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 claims1-3, wherein the detection probe directly hybridizes to an intermediate probe that directly hybridizes to the target sequence.

6. The method of any one of claims 1-5, 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.

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

8. The method of any one of claims 1-7, 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.

9. The method of claim 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.

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

11. The method of any one of claims 1-10, further comprising imaging the biological sample to detect a complex comprising multiple molecules of the detection probe *in situ* in the biological sample.

12. The method of any one of claims 1-11, 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.

13. The method of claim 12, further 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.

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

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.

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.

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.

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

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

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