CLAIMS

1. A method of detecting an analyte in a sample, said method comprising:

(i) performing a linear oligo hybridization chain reaction (LO-HCR), wherein an initiator is contacted with a plurality of LO-HCR monomers ~~of at least~~ comprising a first and a second species to generate a polymeric LO-HCR product hybridized to a target nucleic acid molecule, wherein the polymeric LO-HCR product comprises a plurality of the first species and a plurality of the second species,

wherein the first species comprises a first hybridization region complementary to the initiator and a second hybridization region complementary to the second species,

wherein the first species and the second species are linear, single-stranded nucleic acid molecules,

wherein the target nucleic acid molecule is a rolling circle amplification product (RCP) generated from the analyte or from a probe that direct or indirectly binds to the analyte, and

wherein the initiator is provided in one or more parts, and ~~hybridizes~~ directly or indirectly binds to or is comprised in the target nucleic acid molecule; and

(ii) detecting the polymeric LO-HCR product in the sample, thereby detecting the analyte in the sample.

2. The method of claim 1, wherein performing the linear oligo hybridization chain reaction comprises contacting the target nucleic acid molecule with the initiator to provide the initiator hybridized to the target nucleic acid molecule.

3. The method of claim 1 or claim 2, wherein the first species and/or the second species do not comprise a hairpin structure.

4. The method of any of claims 1 to 3, wherein the plurality of LO-HCR monomers do not comprise a metastable secondary structure.

5. The method of any of claims 1 to 4, wherein the plurality of LO-HCR monomers do not comprise a repeating sequence of more than about 5, about 10, or about 20 nucleotides in length.

6. The method of any one of claims 1 to 5, wherein the LO-HCR polymer does not comprise a branched structure.

7. The method of any of claims 1 to 6, wherein the LO-HCR monomers are between about 10 and about 100 nucleotides in length.

8. The method of any of claims 1 to 7, wherein an LO-HCR monomer of the first species hybridizes to an LO-HCR monomer of the second species, which in turn hybridizes to an LO-HCR monomer of the first species.

9. The method of any of claims 1 to 7, wherein an LO-HCR monomer of the first species hybridizes to an LO-HCR monomer of the second species, which in turn hybridizes to an LO-HCR monomer of a third species, which in turn hybridizes to an LO-HCR monomer of a fourth species, which in turn hybridizes to an LO-HCR monomer of the first species.

10. The method of any of claims 1 to 9, wherein the analyte is a cellular nucleic acid molecule ~~and wherein the target nucleic acid molecule is the cellular nucleic acid molecule or a nucleic acid molecule generated from the cellular nucleic acid molecule~~.

11. The method of claim 10, wherein the target nucleic acid molecule comprises ~~a reporter comprising~~ a marker sequence indicative of said cellular nucleic acid molecule.

12. The method of claim 11, wherein the initiator hybridizes ~~directly or indirectly~~ to or is comprised in the marker sequence.

13. The method of any one of claims 10 to 12, wherein the cellular nucleic acid molecule is a DNA or a RNA molecule~~, including gDNA, mRNA, miRNA, lncRNA~~.

14. The method of any one of claims 10 to 13, wherein the <u>cellular</u> nucleic acid molecule is a cDNA ~~molecule generated from the cellular nucleic acid molecule, an amplified product comprising a sequence of said cellular nucleic acid molecule, or a nucleic acid probe or component of a probe that targets said cellular nucleic acid molecule~~<u>, gDNA, mRNA, miRNA, or lncRNA</u>.

15. The method of any of claims 10 to 12, wherein the analyte is a non-nucleic acid analyte, optionally a lipid or a protein, and wherein the target nucleic acid molecule comprises ~~a reporter comprising~~ a marker sequence indicative of said non-nucleic acid analyte.

16. The method of any one of claims 1 to 15, wherein the sample comprises cells, wherein optionally the cells are immobilized, fixed or in suspension.

17. The method of any one of claims 1 to 16, wherein the analyte is detected *in situ* in a tissue sample, optionally in single cells.

18. The method of any one of claims 1 to 17, wherein the method is used to detect multiple ~~target~~ analytes in a sample, and at least one target nucleic acid molecule is provided for each ~~target~~ analyte.

19. The method of claim 18, wherein multiple polymeric LO-HCR products are generated for each target ~~analyte~~ nucleic acid molecule.

20. The method of any one of claims 11 to ~~17~~19, wherein the target nucleic acid molecule comprises multiple copies of the marker sequence, such that multiple LO-HCR reactions can be initiated from each target nucleic acid molecule, and/or wherein multiple target nucleic acid molecules are provided for each ~~target~~ analyte.

