

Claims

1. A method for detection of a target ~~nucleic acid~~ RNA in a sample, comprising:
 - (a) contacting said sample with a hairpin probe comprising:
 - (i) a first domain capable of hybridizing to a target sequence in said target ~~nucleic acid~~ RNA; and
 - (ii) a second domain capable of hybridizing to a ~~circular or~~ circularizable probe;

wherein the second domain is at least partly comprised within a duplex region of the hairpin probe, and

wherein hybridization of said first domain to said target sequence causes the duplex region to open, thereby exposing said second domain or part thereof;
 - (b) contacting said sample with the ~~circular or~~ circularizable probe, thereby hybridizing the ~~circular or~~ circularizable probe to the second domain;

wherein the circularizable probe comprises 3' and 5' end regions complementary to sequences in the second domain such that said end regions are hybridized in juxtaposition for ligation, directly or indirectly, to each other to generate a circularized probe;
 - (c) performing a rolling circle amplification (RCA) reaction using the ~~circular probe or a circularized probe comprising a sequence of the circularizable probe of (b);~~ and
 - (d) detecting a product of said RCA reaction.
2. ~~The method of claim 1, wherein the target nucleic acid is a target RNA.~~
- [[3]]2. The method of claim 1 ~~or claim 2~~, wherein step (a) comprises contacting said sample with a probe set comprising two or more hairpin probes, and wherein the first domain of each hairpin probe in the probe set comprises a region of complementarity to a different target sequence in the target ~~nucleic acid~~ RNA.
- [[4]]3. The method of ~~any one of claims 1-3~~ claim 1 or claim 2, wherein step (a) comprises contacting the sample with a probe set comprising two or more hairpin probes, and wherein the first domain of each hairpin probe in the probe set comprises a region of complementarity to a target sequence in a different target ~~nucleic acid~~ RNA.

[[5]]4. The method of any one of claims [[1-4]]1-3, wherein the hairpin probe comprises 5' to 3':

- (i) a single stranded region at the 5' end;
- (ii) a first strand of the duplex region;
- (iii) a single stranded loop region; and
- (iv) a second strand of the duplex region at the 3' end.

[[6]]5. The method of claim 4 ~~or claim 5~~, wherein the first domain comprises all or a portion of the single stranded region at the 5' end and all or a portion of the first strand of the duplex region, and wherein the single stranded region at the 5' end acts as a toehold for hybridizing to the target ~~nucleic acid~~ RNA.

[[7]]6. The method of ~~any one of claims 4 to 6~~ claim 4 or claim 5, wherein the second domain comprises all or a portion of the second strand of the duplex region at the 3' end.

[[8]]7. The method of any one of claims [[4 to 7]]4 to 6, wherein the second domain comprises all or a portion of the single stranded loop region.

[[9]]8. The method of any one of claims 1-[[4]]3, wherein the hairpin probe comprises from 5' to 3':

- (i) a first strand of the duplex region at the 5' end;
- (ii) a single stranded loop region; and
- (iii) a second strand of the duplex region at the 3' end.

[[10]]9. The method of claim [[9]]8, wherein the first domain lies in the single stranded loop region and the second domain lies in the second strand of the duplex region at the 3' end.

[[11]]10. The method of any one of claims 1 to [[10]]9, wherein the duplex region comprises one or more mismatches.

[[12]]11. The method of any one of claims 1 to [[11]]10, wherein step (b) comprises contacting said sample with two or more ~~circular or~~ circularizable probes, each ~~circular or~~ circularizable probe being cognate for a different hairpin probe.

[[13]]12. The method of any one of claims 1 to [[12]]11, wherein step (b) comprises contacting the sample with two or more ~~circular or~~ circularizable probes that hybridize to the same second domain of each member of a set of hairpin probes.

[[14]]13. The method of any one of claims 1 to [[13]]12, wherein step (b) comprises contacting said sample with two or more ~~circular or~~ circularizable probes, each ~~circular or~~ circularizable probe being cognate for a different set of hairpin probes.

[[15]]14. The method of any one of claims 1 to [[14]]13, wherein the target ~~nucleic acid~~ RNA is mRNA.

