

Claims

1. ~~1. A method of detecting a nucleic acid molecule, wherein the method comprises:~~

5 ~~(a) assigning a unique signal code sequence to the nucleic acid molecule, wherein the signal code sequence is specific to that molecule, wherein the signal code sequence may be derived by interrogating a target nucleotide sequence within the nucleic acid molecule with a sequence of detection probes, each detection probe yielding a signal and the signals together make up the signal code sequence, and wherein the target nucleotide sequence~~
10 ~~comprises first and second domains, each domain comprising a first subunit and a second subunit, wherein the second subunit of the first domain at least partially overlaps with the first subunit of the second domain in the target nucleotide sequence;~~

15 ~~(b) contacting the nucleic acid molecule with a first detection probe for generating a signal code for the first position of the signal code sequence, and allowing the detection probe to hybridise to the nucleic acid molecule, wherein the detection probe comprises a recognition sequence that is complementary to the first and second subunits of the first domain and a reporter;~~

~~(c) detecting a signal from the reporter of the first detection probe which has hybridized to the nucleic acid molecule at the first domain;~~

20 ~~(d) identifying the signal code for the first position of the signal code sequence from the signal detected in (c);~~

~~(e) contacting the nucleic acid molecule with a subsequent detection probe for generating a signal code for the next position of the signal code sequence, and allowing the detection probe to hybridise to the nucleic acid molecule, wherein the subsequent detection probe comprises a recognition sequence that is complementary to the first and second subunits of the second domain, and a reporter, and wherein the subsequent detection probe hybridises to a sequence in the second subunit of the preceding domain, and thereby initiates a strand displacement reaction which displaces the hybridised preceding detection probe;~~

30 ~~(f) detecting a signal from the reporter of the subsequent detection probe which has hybridized to the nucleic acid molecule at the adjacent domain;~~

~~(g) identifying the signal code for the next position of the signal code sequence from the signal detected in (f);~~

35 ~~(h) repeating steps (e)–(g) to identify signal codes for subsequent signal code sequence positions until sufficient signal codes have been identified to detect the nucleic acid molecule, wherein in repeated steps the detection probes for identifying the subsequent signal codes in the signal code sequence respectively have recognition sequences that are~~

~~complementary to the subunits that are present at the first and second domains of the target nucleotide sequence such that detection probes successively hybridise to and displace preceding detection probes from the first and second domains;~~

~~wherein the first domain can be the 5' or the 3' domain in the target nucleotide sequence.~~

~~2. — The method of claim 1, wherein the target nucleotide sequence is a specific target sequence which occurs within a DNA or RNA molecule to be detected.~~

~~3. — The method of claim 2, wherein the target nucleotide sequence is a specific target sequence which is present in a native genomic DNA or in a naturally occurring RNA molecule, or in a cDNA, or in an amplification product generated from any of the foregoing nucleic acid molecules.~~

~~4. — The method of any one of claims 1 to 3, wherein the second subunit from each domain fully overlaps with the first subunit of the adjacent domain.~~

~~5. — The method of any one of claims 1 to 4 for detecting different nucleic acid molecules present in a sample, wherein each nucleic acid molecule comprises a different target nucleotide sequence, and wherein a different series of first and subsequent detection probes is provided for each different target nucleotide sequence, each different series of detection probes yielding a different signal code sequence.~~

~~6. — The method of claim 5, wherein each target nucleotide sequence comprises: (i) a first common region adjacent to the first subunit of the first domain, wherein the recognition sequence of the first detection probe comprises a sequence which is complementary to the first common region; and (ii) a second common region adjacent to the second subunit of the second domain, wherein the recognition sequence of the subsequent detection probe comprises a sequence which is complementary to the second common region.~~

~~7. — The method of any one of claims 1 to 6, wherein the first detection probes and the subsequent detection probes each comprise a displacer toehold overhang region.~~

~~8. — The method of claim 7, wherein all of the detection probes which comprise a recognition sequence which is complementary to the first domain comprise a first displacer toehold overhang sequence, and all of the detection probes which comprise a recognition~~

~~sequence which is complementary to the second domain comprise a second displacer
toehold overhang sequence.~~

9. ~~The method of claim 8, wherein step (e) further comprises contacting the
nucleic acid molecule with either a first displacer probe which comprises a displacer toehold
binding region complementary to the first displacer toehold overhang and a sequence
complementary to the first common region; or a second displacer probe which comprises a
displacer toehold binding region complementary to the second displacer toehold overhang
and a sequence complementary to the second common region.~~

10. ~~The method of any one of claims 1 to 9, wherein the target nucleotide
sequence is a nucleotide barcode sequence provided within the nucleic acid molecule.~~

11. ~~The method of any one of claims 1 to 10, wherein the nucleic acid molecule is
linked to an antibody.~~

