hybridizes to an intermediate probe that directly hybridizes to the target sequence.

~~7. The method of any one of claims 1-6, wherein the detection probe comprises a single-stranded region and optionally a double-stranded region.~~

~~8~~6. The method of any one of claims 1-~~7~~5, wherein the sequence of nucleobases on the synthetic backbone is a first probe sequence, and the detection probe further comprises a second probe sequence comprising nucleobases on a sugar-phosphate backbone.

~~9. The method of claim 8, wherein the first probe sequence is 5' or 3' to the second probe sequence.~~

~~10. The method of claim 8 or 9, wherein the detection probe comprises one or more first probe sequences and/or one or more second probe sequences.~~

~~11. The method of any one of claims 8-10, wherein the second probe sequence is a DNA sequence, an RNA sequence, or a combination thereof.~~

~~12. The method of any one of claims 1-11, wherein the detection probe comprises nucleobases A, T, C, G, or U, or variants thereof, in any suitable order and combination.~~

~~13. The method of any one of claims 1-12, wherein the detection probe is between about 5 and about 50 nucleobases in length.~~

~~14. The method of any one of claims 1-13, wherein the detection probe comprises phosphorodiamidate linkages in the synthetic backbone.~~

~~15. The method of any one of claims 1-14, wherein the detection probe comprises morpholine rings in the synthetic backbone.~~

~~16. The method of any one of claims 1-15, wherein the detection probe is a morpholino.~~

~~17. The method of any one of claims 1-16, wherein the detection probe is uncharged or positively charged.~~

~~18~~7. The method of any one of claims 1-~~17~~6, wherein the DNA concatemer comprises one or more barcode sequences~~, optionally wherein the target sequence comprises a barcode sequence~~ corresponding to an analyte in the biological sample.

~~19. The method of any one of claims 1-18, wherein the DNA concatemer is an extension product, a replication product, a reverse transcription product, and/or an amplification product of an endogenous nucleic acid molecule in the biological sample.~~

~~20. The method of any one of claims 1-18, wherein the DNA concatemer is an extension product, a replication product, a reverse transcription product, and/or an amplification product of a labelling agent that directly or indirectly binds to an analyte in the biological sample.~~

~~21. The method of claim 20, wherein the labelling agent comprises a reporter oligonucleotide, optionally wherein the reporter oligonucleotide comprises one or more~~

~~barcode sequences and the DNA concatemer comprises one or a plurality of copies of the one or more barcode sequences.~~

~~22~~8. The method of any one of claims 1-~~21~~7, wherein the DNA concatemer is a rolling circle amplification (RCA) product of a circular or circularizable probe or probe set that hybridizes to a DNA or RNA molecule in the biological sample.

~~23~~9. The method of claim ~~22~~8, wherein DNA concatemers generated from a plurality of different mRNA and/or cDNA molecules are analyzed, a barcode sequence in a particular circular or circularizable probe or probe set uniquely corresponds to a particular mRNA or cDNA molecule, and the particular circular or circularizable probe or probe set further comprises an anchor sequence that is common among circular or circularizable probes or probe sets for a subset of the plurality of different mRNA and/or cDNA molecules.

~~24. The method of any of claims 20-23, wherein the labelling agent comprises a binding moiety that directly or indirectly binds to a non-nucleic acid analyte in the biological sample, and the reporter oligonucleotide in the labelling agent identifies the binding moiety and/or the non-nucleic acid analyte.~~

~~25. The method of claim 24, wherein the binding moiety of the labelling agent comprises an antibody or antigen binding fragment thereof that directly or indirectly binds to a protein analyte, and the DNA concatemer is a rolling circle amplification (RCA) product of a circular or circularizable probe or probe set that hybridizes to a reporter oligonucleotide of the labelling agent.~~

~~26. The method of any one of claims 1-25, wherein the DNA concatemer is in the form of a nanoball having a diameter of between about 0.1 µm and about 3 µm, e.g., between about 0.1 µm and about 0.5 µm, between about 0.5 µm and about 1 µm, between about 0.8 µm and about 1.3 µm, or between about 1 µm and about 1.5 µm.~~