~~21. The method of any one of claims 1 to 20, wherein the target nucleic acid molecule is a hybridization probe which is capable of hybridizing to the analyte.~~

~~22. The method of claim 21, wherein the hybridization probe comprises a target binding domain, which is capable of hybridizing to the target analyte, and an initiator binding domain which comprises at least one copy of a marker sequence indicative of said analyte.~~

~~23. The method of any one of claims 1 to 22, wherein the target nucleic acid molecule is a ligatable probe provided in one or more parts, including a padlock probe or a probe comprising multiple component parts which are capable of hybridizing to a target analyte such that they can be ligated together.~~

~~24. The method of any one of claims 1 to 23, wherein the target nucleic acid molecule is a rolling circle amplification product (RCP) generated from the analyte or from a reporter for the target analyte.~~

~~25~~21. The method of any one of claims 1 to ~~24~~20, wherein the initiator is provided in two or more parts.

~~26~~22. The method of any one of claims 1 to ~~25~~21, wherein the initiator is in the form of an initiator complex which is capable of supporting multiple LO-HCR reactions, such that multiple LO-HCR reactions can be initiated from each marker sequence.

~~27~~23. The method of any one of claims 1 to ~~26~~22, wherein performing the LO-HCR reaction comprises:

(a) contacting the target nucleic acid molecule with the initiator to provide the initiator hybridized to the target nucleic acid molecule, wherein the initiator comprising an output domain complementary to the first hybridization region of the first species LO-HCR monomer;

(b) hybridizing the first species of LO-HCR monomer to the initiator, wherein the first species optionally comprises a detectable label;

(c) hybridizing the second species of LO-HCR monomer to the second hybridization region of the first species of LO-HCR monomer, wherein the second species optionally comprises a detectable label; and

(d) repeating steps (b) to (c) one or more times to generate a detectable polymeric LO-HCR product.

~~28~~24. The method of any one of claims 1 to ~~27~~23, wherein performing the LO-HCR reaction comprises a step of contacting the target nucleic acid molecule simultaneously with the at least first and second species of LO-HCR monomers.

~~29~~25. The method of any one of claims 1 to ~~28~~24, wherein at least a fraction of the plurality of LO-HCR monomers in the LO-HCR reaction are labelled with a detectable label, optionally wherein the first species of LO-HCR monomer is provided with a detectable label.

~~30~~26. The method of claim ~~29~~25, wherein the detectable label is a fluorescent label.

~~31~~27. The method of any one of claims 1 to ~~30~~26, wherein the first and/or second species of LO-HCR monomers comprise an overhang region capable of facilitating a displacement reaction to depolymerize the polymeric LO-HCR product.

~~32~~28. The method of any one of claims 1 to ~~31~~27, wherein for each target nucleic acid molecule, multiple sequential LO-HCR reactions are performed, and wherein detection of the analyte comprises detection of signals from the multiple sequential LO-HCR reactions.

~~33~~29. The method of claim ~~32~~28, wherein sequential LO-HCR reactions are performed using differentially labelled LO-HCR monomer sets, such that each target nucleic acid molecule is combinatorially labelled to detect the analyte.

~~34~~30. A kit for detecting a nucleic acid or non-nucleic acid ~~target~~ analyte in a sample, said kit comprising:

(i) a set of LO-HCR monomers for assembly into an LO-HCR product comprising at least first and second LO-HCR monomers, wherein the first LO-HCR monomers have an input domain complementary to the initiator domain of an initiator and the output domain of the second LO-HCR monomer, and an output domain complementary to the input domain of the second LO-HCR monomer; and wherein the second LO-HCR monomers have an input domain complementary to the output domain of the first LO-HCR monomer and an output domain complementary to the input domain of the first or a subsequent LO-HCR monomer, said LO-HCR monomers being capable of being hybridized together to form an LO-HCR product,

wherein said LO-HCR monomers each is a single-stranded linear oligonucleotide having no metastable secondary structure; and

(ii) a nucleic acid probe that directly or indirectly binds to the analyte, wherein the nucleic acid probe comprises a marker sequence or a complement thereof indicative of said analyte,

wherein the nucleic acid probe is a circular or circularizable nucleic acid molecule.

~~35~~31. The kit of claim ~~34~~30, wherein said kit further comprises ~~one or more of~~:

~~(ii) a nucleic acid probe for detecting a target analyte, wherein the probe comprises a marker sequence indicative of said analyte;~~

~~(iii)~~ an HCR initiator in one or more parts which is capable of hybridizing to ~~a marker sequence in the probe or in the target analyte~~ the marker sequence or complement thereof; and/or

~~(iv)~~ reagents for performing an RCA reaction.

~~36. The kit of claim 34 or 35, wherein the nucleic acid probe is a circular or circularizable nucleic acid molecule.~~

32. The method of any one of claims 1 to 29, wherein the sample is a cell or tissue sample, and the analyte is detected in or at its location in the sample.