12. ~~A method of decoding a nucleotide barcode sequence in a nucleic acid
molecule, wherein the nucleotide barcode sequence corresponds to a specific signal code
sequence that may be derived by interrogating the nucleotide barcode sequence with
sequential detection probes each yielding a signal and the signals together make up the
signal code sequence, and wherein the nucleotide barcode sequence comprises at least two
adjacent barcode positions, each barcode position comprising a barcode subunit pair
comprising a first barcode subunit and a second barcode subunit, wherein the second
barcode subunit from each barcode position at least partially overlaps with the first barcode
subunit of the adjacent barcode position in the sequence, said method comprising;~~

(i) ~~contacting the nucleic acid molecule with a first detection probe for generating a
signal code for the first position of the signal code sequence, and allowing the detection
probe to hybridise to the nucleic acid molecule, wherein the detection probe comprises a
recognition sequence that is complementary to the barcode subunit pair that is present at the
first barcode position and a reporter;~~

(ii) ~~detecting a signal from the reporter of the first detection probe which has hybridized
to the nucleotide barcode sequence at the first barcode position;~~

(iii) ~~identifying the signal code for the first position of the signal code sequence from
the signal detected in (ii);~~

(iv) ~~contacting the nucleotide barcode sequence with a subsequent detection probe for
generating a signal code for the next position of the signal code sequence, and allowing the
detection probe to hybridise to the nucleic acid molecule, wherein the subsequent detection~~

probe comprises a recognition sequence that is complementary to the barcode subunit pair that is present at the adjacent barcode position, and a reporter, and wherein the subsequent detection probe which hybridises to the barcode subunit at the adjacent position hybridises to a sequence within the overlapping portion of the preceding barcode position, and thereby initiates a strand displacement reaction which displaces the hybridised preceding detection probe;

(v) detecting a signal from the reporter of the subsequent detection probe which has hybridized to the nucleotide barcode sequence at the adjacent barcode position;

(vi) identifying the signal code for the next position of the signal code sequence from the signal detected in (v);

(vii) repeating steps (iv) – (vi) to identify signal codes for subsequent signal code sequence positions until sufficient signal codes have been identified to decode the nucleotide barcode sequence, wherein detection probes for identifying the subsequent signal codes in the signal code sequence have (a) recognition sequences that are complementary to barcode subunit pairs that are present at subsequent sequential barcode positions in a nucleotide barcode sequence comprising multiple sequential adjacent barcode positions, or (b) recognition sequences that are complementary to the barcode subunit pairs that are present at the first and second barcode positions of a nucleotide barcode sequence comprising two adjacent barcode positions, and a reporter;

wherein the first barcode position can be any barcode position in the nucleotide barcode sequence.

13. — The method of claim 12, wherein each nucleotide barcode sequence comprises unique barcode subunits.

14. — The method of claim 12 or claim 13, wherein the nucleotide barcode sequence comprises first and second adjacent barcode positions, each barcode position comprising a barcode subunit pair comprising a first barcode subunit and a second barcode subunit, wherein the second barcode subunit from the first barcode position at least partially overlaps with the first barcode subunit of the second barcode position, said method comprising;

(i) contacting the nucleic acid molecule with a first detection probe for generating a signal code for the first position of the signal code sequence, and allowing the detection probe to hybridise to the nucleic acid molecule, wherein the detection probe comprises a recognition sequence that is complementary to the barcode subunit pair that is present at the first barcode position and a reporter;

(ii) detecting a signal from the reporter of the first detection probe which has hybridized to the nucleotide barcode sequence at the first barcode position;

(iii) identifying the signal code for the first position of the signal code sequence from the signal detected in (ii);

(iv) contacting the nucleotide barcode sequence with a subsequent detection probe for generating a signal code for the next position of the signal code sequence, and allowing the detection probe to hybridise to the nucleic acid molecule, wherein the detection probe comprises a recognition sequence that is complementary to the barcode subunit pair that is present at the second barcode position and a reporter, and wherein the subsequent detection probe which hybridises to the barcode subunit at the adjacent position hybridises to a sequence in the second barcode subunit of the preceding barcode position, and thereby initiates a strand displacement reaction which displaces the hybridised preceding detection probe;

(v) detecting a signal from the reporter of the subsequent detection probe which has hybridized to the nucleotide barcode sequence at the adjacent barcode position;

(vi) identifying the signal code for the next position of the signal code sequence from the signal detected in (v);

(vii) repeating steps (iv) – (vi) to identify signal codes for subsequent signal code sequence positions until sufficient signal codes have been identified to decode the nucleotide barcode sequence, wherein in repeated steps the detection probes for identifying the subsequent signal codes in the signal code sequence respectively have recognition sequences that are complementary to the barcode subunit pairs that are present at the first and second barcode positions of the nucleotide barcode sequence such that detection probes successively hybridise to and displace preceding detection probes from the first and second barcode positions;

wherein the first barcode position can be the 5' or the 3' barcode position in the nucleotide barcode sequence.

15. — The method of claim 14, wherein the nucleic acid molecule further comprises general regions flanking the nucleotide barcode sequence, and wherein the detection probes hybridise to the general regions in addition to their respective barcode positions.