~~27. The method of any one of claims 1-26, wherein the DNA concatemer is between about 1 and about 15 kilobases, between about 15 and about 25 kilobases, between about 25 and about 35 kilobases, between about 35 and about 45 kilobases, between about 45 and about 55 kilobases, between about 55 and about 65 kilobases, between about 65 and about 75 kilobases, or more than 75 kilobases in length.~~

~~28. The method of any one of claims 1-27, wherein the DNA concatemer comprises between about 10 and about 100, between about 100 and about 1,000, between about 1,000 and about 5,000, between about 5,000 and about 10,000, or more than 10,000 copies of the target sequence.~~

~~29. The method of claim 28, wherein at least about 10%, at least about 25%, at least about 50%, at least about 75%, or at least about 90% of the copies of the target sequence in the DNA concatemer are bound directly or indirectly to one or more molecules of the plurality of detection probes.~~

~~30~~10. The method of any one of claims 1-~~29~~19, wherein the DNA concatemer is generated *in situ*.

~~31. The method of any one of claim 1-30, wherein the DNA concatemer is immobilized in the biological sample.~~

~~32. The method of any one of claim 1-31, wherein the DNA concatemer is crosslinked to one or more other molecules in the biological sample.~~

~~33~~11. The method of any one of claim~~s~~ 1-~~32~~10, further comprising imaging the biological sample to detect ~~wherein~~ a complex comprising multiple molecules of the detection probe ~~is detected~~ *in situ* in the biological sample.

~~34. The method of claim 33, wherein the method comprises imaging the biological sample to detect the complex, optionally using fluorescent microscopy.~~

~~35. The method of any one of claim 1-34, wherein a sequence of the DNA concatemer molecule is analyzed *in situ* in the biological sample.~~

~~36. The method of any one of claim 1-35, wherein the detection probe is for analyzing a sequence of the DNA concatemer by sequential hybridization, sequencing by hybridization, sequencing by ligation, sequencing by synthesis, sequencing by binding, or a combination thereof.~~

~~37. The method of claim 35 or 36, wherein the sequence to be analyzed comprises a barcode sequence corresponding to an analyte or a portion thereof in the biological sample.~~

~~38. The method of any one of claims 1-37, further comprising removing one or more molecules of the detection probe that have not bound to the target sequence.~~

~~39. The method of any one of claims 1-33, wherein the detection probe of the plurality of detection probes is a first detection probe and the method further comprises removing molecules of the first detection probe bound to the target sequence, prior to contacting the biological sample with a plurality of second detection probes comprising a sequence of nucleobases on a synthetic backbone.~~

~~40. The method of claim 39, wherein the target sequence is a first target sequence, and a second detection probe of the plurality of second detection probes directly or indirectly binds to a second target sequence in the DNA concatemer, wherein the first and second target sequences are the same or different.~~

~~41~~12. The method of any one of claims 1-~~33~~11, wherein the detection probe is a first detection probe and hybridizes to a first intermediate probe which hybridizes to the target sequence, optionally wherein the first intermediate probe comprises a sequence of nucleobases on a synthetic backbone.

~~42. The method of claim 41, wherein the method further comprises removing molecules of the first detection probe and/or the first intermediate probe bound to the target sequence.~~

~~43~~13. The method of claim ~~41 or 42~~12, ~~wherein the method~~ further ~~comprises~~ comprising contacting the biological sample with a plurality of second detection probes comprising a sequence of nucleobases on a synthetic backbone and a second intermediate probe, wherein the plurality of second detection probes hybridize to the second intermediate probe which hybridizes to the target sequence, optionally wherein the first intermediate probe comprises a sequence of nucleobases on a synthetic backbone.

~~44. The method of any one of claims 1-43, wherein the biological sample is a processed or cleared biological sample.~~

~~45~~15. The method of any one of claims 1-~~44~~13, wherein the biological sample is a tissue sample.

