PROBES COMPRISING A SPLIT BARCODE REGION AND METHODS OF USE

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Patent Application No. 63/233,599, filed August 16, 2021, entitled "PROBES COMPRISING A SPLIT BARCODE REGION AND METHODS OF USE," which is herein incorporated by reference in its entirety for all purposes.

REFERENCE TO AN ELECTRONIC SEQUENCE LISTING

[0002] The contents of the electronic sequence listing (202412006740SEQLIST.xml; Size: 4,665 bytes; and Date of Creation: August 8, 2022) is herein incorporated by reference in its entirety.

FIELD

[0003] The present disclosure generally relates to methods and compositions for *in situ* analysis for detection of analytes in a sample.

BACKGROUND

[0004] Methods are available for analyzing nucleic acids in a biological sample *in situ*, such as a cell or a tissue. For instance, advances in single molecule fluorescent hybridization (smFISH) have enabled nanoscale-resolution imaging of RNA in cells and tissues. However, oligonucleotide probe-based assay methods for *in situ* analysis may suffer from low sensitivity, specificity, and/or detection efficiency and may require careful and laborious optimization. Improved methods for *in situ* analysis are needed. The present disclosure addresses these and other needs.

BRIEF SUMMARY

[0004a] In some embodiments, disclosed herein is a method for analyzing a biological sample, comprising: (a) contacting the biological sample with: (i) a first probe comprising a first hybridization region and a first portion of a barcode region, and (ii) a second probe comprising a second hybridization region and a second portion of the barcode region, wherein the first and second hybridization regions are complementary to target sequences in a target nucleic acid molecule in the biological sample, and wherein the barcode region comprises one or more barcode sequences corresponding to the target nucleic acid molecule; (b) connecting the first and second probes hybridized to the target nucleic acid molecule to form a composite probe by connecting the first and second portions of the

barcode region; and (c) contacting the biological sample with one or more detectable probes that hybridize to the barcode region at sequences corresponding to both the first portion of the barcode region and the second portion of the barcode region, or a complement thereof, wherein a signal associated with the one or more detectable probes is detected in the biological sample, thereby detecting the target nucleic acid molecule in the biological sample.

[0004b] In some embodiments, disclosed herein is a method for analyzing a biological sample, comprising: (a) contacting the biological sample with: (i) a first probe comprising a first hybridization region and a first barcode sequence, and (ii) a second probe comprising a second hybridization region and a second barcode sequence, wherein the first and second hybridization regions are complementary to adjacent target sequences in a target RNA molecule in the biological sample, and wherein the first and second barcode sequences each corresponds to the target RNA molecule; (b) ligating the first and second probes hybridized to the target RNA molecule to form a circular probe, wherein the ligating comprises: (i) ligating the first and second hybridization regions hybridized to the adjacent target sequences using the target RNA molecule as a template, and (ii) ligating the first and second probes to form a composite probe comprising the first and second barcode sequences corresponding to the target RNA molecule; and (c) generating a rolling circle amplification product of the composite probe; (d) contacting the biological sample with a detectable probe that hybridizes to the composite probe or a complement thereof in the rolling circle amplification product at sequences corresponding to both the first and second barcode sequences, wherein a signal associated with the detectable probe is detected in the biological sample, thereby detecting the target RNA molecule in the biological sample.

[0004c] In some embodiments, disclosed herein is a kit for analyzing a biological sample, comprising: (a) a first probe comprising a first hybridization region and a first portion of a barcode sequence, and (b) a second probe comprising a second hybridization region and a second portion of the barcode sequence, wherein the first and second hybridization regions are complementary to adjacent target sequences in a target RNA molecule, and the barcode sequence corresponds to the target RNA molecule, and wherein the kit comprises: (c) a splint that hybridizes to the first and second probes for ligation of the first and second portions to form a composite probe comprising the barcode sequence, optionally wherein: (i) the first and second portions of the barcode sequence are no more than 10 nucleotides in length, and the barcode sequence is at least 15 nucleotides in length, and/or (ii) the splint is single-stranded.

[0005] In some embodiments, disclosed herein is a method for analyzing a biological sample, comprising: (a) contacting the biological sample with: (i) a first probe comprising a first hybridization region and a first portion of a barcode region, and (ii) a second probe comprising a second hybridization region and a second portion of the barcode region, wherein the first and second hybridization regions are complementary to target sequences in a target nucleic acid molecule in the biological sample. In some embodiments, the barcode region comprises one or more barcode sequences corresponding to the target nucleic acid molecule. For instance, the first and second probes comprise a split barcode sequence, a portion of which is provided in the first portion of the barcode region, whereas