16. — The method of any one of claims 12 to 15 for decoding different nucleotide barcode sequences in multiple different nucleic acid molecules present in a sample, wherein a different series of first and subsequent detection probes is provided for each different nucleotide barcode sequence, each different series of detection probes yielding a different signal code sequence.

17. ~~— A method of detecting a nucleic acid molecule, wherein the method comprises;~~

5 ~~(a) — assigning a unique signal code sequence to the nucleic acid molecule, wherein the signal code sequence is specific to that molecule;~~

10 ~~(b) — providing the nucleic acid molecule with a nucleotide barcode sequence that corresponds to the signal code sequence, wherein the signal code sequence may be derived by interrogating the nucleotide barcode sequence with sequential detection probes each yielding a signal and the signals together make up the signal code sequence, and wherein the nucleotide barcode sequence comprises at least two adjacent barcode positions, each barcode position comprising a barcode subunit pair comprising a first barcode subunit and a second barcode subunit, wherein the second barcode subunit from each barcode position at least partially overlaps with the first barcode subunit of the adjacent barcode position in the sequence;~~

15 ~~(c) — performing steps (i) to (vii) as defined in any one of claims 12 to 23 to generate a signal code sequence to decode the nucleotide barcode sequence and detect the nucleic acid molecule.~~

20 ~~18. — The method of any one of claims 12 to 17, wherein each nucleotide barcode sequence comprises: (i) a first common region adjacent to the first barcode subunit of the first barcode position, wherein the recognition sequence of the first detection probe comprises a sequence which is complementary to the first common region; and (ii) a second common region adjacent to the second barcode subunit of the second barcode position, wherein the recognition sequence of the subsequent detection probe comprises a sequence~~
25 ~~which is complementary to the second common region.~~

19. ~~— The method of any one of claims 12 to 18, wherein the first detection probes and the subsequent detection probes each comprise a displacer toehold overhang region.~~

30 ~~20. — The method of claim 19, wherein step (iv) further comprises contacting the nucleotide barcode sequence with a set of displacer probes, wherein each displacer probe comprises a displacer toehold binding region and a sequence complementary to that of a different detection probe.~~

35 ~~21. — The method of claim 19, wherein all of the detection probes which comprise a recognition sequence which is complementary to the first barcode position comprise a first displacer toehold overhang sequence, and all of the detection probes which comprise a~~

recognition sequence which is complementary to the second barcode position comprise a second displacer toehold overhang sequence.

22. The method of claim 21, wherein step (iv) further comprises contacting the nucleotide barcode sequence with either a first displacer probe, which comprises a displacer toehold binding region complementary to the first displacer toehold overhang and a sequence complementary to the first common region; or a second displacer probe, which comprises a displacer toehold binding region complementary to the second displacer toehold overhang and a sequence complementary to the second common region.

23. A method of decoding a nucleotide barcode sequence in a nucleic acid molecule to differentiate said nucleotide barcode sequence from other nucleotide barcode sequences, wherein a nucleotide barcode sequence comprises multiple sequential barcode positions, each barcode position partially but not fully overlapping the adjacent barcode position in the sequence and each barcode position comprising at least one barcode subunit, and wherein each barcode subunit is selected from a panel of barcode subunits, such that the order of barcode subunits in each nucleotide barcode sequence defines a signal code sequence which comprises a signal code corresponding to each barcode position, and which is distinct from the signal code sequences of other nucleotide barcode sequences and identifies a given nucleotide barcode sequence, said method comprising;

(i) contacting the nucleic acid molecule with a first set of first detection probes for decoding the nucleotide barcode sequence at the first sequential barcode position and allowing the detection probes to hybridise to the nucleic acid molecule, wherein each detection probe in the set comprises a sequence complementary to a different barcode subunit within the panel and a reporter, such that each detection probe is capable of hybridising to and detecting a different barcode subunit at said first barcode position;

(ii) detecting a signal from the reporter of the first detection probe which has hybridized to the nucleotide barcode sequence at the first sequential barcode position;

(iii) identifying the signal code for the first barcode position from the signal detected in (ii);

(iv) contacting the nucleotide barcode sequence with a next set of subsequent detection probes for decoding the nucleotide barcode sequence at the next sequential barcode position and allowing the detection probes to hybridise to the nucleic acid molecule, wherein each detection probe comprises a sequence complementary to a different barcode subunit within the panel and a reporter, such that each detection probe is capable of hybridising to and detecting a different barcode subunit at said barcode position, and wherein the subsequent detection probe which hybridises to the barcode subunit at the next

position hybridises to a sequence within the overlapping portion of the preceding barcode position, and thereby initiates a strand displacement reaction which displaces the hybridised preceding detection probe;

(v) detecting a signal from the reporter of the subsequent detection probe which has hybridized to the nucleotide barcode sequence at the next sequential barcode position;

(vi) identifying the signal code for the next barcode position from the signal detected in (v);

(vii) repeating steps (iv)–(vi) to identify signal codes for subsequent sequential barcode positions until sufficient signal codes have been identified to decode the nucleotide barcode sequence;

wherein the first sequential barcode position can be any barcode position in the nucleotide barcode sequence.