~~46. The method of claim 45, wherein the tissue sample is a tissue slice between about 1 μm and about 50 μm in thickness, optionally wherein the tissue slice is between about 5 μm and about 35 μm in thickness.~~

~~47. The method of claim 45 or 46, wherein the tissue sample is embedded in a hydrogel.~~

~~48. The method of claim 47, wherein the analyte or the DNA concatemer is crosslinked to the hydrogel.~~

~~49. The method of claim 48, wherein the analyte or the DNA concatemer comprises one or more functional groups for attachment to the hydrogel.~~

~~50. The method of claim 48 or 49, wherein the analytes comprise biomolecules and/or products thereof from a tissue sample.~~

~~51~~15. A method of analyzing a biological sample, comprising:

(a) contacting a biological sample with a plurality of fluorescently labeled detection probes, wherein:

the biological sample comprises a rolling circle amplification (RCA) product immobilized therein, wherein the RCA product comprises multiple copies of a target sequence,

a fluorescently labeled detection probe of the plurality of fluorescently labeled detection probes comprises a sequence of nucleobases on a synthetic backbone which hybridizes to the target sequence or an intermediate probe that hybridizes to the target sequence, wherein the fluorescently labeled detection probe and/or the intermediate probe are morpholino;

(b) optionally removing molecules of the fluorescently labeled detection probe and/or the intermediate probe that have not hybridized to the RCA product; and

(c) detecting molecules of the fluorescently labeled detection probe bound to the RCA product in the biological sample,

wherein the signals generated by the fluorescently labeled detection probes hybridized to the RCA product is greater than a plurality of reference detection probes having the same fluorescent label and sequence of nucleobases on a sugar-phosphate backbone.

~~52. The method of claim 51, further comprising prior to step (a):~~

~~contacting the biological sample with a circular or circularizable probe or probe set that directly or indirectly binds to an analyte in the biological sample, and~~

~~generating the RCA product using the circular or circularizable probe or probe set as template.~~

~~53. The method of claim 51 or 52, wherein the fluorescently labeled detection probe is uncharged or less electrostatically negative than a reference detection probe having the same fluorescent label and sequence of nucleobases on a sugar-phosphate backbone.~~

~~54. The method of any one of claims 52-53, wherein the circular or circularizable probe or probe set hybridizes to a nucleic acid sequence of the analyte.~~

~~55. The method of any one of claims 52-53, wherein the circular or circularizable probe or probe set hybridizes to a reporter oligonucleotide of a labelling agent, wherein the labelling agent further comprises a binding moiety that directly or indirectly binds to the analyte, wherein the reporter oligonucleotide corresponds to the binding moiety and/or the analyte.~~

~~56. The method of any one of claims 51-55, wherein the intermediate probe comprises a sequence of nucleobases on a synthetic backbone which hybridizes to the target sequence, optionally wherein there are more molecules of the intermediate probe hybridized in the interior of the RCA product than a reference intermediate probe having the same sequence of nucleobases on a sugar-phosphate backbone.~~

~~57. The method of any one of claims 51-56, wherein the fluorescently labeled detection probe and/or the intermediate probe are morpholino.~~

~~58. The method of any one of claims 51-57, wherein the RCA product has a diameter of less than about 1.5 μm.~~

~~59. The method of any one of claims 51-58, wherein in the contacting step, the number of molecules of the fluorescently labeled detection probe is no more than 5, no more than 4, no more than 3, no more than 2, or no more than 1.5 times the number of copies of the target sequence in the RCA product.~~

~~60. The method of any one of claims 51-59, wherein at least about 10%, at least about 25%, at least about 50%, at least about 75%, or at least about 90% of the copies of the target~~

~~sequence in the RCA product are bound directly or indirectly to one or more molecules of the intermediate probe and/or the fluorescently labeled detection probe.~~

~~61. The method of any one of claims 51-60, wherein the biological sample is contacted with multiple molecules of the reference detection probe, and more molecules of the fluorescently labeled detection probe than the reference detection probe bind to the RCA product in the biological sample.~~

~~62. The method of any one of claims 51-61, further comprising:~~

~~removing molecules of the fluorescently labeled detection probe and the intermediate probe bound to the RCA product in the biological sample.~~

~~63. The method of any one of claims 51-62, wherein the fluorescently labeled detection probe is a first fluorescently labeled detection probe and the intermediate probe is a first intermediate probe, and the method further comprises contacting the biological sample with a second fluorescently labeled detection probe, wherein:~~

