CLAIMS

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

a) contacting the biological sample with a circularizable probe, wherein:

the circularizable probe comprises a 5' hybridization region and a 3' hybridization region that hybridize to adjacent first and second target regions, respectively, in a target nucleic acid in the biological sample,

the 5' hybridization region and the 3' hybridization region are different in lengths,

the 5' hybridization region or the 3' hybridization region comprises an interrogatory sequence complementary to a sequence of interest in the first or second target region, respectively, and

the interrogatory sequence is an internal sequence in the 5' hybridization region or the 3' hybridization region and does not comprise a ligatable end;

b) circularizing the circularizable probe hybridized to the target nucleic acid to form a circularized probe; and

c) detecting the circularized probe or a product thereof in the biological sample.

2. The method of claim 1, wherein the target nucleic acid comprises RNA.

3. The method of any of claims 1-2, wherein the circularizable probe comprises DNA.

4. The method of any of claims 1-3, wherein the circularizable probe comprises one or more ribonucleotide residues, optionally wherein the one or more ribonucleotides are at and/or near a ligatable 3' end of the circularizable probe.

5. The method of any of claims 1-4, wherein the circularizable probe comprises a 3' terminal ribonucleotide.

6. The method of any of claims 1-5, wherein the interrogatory sequence is in the shorter one of the 5' hybridization region and the 3' hybridization region.

7. The method of any of claims 1-5, wherein the interrogatory sequence is in the longer one of the 5' hybridization region and the 3' hybridization region.

8. The method of any of claims 1-7, wherein the 5’ hybridization region and the 3’ hybridization region are independently 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35 nucleotides in length.

9. The method of any of claims 1-8, wherein the lengths of the 5’ hybridization region and the 3’ hybridization region differ by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides.

10. The method of claim 9, wherein the 5’ hybridization region comprises the interrogatory sequence and is shorter than the 3’ hybridization region by about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 nucleotides, optionally wherein the 5’ hybridization region is about 10 nucleotides shorter than the 3’ hybridization region.

11. The method of any of claims 1-10, wherein the interrogatory sequence in the circularizable probe is one, two, three, four, or five nucleotides in length.

12. The method of claim 11, wherein the interrogatory sequence is an interrogatory nucleotide, and the sequence of interest is a single nucleotide of interest selected from the group consisting of a single-nucleotide polymorphism (SNP), a single-nucleotide variant (SNV), a single-nucleotide substitution, a point mutation, a single-nucleotide deletion, and a single-nucleotide insertion.

13. The method of claim 12, wherein the interrogatory nucleotide is the second, third, fourth, fifth, sixth, seventh, eighth, ninth, or tenth nucleotide of the 5’ hybridization region, with the 5’ terminal nucleotide of the circularizable probe being the first nucleotide of the 5’ hybridization region, optionally wherein the interrogatory nucleotide is the fourth, fifth, or sixth nucleotide of the 5’ hybridization region.

14. The method of claim 12, wherein the interrogatory nucleotide is the second, third, fourth, fifth, sixth, seventh, eighth, ninth, or tenth nucleotide of the 3’ hybridization region, with the 3’ terminal nucleotide of the circularizable probe being the first nucleotide of the 3’ hybridization region.

15. The method of any of claims 1-14, wherein a 3’ end and a 5’ end of the circularizable probe are ligated using the target nucleic acid as a template.

16. The method of any of claims 1-15, further comprising prior to the circularizing step, a step of removing molecules of the circularizable probe that are not bound to the target nucleic acid from the biological sample.

17. The method of any of claims 1-16, further comprising prior to the circularizing step, a step of removing molecules of the circularizable probe that are bound to the target nucleic acid but comprise one or more mismatches in the interrogatory sequence from the biological sample, and/or allowing the molecules (or portions thereof) comprising one or more mismatches to dissociate from the target nucleic acid while the molecules comprising no mismatch in the interrogatory sequence remain bound to the target nucleic acid.

18. The method of any of claims 1-17, further comprising generating the product of the circularized probe *in situ* in the biological sample, optionally wherein the product is generated using rolling circle amplification (RCA).

19. The method of any of claims 1-18, wherein the method comprises imaging the biological sample to detect the circularized probe or product thereof.

20. The method of claim 19, wherein the imaging comprises detecting a signal associated with a fluorescently labeled probe that directly or indirectly binds to a rolling circle amplification product of the circularized probe, optionally wherein a sequence of the rolling circle amplification product is analyzed *in situ* in the biological sample.

21. The method of claim 20, wherein the detecting comprises:

contacting the biological sample with one or more detectably-labeled probes that directly or indirectly hybridize to the rolling circle amplification product, and

dehybridizing the one or more detectably-labeled probes from the rolling circle amplification product.

22. The method of claim 20, wherein the detecting comprises:

contacting the biological sample with one or more intermediate probes that directly or indirectly hybridize to the rolling circle amplification product, wherein the one or more intermediate probes are detectable using one or more detectably-labeled probes, and

dehybridizing the one or more intermediate probes and/or the one or more detectably-labeled probes from the rolling circle amplification product.

23. A method for analyzing a biological sample, comprising:

a) contacting the biological sample with a circularizable probe, wherein:

the circularizable probe comprises a 5' hybridization region and a 3' hybridization region that hybridize to adjacent first and second target regions, respectively, in a target RNA in the biological sample,

the 5' hybridization region is shorter than the 3' hybridization region and comprises an interrogatory nucleotide complementary to a single nucleotide of interest in the first target region of a first molecule of the target RNA, wherein a second molecule of the target RNA comprises a mismatch with the interrogatory nucleotide, and

the interrogatory nucleotide is any one of the second to the tenth nucleotides from the 5' end of the circularizable probe;

b) allowing the 5' hybridization region to dissociate from the second molecule of the target RNA, wherein under the same conditions, the 5' hybridization region remains hybridized to the first molecule of the target RNA;

c) ligating the ends of the circularizable probe hybridized to the first molecule of the target RNA to form a circularized circularizable probe with or without processing of the circularizable probe;

d) generating a rolling circle amplification product of the circularized circularizable probe; and

e) detecting a signal associated with the rolling circle amplification product in the biological sample, wherein a signal associated with the second molecule of the target RNA is not detected.

24. The method of any of claims 1-23, wherein the biological sample is a fixed and/or permeabilized biological sample.

25. The method of any of claims 1-24, wherein the biological sample is a formalin-fixed, paraffin-embedded (FFPE) tissue sample, a frozen tissue sample, or a fresh tissue sample.

26. The method of any of claims 1-25, wherein the biological sample is crosslinked.

27. A kit for analyzing a biological sample, comprising a circularizable probe comprising a 5' hybridization region and a 3' hybridization region that hybridize to adjacent first and second target regions, respectively, in a target nucleic acid, wherein:

the 5' hybridization region and the 3' hybridization region are different in lengths,

the 5' hybridization region or the 3' hybridization region comprises an interrogatory sequence complementary to a sequence of interest in the first or second target region, respectively, and

the interrogatory sequence does not comprise a ligatable end with or without processing of the circularizable probe.