24. — The method of claim 23, wherein the first detection probes and the subsequent detection probes each comprise a displacer toehold overhang region.

25. — The method of claim 24, wherein step (iv) further comprises contacting the nucleotide barcode sequence with a set of displacer probes, wherein each displacer probe comprises a displacer toehold binding region and a sequence complementary to that of a different detection probe.

26. — The method of any one of claims 12 to 25, wherein the first sequential barcode position is one of the terminal barcode positions of the nucleotide barcode sequence.

27. — The method of any one of claims 1 to 26, wherein the reporter of each detection probe comprises a fluorophore.

28. — The method of claim 28, wherein the detection probes comprise an overhang region comprising a binding site capable of hybridising to a reporter probe comprising a fluorophore.

29. — The method of claim 27 or claim 28, wherein the signal code for each domain or barcode position is the colour of the fluorophore of the reporter of the detection probe that hybridises to that domain or barcode position.

30. ~~The method of any one of claims 23 to 29, wherein each barcode position comprises a first spacer sequence, a barcode subunit, and a second spacer sequence, wherein the second spacer sequence from each barcode position at least partially overlaps with the first spacer sequence of the adjacent barcode position in the sequence, and wherein each barcode subunit is selected from a panel of barcode subunits, such that the order of barcode subunits in each barcode sequence is distinct from that of other barcode sequences and identifies a given barcode sequence, said method comprising;~~

~~(i) contacting the nucleic acid molecule with a first set of first detection probes for decoding the nucleotide barcode sequence at the first sequential barcode position and allowing the detection probes to hybridise to the nucleic acid molecule, wherein each detection probe comprises a first flanking sequence complementary to at least a portion of the first spacer sequence of the first barcode position, a sequence complementary to a barcode subunit, a second flanking sequence complementary to at least a portion of the second spacer sequence of the first barcode position and a reporter, wherein each detection probe comprises a sequence complementary to a different barcode subunit within the panel, such that each detection probe is capable of hybridising to and detecting a different barcode subunit at said first barcode position;~~

~~(ii) detecting a signal from the reporter of the first detection probe which has hybridized to the nucleotide barcode sequence at the first sequential barcode position;~~

~~(iii) determining the identity of the first barcode subunit from the signal detected in (ii);~~

~~(iv) contacting the nucleotide barcode sequence with a next set of subsequent detection probes for decoding the nucleotide barcode sequence at the next sequential barcode position and allowing the detection probes to hybridise to the nucleic acid molecule, wherein each detection probe comprises a first flanking sequence complementary to at least a portion of the first spacer sequence of the subsequent barcode position, a sequence complementary to a barcode subunit, a second flanking sequence complementary to at least a portion of the second spacer sequence of the subsequent barcode position and a reporter, wherein each detection probe comprises a sequence complementary to a different barcode subunit within the panel, such that each detection probe is capable of hybridising to and detecting a different barcode subunit at said barcode position, and wherein the subsequent detection probe which hybridises to the barcode subunit at the next position hybridises to at least a portion of the second spacer sequence of the preceding barcode position, and thereby initiates a strand displacement reaction which displaces the hybridised preceding detection probe;~~

~~(v) detecting a signal from the reporter of the subsequent detection probe which has hybridized to the nucleotide barcode sequence at the next sequential barcode position;~~

~~(vi) determining the identity of the next barcode subunit from the signal detected in (v);~~

(vii) repeating steps (iv)–(vi) to decode subsequent sequential barcode positions until the identity of sufficient positions of the subunit barcode sequence has been determined to decode the nucleotide barcode sequence,

wherein the first sequential barcode position can be any barcode position in the nucleic acid molecule.

31. — The method of claim 30, wherein each barcode subunit is 3 nucleotides in length.

32. — The method of claim 30 or claim 31, wherein each barcode subunit comprises at least 2 mismatches relative to every other barcode subunit.

33. — The method of any one of claims 30 to 32, wherein the spacer sequences are between 2 and 20 nucleotides in length.

34. — The method of any one of claims 30 to 33, wherein the spacer sequences are all the same length.

35. — The method of any one of claims 30 to 34, wherein the second spacer sequence of each barcode position fully overlaps with the first spacer sequence of the adjacent barcode position.

36. — The method of any one of claims 30 to 35, wherein the first flanking sequence of each detection probe has a higher affinity for the first spacer sequence of the corresponding barcode position than the second flanking sequence of the detection probe has for the second spacer sequence of the corresponding barcode position.

37. — The method of any one of claims 30 to 36, wherein in each detection probe, the first flanking sequence is longer than the second flanking sequence.