~~the second fluorescently labeled detection probe comprises a sequence of nucleobases on a synthetic backbone which hybridizes to a second intermediate probe that hybridizes to the target sequence, and~~

~~molecules of the second fluorescently labeled detection probe bound to the RCA product in the biological sample are detected.~~

~~64. The method of claim 63, wherein the second intermediate probe comprises a sequence of nucleobases on a synthetic backbone which hybridizes to the target sequence, optionally wherein there are more molecules of the second intermediate probe hybridized in the interior of the RCA product than a reference intermediate probe having the same sequence of nucleobases on a sugar-phosphate backbone.~~

~~65~~16. A composition, comprising:

a DNA concatemer comprising multiple copies of a target sequence,

a plurality of intermediate probes that hybridize to the target sequence, and

a plurality of fluorescently labeled detection probes each comprising a sequence of nucleobases on a synthetic backbone,

wherein the DNA concatemer, at least one molecule of the intermediate probe and at least one molecule of the fluorescently labeled detection probe form a hybridization complex,

wherein the fluorescently labeled detection probe and/or the intermediate probe are morpholino,

optionally wherein the hybridization complex comprises multiple molecules of the intermediate probe and multiple molecules of the fluorescently labeled detection probe.

~~66. The composition of claim 65, wherein the DNA concatemer is a rolling circle amplification (RCA) product.~~

~~67. The composition of claim 66, wherein the RCA product is generated *in situ* in a biological sample, using a circular or circularizable probe or probe set that directly or indirectly binds to an analyte in the biological sample as template.~~

~~68. The composition of any one of claims 65-67, wherein the fluorescently labeled detection probe is uncharged or less electrostatically negative than a reference detection probe having the same fluorescent label and sequence of nucleobases on a sugar-phosphate backbone.~~

~~69. The composition of any one of claims 65-68, wherein the intermediate probe comprises a sequence of nucleobases on a synthetic backbone which hybridizes to the target sequence, optionally wherein the intermediate probe is uncharged or less electrostatically negative than a reference intermediate probe having the same sequence of nucleobases on a sugar-phosphate backbone.~~

~~70. The composition of any one of claims 65-69, wherein at least a portion of the fluorescently labeled detection probe hybridizes to the intermediate probe.~~

~~71. The composition of any one of claims 65-70, wherein the fluorescently labeled detection probe and/or the intermediate probe are morpholino.~~

~~72. The composition of any one of claims 65-71, wherein the DNA concatemer has a diameter of less than about 1.5 μm.~~

~~73. The composition of any one of claims 65-72, wherein at least about 10%, at least about 25%, at least about 50%, at least about 75%, or at least about 90% of the copies of the target sequence in the DNA concatemer are bound directly or indirectly to one or more molecules of the intermediate probe and/or the fluorescently labeled detection probe.~~

~~74~~17. A kit, comprising:

a fluorescently labeled detection probe comprising a sequence of nucleobases on a synthetic backbone, wherein at least a portion of the fluorescently labeled detection probe hybridizes to an intermediate probe, and

the intermediate probe which comprises a sequence of nucleobases on a synthetic backbone that hybridizes to a target sequence, wherein the fluorescently labeled detection probe and/or the intermediate probe are morpholino.

~~75. The kit of claim 74, wherein the fluorescently labeled detection probe and the intermediate probe are morpholino.~~

~~76. The kit of claim 74 or 75, further comprising a circular or circularizable probe or probe set comprising the target sequence or a complement thereof, wherein the probe or probe set is capable of directly or indirectly binding to an analyte in a biological sample and generating a rolling circle amplification product using the probe or probe set as template.~~

~~78~~18. The method of any one of claims 1-~~64~~15, wherein the binding of the plurality of detection probes occurs in the presence of a hybridization buffer.

~~79. The method of claim 78, wherein the hybridization buffer comprises a reagent that increases the stability of A:T base pairs to approximately that of G:C pairs.~~

~~80~~19. The method of claim ~~79~~18, wherein the hydridization buffer comprises a reagent which comprises ammonium ions.

~~81~~20. The method of claim ~~80~~19, wherein the reagent is tetramethylammonium chloride.