

binding to fluorescently-labelled probes as well as the barcode sequences and/or complements thereof.

[0032a] In some aspects, provided herein is a method for analyzing a biological sample, comprising: (a) contacting a biological sample with a circular probe or circularizable probe or probe set that hybridizes to a target nucleic acid in the biological sample, wherein the circular probe or circularizable probe or probe set comprises a first hybridizing region and a second hybridizing region capable of hybridizing to each other; (b) generating a rolling circle amplification product (RCP) at a location in the biological sample using the circular probe or a circularized probe generated from the circularizable probe or probe set as a template, wherein the RCP comprises multiple copies of complementary sequences of the first and second hybridizing regions, and wherein the complementary sequences of the first and second hybridizing regions from different copies hybridize to one another, thereby compacting and/or stabilizing the RCP at the location; and (c) detecting the RCP at the location in the biological sample.

[0032b] In some aspects, provided herein is a method for analyzing a biological sample, comprising: (a) contacting the biological sample with a padlock probe comprising a sequence that hybridizes to a target nucleic acid in the biological sample, wherein the padlock probe comprises: (i) a first hybridizing region and a second hybridizing region capable of hybridizing to each other, and (ii) one or more barcode sequences, wherein the first and second hybridizing regions are linked by a loop region, optionally wherein the loop region comprises the one or more barcode sequences, and optionally wherein the target nucleic acid is a cDNA or an mRNA; (b) circularizing the padlock probe to generate a circularized padlock probe using the target nucleic acid/or a splint as template, and generating a rolling circle amplification product (RCP) at a location in the biological sample using the circularized padlock probe as a template, wherein the RCP comprises multiple copies of complementary sequences of the first and second hybridizing regions, and wherein the complementary sequences of the first and second hybridizing regions from different copies hybridize to one another, thereby compacting and/or stabilizing the RCP at the location in the biological sample; and (c) detecting the RCP at the location in the biological sample.

[0032c] In some aspects, provided herein is a method for analyzing a biological sample, comprising: (a) contacting the biological sample with a padlock probe comprising a sequence that hybridizes to a target nucleic acid in the biological sample, wherein the padlock

probe comprises: (i) a first hybridizing region and a second hybridizing region capable of hybridizing to each other, wherein the first and second hybridizing regions are linked by a first loop region; (ii) a third hybridizing region and a fourth hybridizing region capable of hybridizing to each other, wherein the third and fourth hybridizing regions are linked by a second loop region; and (iii) one or more barcode sequences in the first and/or second loop regions; (b) circularizing the padlock probe to generate a circularized padlock probe using the target nucleic acid/or a splint as template, and generating a rolling circle amplification product (RCP) at a location in the biological sample using the circularized padlock probe as a template, wherein the RCP comprises multiple copies of complementary sequences of the first, second, third, and fourth hybridizing regions, and wherein the complementary sequences of the first and second hybridizing regions from two or more different copies hybridize to one another and the complementary sequences of the third and fourth hybridizing regions from two or more different copies hybridize to one another, thereby compacting and/or stabilizing the RCP at the location in the biological sample; and (c) detecting the RCP at the location in the biological sample.

[0032d] In some aspects, provided herein is a method for analyzing a biological sample, comprising: (a) contacting the biological sample with a padlock probe comprising a sequence that hybridizes to a target nucleic acid in the biological sample, wherein the padlock probe comprises: (i) a first hybridizing region and a second hybridizing region capable of hybridizing to each other, wherein the first and second hybridizing regions are linked by a loop region; and (ii) one or more barcode sequences; (b) circularizing the padlock probe to generate a circularized padlock probe using the target nucleic acid/or a splint as template, and generating a rolling circle amplification product (RCP) at a location in the biological sample using the circularized padlock probe as a template, wherein the RCP comprises multiple copies of a unit sequence comprising complementary sequences of the first and second hybridizing regions, and wherein the complementary sequence of the first hybridizing region from a first copy hybridizes to the complementary sequence of the second hybridizing region from a second copy, and the complementary sequence of the second hybridizing region from the first copy hybridizes to the complementary sequence of the first hybridizing region from the second copy, thereby compacting and/or stabilizing the RCP at the location in the biological sample; and (c) detecting the RCP at the location in the biological sample.

[0032e] In some aspects, provided herein is a method for analyzing a biological sample, comprising: (a) contacting a biological sample with a circular probe or circularizable probe or probe set that hybridizes to a target nucleic acid in the biological sample, wherein the circular probe or circularizable probe or probe set comprises a palindromic sequence; (b) generating a rolling circle amplification product (RCP) at a location in the biological sample using the circular probe or a circularized probe generated from the circularizable probe or probe set as a template, wherein the RCP comprises multiple copies of a unit sequence comprising a complementary sequence of the palindromic sequence, and wherein the complementary sequences of the palindromic sequence from different copies of the unit sequence hybridize to one another, thereby compacting and/or stabilizing the RCP at the location; and (c) detecting the RCP at the location in the biological same.

BRIEF DESCRIPTION OF THE DRAWINGS

[0033] The drawings illustrate certain embodiments of the features and advantages of this disclosure. These embodiments are not intended to limit the scope of the appended claims in any manner.

[0034] **FIGS. 1A-1C** each depicts an exemplary circular probe or circularizable probe or probe set, comprising a first hybridizing region and a second hybridizing region capable of hybridizing to each other (**FIG. 1A**), a palindromic sequence (**FIG. 1B**), or a first hybridizing region and a second hybridizing region capable of hybridizing to each other and a third hybridizing region and a fourth hybridizing region capable of hybridizing to each other (**FIG. 1C**). The hybridizing regions may but do not need to be palindromic sequences. The first and second hybridizing regions are of the same sequence when they are palindromic. The circular probe or circularizable probe or probe set may comprise one or more barcode sequences that can be used to identify the target nucleic acid during downstream analyses.

[0035] **FIGS. 2A-2C** illustrate exemplary conformations of RCP comprising complementary hybridizing regions.

[0036] **FIG. 3** shows exemplary configurations of the hybridization of hybridizing regions in an RCP.

[0037] **FIG. 4A** depicts an exemplary circularized probe with a hairpin stem region .

[0038] **FIG. 4B** depicts an exemplary circularized probe with a dumbbell stem region.

[0039] **FIG. 5** shows the results from an assay evaluating the compaction of RCPs generated from circularized probes having a dumbbell structure.

[0040] **FIG. 6** shows the results from an assay evaluating the compaction of RCPs generated from circularized probes having a hairpin structure.

[0041] **FIG. 7** depicts RCP feature density plotted against RCP size detected in fresh frozen mouse brain samples, using a circularizable probe comprising a palindromic sequence (“Palindromic”) or using a circularizable probe comprising no palindromic sequence (“Control”).

[0042] **FIGS. 8A-8B** show RCP size distributions in an FFPE tissue sample (**FIG. 8A**) and in an FF huTonsil tissue sample (**FIG. 8B**), detected using a circularizable probe