38. — The method of any one of claims 23 to 29, wherein each barcode position comprises a barcode subunit pair comprising a first barcode subunit and a second barcode subunit, wherein the second barcode subunit from each barcode position at least partially overlaps with the first barcode subunit of the adjacent barcode position in the sequence, such that the order of barcode subunit pairs in each nucleotide barcode sequence defines a signal code sequence which comprises a signal code corresponding to each barcode subunit pair, and which is distinct from the signal code sequences of other nucleotide

barcode sequences and identifies a given nucleotide barcode sequence, said method comprising;

(i) contacting the nucleic acid molecule with a first set of first detection probes for decoding the nucleotide barcode sequence at the first sequential barcode position and allowing the detection probes to hybridise to the nucleic acid molecule, wherein each detection probe comprises a sequence that is complementary to a different barcode subunit pair and a reporter, such that each detection probe is capable of hybridising to and detecting a different barcode subunit pair at said first barcode position;

(ii) detecting a signal from the reporter of the first detection probe which has hybridized to the nucleotide barcode sequence at the first sequential barcode position;

(iii) identifying the signal code for the first barcode subunit pair from the signal detected in (ii);

(iv) contacting the nucleotide barcode sequence with a next set of subsequent detection probes for decoding the nucleotide barcode sequence at the next sequential barcode position and allowing the detection probes to hybridise to the nucleic acid molecule, wherein each detection probe comprises a sequence that is complementary to a different barcode subunit pair and a reporter, such that each detection probe is capable of hybridising to and detecting a different barcode subunit pair at said barcode position, and wherein the subsequent detection probe which hybridises to the barcode subunit at the next position hybridises to a sequence within the overlapping portion of the preceding barcode position, and thereby initiates a strand displacement reaction which displaces the hybridised preceding detection probe;

(v) detecting a signal from the reporter of the subsequent detection probe which has hybridized to the nucleotide barcode sequence at the next sequential barcode position;

(vi) identifying the signal code for the next barcode subunit pair from the signal detected in (v);

(vii) repeating steps (iv) – (vi) to identify signal codes for barcode subunit pairs at subsequent sequential barcode positions until sufficient signal codes have been identified to decode the nucleotide barcode sequence;

wherein the first sequential barcode position can be any barcode position in the nucleotide barcode sequence.

39. The method of claim 38, wherein each nucleotide barcode sequence comprises unique barcode subunits.

40. ~~—The method of any one of claims 12 to 29, 38 or 39, wherein the second barcode subunit from each barcode position fully overlaps with the first barcode subunit of the adjacent barcode position.~~

41. ~~—The method of any one of claims 12 to 40, wherein the nucleic acid molecule containing the nucleotide barcode sequence is a probe molecule or an amplification product of a probe molecule.~~

42. ~~—The method of any one of claims 12 to 41, wherein the nucleic acid molecule containing the nucleotide barcode sequence is a rolling circle amplification product of a circularised padlock probe.~~

43. ~~—A method of detecting a target nucleic acid molecule, wherein the target nucleic acid molecule is detected using a padlock probe specific for said target molecule which is circularised upon hybridisation to a target sequence in the target nucleic acid molecule and which is provided with a nucleotide barcode sequence to identify the padlock probe, and wherein the circularised padlock probe is amplified by rolling circle amplification (RCA) to produce a rolling circle product (RCP), the RCP containing multiple complementary copies of the nucleotide barcode sequence, characterised in that the method comprises;~~

(a) ~~—assigning a unique signal code sequence to the target nucleic acid molecule, wherein the signal code sequence is specific to that molecule;~~

(b) ~~—providing the padlock probe with a nucleotide barcode sequence such that a RCP is formed comprising multiple complementary copies of the nucleotide barcode sequence that each corresponds to the signal code sequence, wherein the signal code sequence may be derived by interrogating the complementary copies of the nucleotide barcode sequence with sequential detection probes each yielding a signal and the signals together make up the signal code sequence;~~

(c) ~~—contacting the RCP with a first detection probe for generating a signal code for the first position of the signal code sequence and allowing the detection probe to hybridise to the RCP at the complementary copies of the nucleotide barcode sequence, wherein the detection probe comprises a recognition sequence complementary to the nucleotide barcode sequence and a reporter;~~

(d) ~~—detecting a signal from the reporter of the first detection probe which has hybridized to the RCP;~~

(e) ~~—identifying the first signal code for the first position in the signal code sequence for the RCP from the signal detected in (d);~~

(f) ~~—removing the first detection probe from the RCP;~~

(g) ~~contacting the RCP with a subsequent detection probe for generating a signal code for the next position of the signal code sequence, and allowing the detection probe to hybridise to the nucleic acid molecule at the complementary copies of the nucleotide barcode sequence, wherein the detection probe comprises a recognition sequence complementary to the nucleotide barcode sequence and a reporter;~~

(h) ~~detecting a signal from the reporter of the subsequent detection probe which has hybridized to the RCP;~~

(i) ~~identifying the signal code for the next position in the signal code sequence for the RCP from the signal detected in (g);~~

(j) ~~removing the subsequent detection probe from the RCP;~~

(k) ~~repeating steps (g) – (j) to identify signal codes for subsequent signal code positions until sufficient signal codes have been identified to identify the nucleotide barcode sequence and thereby to detect the nucleic acid molecule.~~

