

Claims

1. A method of analyzing a sample, comprising:
 - a) contacting the sample with a probe that binds to a target nucleic acid sequence, wherein the probe comprises a complement of a barcode sequence that identifies the target nucleic acid sequence, and wherein the sample is a cell or tissue sample;
 - b) circularizing the probe;
 - c) amplifying the circularized probe to produce a rolling circle amplification (RCA) product in the sample, the RCA product comprising multiple copies of the barcode sequence;
 - d) contacting the sample with a first detection probe and a first reporter probe to generate a first complex comprising the first detection probe hybridized to the RCA product and the first reporter probe hybridized to the first detection probe,
wherein the first detection probe comprises (i) a recognition sequence complementary to the barcode sequence and (ii) a first overhang sequence, and
wherein the first reporter probe comprises (i) a sequence complementary to the first overhang sequence and (ii) a first optically detectable moiety;
 - e) imaging the sample to detect a first signal from the first optically detectable moiety, wherein the first signal corresponds to a first signal code in the signal code sequence;
 - f) contacting the sample with a second detection probe and a second reporter probe to generate a second complex comprising the second detection probe hybridized to the RCA product and the second reporter probe hybridized to the second detection probe,
wherein the second detection probe comprises (i) a recognition sequence complementary to the barcode sequence and (ii) a second overhang sequence, and
wherein the second reporter probe comprises (i) a sequence complementary to the second overhang sequence and (ii) a second optically detectable moiety; and
 - g) imaging the sample to detect a second signal from the second optically detectable moiety, wherein the second signal corresponds to a second signal code in the signal code sequence,
wherein the signal code sequence comprising at least the first signal code and the second signal code is determined at a location in the sample, thereby decoding the barcode sequence and identifying the target nucleic acid sequence at the location in the sample.

2. The method of claim 1, wherein the target nucleic acid is a genomic DNA, an RNA, or a cDNA.
3. The method of claim 1, wherein the target nucleic acid is targeted by multiple hybridization probes each comprising a complement of a different barcode sequence.
4. The method of claim 1, wherein the target nucleic acid is linked to a binding partner having affinity for a non-nucleic acid analyte.
5. The method of claim 1, wherein the barcode sequence comprises one or more barcode positions each comprising one or more barcode subunits.
6. The method of claim 5, wherein a barcode position in the barcode sequence partially overlaps an adjacent barcode position in the barcode sequence.
7. The method of claim 5, wherein a barcode position in the barcode sequence comprises a barcode subunit flanked by spacer sequences.
8. The method of claim 7, wherein the first detection probe and the second detection probe comprise the same recognition sequence, and wherein the first overhang sequence and the second overhang sequence are the same or different.
9. The method of claim 1, wherein the first overhang sequence and the second overhang sequence are the same.
10. The method of claim 1, wherein the first overhang sequence and the second overhang sequence are different.
11. The method of claim 1, wherein the optically detectable moiety is a fluorophore and the signal code sequence is a fluorophore sequence uniquely assigned to the target nucleic acid.
12. The method of claim 1, wherein the first detection probe, the second detection probe, and one or more subsequent detection probes are contacted with the sample sequentially in a pre-determined sequence which corresponds to the signal code sequence assigned to the barcode sequence, wherein the one or more subsequent detection probes each comprises (i) a recognition sequence complementary to the barcode sequence and (ii) an overhang sequence complementary to a reporter probe of a universal pool of reporter probes.

13. The method of claim 12, wherein the first reporter probe and the second reporter probe are in the universal pool of reporter probes.
14. The method of claim 13 wherein the sample is contacted with the universal pool of reporter probes in d) and with the universal pool of reporter probes in g).
15. The method of claim 12, wherein the one or more subsequent detection probes are contacted with the sample to determine signal codes in the signal code sequence until sufficient signal codes have been determined to decode the barcode sequence, thereby identifying the target nucleic acid.
16. The method of claim 14, wherein between the imaging in e) and the contacting in f) the method comprises a step of removing the first detection probe from the RCA product.
17. The method of claim 15, wherein after the imaging in g) the method comprises a step of removing the second detection probe from the RCA product, before contacting the sample with a subsequent detection probe.
18. The method of claim 1, wherein the barcode sequence associated with the target nucleic acid is selected from a plurality of barcode sequences, and wherein the contacting in d) comprises contacting the sample with a first pool of detection probes and a universal pool of reporter probes,
wherein the first pool of detection probes comprises the first detection probe and the universal pool of reporter probes comprises the first reporter probe and the second reporter probe,
wherein each detection probe in the first pool of detection probes comprises (i) a recognition sequence complementary to one of the plurality of barcode sequences and (ii) an overhang sequence complementary to a reporter probe of the universal pool of reporter probes;
and
wherein the contacting in f) comprises contacting the sample with a second pool of detection probes and the universal pool of reporter probes,
wherein the second pool of detection probes comprises the second detection probe, and
wherein each detection probe in the second pool of detection probes comprises (i) a recognition sequence complementary to one of the plurality of barcode sequences and (ii) an overhang sequence complementary to a reporter probe of the universal pool of reporter probes.

19. The method of claim 18, comprising identifying multiple different target nucleic acids present at locations in the sample, wherein each different target nucleic acid is assigned a different signal code sequence and is targeted by a padlock probe comprising a complement of a different barcode sequence of the plurality of barcode sequences.
20. The method of claim 18, wherein the number of different detection probes in each pool of detection probes is greater than the number of different reporter probes in the universal pool of reporter probes.
21. The method of claim 20, wherein the number of different reporter probes in the universal pool of reporter probes is four.
22. The method of claim 21, wherein the number of different detection probes in each pool of detection probes is 50 or more.
23. The method of claim 1, wherein the signals or are detected *in situ* in the sample.