[[16]]15. The method of any one of claims 1 to [[15]]14, wherein the ~~circular or~~ circularizable probes for each target ~~nucleic acid~~ RNA comprise a different reporter domain, and optionally wherein the reporter domain in each ~~circular or~~ circularized probe is separate from a region complementary to the second domain.

[[17]]16. The method of any one of claims 1 to [[16]]15, wherein the ~~circular or~~ circularizable probe for each target sequence in a given target ~~nucleic acid~~ RNA comprises a different reporter domain or the same reporter domain.

[[18]]17. The method of any one of claims 1 to [[17]]16, wherein the RCA product is detected using a detection oligonucleotide which hybridizes specifically to the RCA product, by using a nucleic acid stain or by using labelled nucleotides for incorporation into the RCA product, and wherein the detection oligonucleotide is directly or indirectly detectable.

19. — ~~The method of any one of claims 1 to 18, wherein the circularizable probe comprises 3' and 5' end regions complementary to sequences in the second domain such that said ends are hybridized in juxtaposition for ligation, directly or indirectly, to each other to generate the circularized probe.~~

[[20]]18. The method of any one of claims 1 to [[19]]17, wherein the second domain or part thereof is not capable of binding to said ~~circular or~~ circularizable probe and/or priming the RCA reaction until said duplex region is opened to expose the second domain or part thereof.

[[21]]19. The method of any one of claims 1 to [[20]]18, wherein the second domain or part thereof is not capable of hybridizing to the target sequence in the target ~~nucleic acid~~ RNA.

[[22]]20. The method of any one of claims 1 to [[21]]19, wherein:
the sample is [[a cell or]] a cell or tissue sample,
the ~~circular or~~ circularized probe is barcoded,
the RCA reaction is performed *in situ* in the sample using the ~~circular or~~ circularized probe hybridized to the second domain as a template, and
the RCA reaction is primed by the second domain.

[[23]]21. A hairpin probe for use in detecting a target RNA ~~molecule~~, said hairpin probe comprising:

(i) a first domain which comprises a first region of complementarity to a target sequence in [[a]] the target RNA and is capable of hybridizing to [[said]] the target RNA;

(ii) a second domain which is not capable of hybridizing to the target RNA, which comprises a second region of complementarity to a cognate ~~circular or~~ circularizable probe, and which is capable of acting as a primer for an RCA reaction;

wherein the second domain is at least partly comprised within a hairpin structure of the hairpin probe, such that the second domain is not capable of binding to said cognate ~~circular or~~ circularizable probe and/or priming an RCA reaction until released from said hairpin structure, and wherein the hairpin structure is such that hybridization of said first domain to said target sequence causes the hairpin structure to open and release said second domain.

[[24]]22. A probe set comprising two or more target-specific hairpin probes as defined in claim [[23]]21, each target-specific hairpin probe being specific for a different target sequence in the same target RNA, wherein the first domain of each target-specific hairpin probe in the probe set comprises a region of complementarity to a different target sequence in the target RNA.

[[25]]23. A kit comprising:

(i) a target-specific hairpin probe as defined in claim [[23]]21, or a probe set as defined in claim [[24]]22; and

(ii) a ~~circular or~~ circularizable probe cognate for said hairpin probe, or ~~circular or~~ circularizable probes cognate for the hairpin probes in the probe set, each ~~circular or~~ circularizable probe comprising ~~one or more regions complementary to the second region of complementarity in the second domain of said hairpin probe(s), wherein if the probe is a~~ circularizable probe, it comprises 3' and 5' end regions complementary to the second region of complementarity in the second domain, such that said end regions ~~[[ends]]~~ can hybridize in juxtaposition for ligation, directly or indirectly, to each other.

~~[[26]]~~24. The kit of claim ~~[[25]]~~23, wherein the kit comprises (i) a set of ~~circular or~~ circularizable probes, wherein each member of the ~~circular or~~ circularizable probe set is cognate for a different hairpin probe, or (ii) one or more ~~circular or~~ circularizable probes wherein each circularizable probe is cognate for all members of a given hairpin probe set.