44. ~~The method of claim 43, wherein the reporter of each detection probe is a binding site for a reporter probe comprising a detectable label, said binding site being contained in an overhang region of the detection probe which does not hybridise to the RCP, and wherein the signal is detected from said label.~~

45. ~~The method of claim 44, wherein said detectable label is a fluorophore and the signal code sequence is a unique fluorophore sequence.~~

46. ~~The method of claim 44 or claim 45, wherein the detection probes for generating the signal code sequence for a target nucleic acid molecule are provided as a set of detection probes each comprising the same recognition sequence and a reporter binding site, which may be the same or different, and which is specific for a reporter probe comprising a detectable label which gives rise to a given signal code, and wherein the detection probes are used sequentially in a pre-determined sequence which corresponds to the signal code sequence assigned to the target nucleic acid molecule.~~

47. ~~The method of any one of claims 43 to 46, for detecting multiple different nucleic acid molecules present in a sample, wherein each different nucleic acid molecule is assigned a different signal code sequence and is detected using a specific padlock probe with a different nucleotide barcode sequence.~~

48. ~~— The method of claim 47, wherein a different set of subsequent detection probes is provided for each different nucleic acid molecule, each different set of detection probes yielding a different signal code sequence.~~

5 49. ~~— The method of any one of claims 1 to 48, wherein said target nucleic acid molecule is detected in situ in a cell sample.~~

50. ~~— The method of claim 49, wherein the target nucleic acid molecule is detected in a cell culture, or in a tissue sample.~~

10 1. A method of analyzing a sample, comprising:

a) producing a rolling circle amplification (RCA) product in the sample, the RCA product comprising multiple copies of a barcode sequence, wherein the barcode sequence is associated with a target analyte and is assigned a signal code sequence, and wherein the sample is a cell or tissue sample;

b) 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,

20 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;

c) 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;

25 d) 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

30 wherein the second reporter probe comprises (i) a sequence complementary to the second overhang sequence and (ii) a second optically detectable moiety; and

e) 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 analyte at the location in the sample.

2. The method of claim 1, wherein the target analyte is a nucleic acid analyte and the RCA product is generated from a hybridization probe, and wherein the hybridization probe comprises a complement of the barcode sequence and hybridizes to the nucleic acid analyte.

3. The method of claim 2, wherein the nucleic acid analyte is a genomic DNA, an RNA, or a cDNA.

4. The method of claim 2, wherein the target analyte is targeted by multiple hybridization probes each comprising a complement of a different barcode sequence.

5. The method of claim 1, wherein the target analyte is a non-nucleic acid analyte and the RCA product is generated from a hybridization probe, and wherein the hybridization probe comprises a complement of the barcode sequence and hybridizes to a nucleic acid molecule linked to a binding partner having affinity for the non-nucleic acid analyte.

6. The method of claim 1, wherein the barcode sequence comprises one or more barcode positions each comprising one or more barcode subunits.

7. The method of claim 6, wherein a barcode position in the barcode sequence partially overlaps an adjacent barcode position in the barcode sequence.

8. The method of claim 6, wherein a barcode position in the barcode sequence comprises a barcode subunit flanked by spacer sequences.

9. The method of claim 8, 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.

10. The method of claim 1, wherein the first overhang sequence and the second overhang sequence are the same.

11. The method of claim 1, wherein the first overhang sequence and the second overhang sequence are different.

12. 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 analyte.

13. 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.

14. The method of claim 13, wherein the first reporter probe and the second reporter probe are in the universal pool of reporter probes.

15. The method of claim 14 wherein the sample is contacted with the universal pool of reporter probes in b) and with the universal pool of reporter probes in e).

16. The method of claim 13, 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 analyte.

17. The method of claim 15, wherein between the imaging in c) and the contacting in d) the method comprises a step of removing the first detection probe from the RCA product.

18. The method of claim 16, wherein after the imaging in e) the method comprises a step of removing the second detection probe from the RCA product, before contacting the sample with a subsequent detection probe.

19. The method of claim 1, wherein the barcode sequence associated with the target analyte is selected from a plurality of barcode sequences, and wherein the contacting in b) 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

5 wherein the contacting in d) 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.

10 20. The method of claim 19, comprising identifying multiple different target analytes present at locations in the sample, wherein each different target analyte 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.

15 21. The method of claim 19, 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.

20 22. The method of claim 21, wherein the number of different reporter probes in the universal pool of reporter probes is four.

23. The method of claim 22, wherein the number of different detection probes in each pool of detection probes is 50 or more.

24. The method of claim 1, wherein the signals or are detected *in situ* in the sample.

25. A method of detecting a nucleic acid molecule, wherein the method comprises:

25 (a) assigning a unique signal code sequence to the nucleic acid molecule, wherein the signal code sequence is specific to that molecule, wherein the signal code sequence may be derived by interrogating a target nucleotide sequence within the nucleic acid molecule with a sequence of detection probes, each detection probe yielding a signal and the signals together make up the signal code sequence, and wherein the target nucleotide sequence
30 comprises first and second domains, each domain comprising a first subunit and a second

subunit, wherein the second subunit of the first domain at least partially overlaps with the first subunit of the second domain in the target nucleotide sequence;

5 (b) contacting the nucleic acid molecule with a first detection probe for generating a signal code for the first position of the signal code sequence, and allowing the detection probe to hybridise to the nucleic acid molecule, wherein the detection probe comprises a recognition sequence that is complementary to the first and second subunits of the first domain and a reporter;

(c) detecting a signal from the reporter of the first detection probe which has hybridized to the nucleic acid molecule at the first domain;

10 (d) identifying the signal code for the first position of the signal code sequence from the signal detected in (c);

15 (e) contacting the nucleic acid molecule with a subsequent detection probe for generating a signal code for the next position of the signal code sequence, and allowing the detection probe to hybridise to the nucleic acid molecule, wherein the subsequent detection probe comprises a recognition sequence that is complementary to the first and second subunits of the second domain, and a reporter, and wherein the subsequent detection probe hybridises to a sequence in the second subunit of the preceding domain, and thereby initiates a strand displacement reaction which displaces the hybridised preceding detection probe;

20 (f) detecting a signal from the reporter of the subsequent detection probe which has hybridized to the nucleic acid molecule at the adjacent domain;

(g) identifying the signal code for the next position of the signal code sequence from the signal detected in (f);

25 (h) repeating steps (e) - (g) to identify signal codes for subsequent signal code sequence positions until sufficient signal codes have been identified to detect the nucleic acid molecule, wherein in repeated steps the detection probes for identifying the subsequent signal codes in the signal code sequence respectively have recognition sequences that are complementary to the subunits that are present at the first and second domains of the target nucleotide sequence such that detection probes successively hybridise to and displace preceding detection probes from the first and second domains;

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wherein the first domain can be the 5' or the 3' domain in the target nucleotide sequence.

26. A method of decoding a nucleotide barcode sequence in a nucleic acid molecule, wherein the nucleotide barcode sequence corresponds to a specific signal code sequence that may be derived by interrogating the nucleotide barcode sequence with sequential detection probes each yielding a signal and the signals together make up the signal code sequence, and wherein the nucleotide barcode sequence comprises at least two adjacent barcode positions, each barcode position comprising a barcode subunit pair comprising a first barcode subunit and a second barcode subunit, wherein the second barcode subunit from each barcode position at least partially overlaps with the first barcode subunit of the adjacent barcode position in the sequence, said method comprising;

(i) contacting the nucleic acid molecule with a first detection probe for generating a signal code for the first position of the signal code sequence, and allowing the detection probe to hybridise to the nucleic acid molecule, wherein the detection probe comprises a recognition sequence that is complementary to the barcode subunit pair that is present at the first barcode position and a reporter;

(ii) detecting a signal from the reporter of the first detection probe which has hybridized to the nucleotide barcode sequence at the first barcode position;

(iii) identifying the signal code for the first position of the signal code sequence from the signal detected in (ii);

(iv) contacting the nucleotide barcode sequence with a subsequent detection probe for generating a signal code for the next position of the signal code sequence, and allowing the detection probe to hybridise to the nucleic acid molecule, wherein the subsequent detection probe comprises a recognition sequence that is complementary to the barcode subunit pair that is present at the adjacent barcode position, and a reporter, and wherein the subsequent detection probe which hybridises to the barcode subunit at the adjacent position hybridises to a sequence within the overlapping portion of the preceding barcode position, and thereby initiates a strand displacement reaction which displaces the hybridised preceding detection probe;

(v) detecting a signal from the reporter of the subsequent detection probe which has hybridized to the nucleotide barcode sequence at the adjacent barcode position;

(vi) identifying the signal code for the next position of the signal code sequence from the signal detected in (v);

(vii) repeating steps (iv) - (vi) to identify signal codes for subsequent signal code sequence positions until sufficient signal codes have been identified to decode the nucleotide barcode sequence, wherein detection probes for identifying the subsequent signal codes in the signal code sequence have (a) recognition sequences that are complementary to barcode subunit pairs that are present at subsequent sequential barcode positions in a nucleotide barcode sequence comprising multiple sequential adjacent barcode positions, or (b) recognition sequences that are complementary to the barcode subunit pairs that are present at the first and second barcode positions of a nucleotide barcode sequence comprising two adjacent barcode positions, and a reporter;

wherein the first barcode position can be any barcode position in the nucleotide barcode sequence.

27. A method of detecting a nucleic acid molecule, wherein the method comprises:

(a) assigning a unique signal code sequence to the nucleic acid molecule,

wherein the signal code sequence is specific to that molecule;

(b) providing the nucleic acid molecule with a nucleotide barcode sequence that corresponds to the signal code sequence, wherein the signal code sequence may be derived by interrogating the nucleotide barcode sequence with sequential detection probes each yielding a signal and the signals together make up the signal code sequence, and wherein the nucleotide barcode sequence comprises at least two adjacent barcode positions, each barcode position comprising a barcode subunit pair comprising a first barcode subunit and a second barcode subunit, wherein the second barcode subunit from each barcode position at least partially overlaps with the first barcode subunit of the adjacent barcode position in the sequence;

(c) performing steps (i) to (vii) as defined in claim 26 to generate a signal code sequence to decode the nucleotide barcode sequence and detect the nucleic acid molecule.

28. A method of decoding a nucleotide barcode sequence in a nucleic acid molecule to differentiate said nucleotide barcode sequence from other nucleotide barcode sequences, wherein a nucleotide barcode sequence comprises multiple sequential barcode positions, each barcode position partially but not fully overlapping the adjacent barcode position in the sequence and each barcode position comprising at least one barcode subunit, and wherein

each barcode subunit is selected from a panel of barcode subunits, such that the order of barcode subunits in each nucleotide barcode sequence defines a signal code sequence which comprises a signal code corresponding to each barcode position, and which is distinct from the signal code sequences of other nucleotide barcode sequences and identifies a given nucleotide barcode sequence, said method comprising;

(i) contacting the nucleic acid molecule with a first set of first detection probes for decoding the nucleotide barcode sequence at the first sequential barcode position and allowing the detection probes to hybridise to the nucleic acid molecule, wherein each detection probe in the set comprises a sequence complementary to a different barcode subunit within the panel and a reporter, such that each detection probe is capable of hybridising to and detecting a different barcode subunit at said first barcode position;

(ii) detecting a signal from the reporter of the first detection probe which has hybridized to the nucleotide barcode sequence at the first sequential barcode position;

(iii) identifying the signal code for the first barcode position from the signal detected in (ii);

(iv) contacting the nucleotide barcode sequence with a next set of subsequent detection probes for decoding the nucleotide barcode sequence at the next sequential barcode position and allowing the detection probes to hybridise to the nucleic acid molecule, wherein each detection probe comprises a sequence complementary to a different barcode subunit within the panel and a reporter, such that each detection probe is capable of hybridising to and detecting a different barcode subunit at said barcode position, and wherein the subsequent detection probe which hybridises to the barcode subunit at the next position hybridises to a sequence within the overlapping portion of the preceding barcode position, and thereby initiates a strand displacement reaction which displaces the hybridised preceding detection probe;

(v) detecting a signal from the reporter of the subsequent detection probe which has hybridized to the nucleotide barcode sequence at the next sequential barcode position;

(vi) identifying the signal code for the next barcode position from the signal detected in (v);

(vii) repeating steps (iv) - (vi) to identify signal codes for subsequent sequential barcode positions until sufficient signal codes have been identified to decode the nucleotide barcode sequence,

wherein the first sequential barcode position can be any barcode position in the nucleotide barcode sequence.

29. A method of detecting a target nucleic acid molecule, wherein the target nucleic acid molecule is detected using a padlock probe specific for said target molecule which is circularised upon hybridisation to a target sequence in the target nucleic acid molecule and which is provided with a nucleotide barcode sequence to identify the padlock probe, and wherein the circularised padlock probe is amplified by rolling circle amplification (RCA) to produce a rolling circle product (RCP), the RCP containing multiple complementary copies of the nucleotide barcode sequence, characterised in that the method comprises;

(a) assigning a unique signal code sequence to the target nucleic acid molecule, wherein the signal code sequence is specific to that molecule;

(b) providing the padlock probe with a nucleotide barcode sequence such that a RCP is formed comprising multiple complementary copies of the nucleotide barcode sequence that each corresponds to the signal code sequence, wherein the signal code sequence may be derived by interrogating the complementary copies of the nucleotide barcode sequence with sequential detection probes each yielding a signal and the signals together make up the signal code sequence;

(c) contacting the RCP with a first detection probe for generating a signal code for the first position of the signal code sequence and allowing the detection probe to hybridise to the RCP at the complementary copies of the nucleotide barcode sequence, wherein the detection probe comprises a recognition sequence complementary to the nucleotide barcode sequence and a reporter;

(d) detecting a signal from the reporter of the first detection probe which has hybridized to the RCP;

(e) identifying the first signal code for the first position in the signal code sequence for the RCP from the signal detected in (d);

(f) removing the first detection probe from the RCP;

(g) contacting the RCP with a subsequent detection probe for generating a signal code for the next position of the signal code sequence, and allowing the detection probe to hybridise to the nucleic acid molecule at the complementary copies of the nucleotide

barcode sequence, wherein the detection probe comprises a recognition sequence complementary to the nucleotide barcode sequence and a reporter;

(h) detecting a signal from the reporter of the subsequent detection probe which has hybridized to the RCP;

5 (i) identifying the signal code for the next position in the signal code sequence for the RCP from the signal detected in (g);

(j) removing the subsequent detection probe from the RCP;

(k) repeating steps (g) - (j) to identify signal codes for subsequent signal code positions until sufficient signal codes have been identified to identify the nucleotide barcode sequence and thereby to detect the nucleic acid molecule.

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