

CLAIMS

1. — A method for pairwise sequencing of first and second regions of a target nucleic acid, wherein the first and second regions are in complementary strands of the target nucleic acid, the method comprising:

- (a) providing an array comprising a plurality of amplification sites,
- wherein the amplification sites comprise (i) a plurality of capture nucleic acids, and (ii) a plurality of clonal single-stranded amplicons,
- wherein each single-stranded amplicon is attached at its 5' end to a capture nucleic acid
- wherein the capture nucleic acids comprise a cleavage site;
- (b) hybridizing a first sequencing primer to a universal sequence present on each single-stranded amplicon;
- (c) carrying out a first sequencing reaction by sequential addition of nucleotides to the first sequencing primer using the single-stranded amplicon as a first template to determine the sequence of a first region and to produce a complementary strand of the first region;
- (d) extending the complementary strand of the first region to form a double-stranded amplicon that comprises the first sequencing primer, nucleotides incorporated during the sequencing reaction, and nucleotides incorporated during the extending;
- (e) cleaving the capture nucleic acid attached to the single-stranded amplicons,
- wherein the cleavage converts the single-stranded amplicons into (i) shortened capture nucleic acids and (ii) unattached first templates that are not attached at the 5' end to the capture nucleic acid;
- (f) carrying out a second sequencing reaction by sequential addition of nucleotides to a second sequencing primer hybridized to the complementary strand and using the complementary strand as a second template to determine the sequence of a second region.

2. — The method of claim 1, wherein the cleavage site permits enzymatic, chemical, or photochemical cleavage.

3. — The method of claim 2, wherein cleavage site is a site for cleavage with a nicking endonuclease.

4. ~~The method of claim 2, wherein the cleaving comprises contacting the array with a composition comprising at least one enzyme to produce an abasic site at the cleavage site, wherein cleavage occurs at the cleavage site.~~
5. ~~The method of claim 4, wherein the capture nucleic acids comprise a uracil base or an 8-oxo-guanine base.~~
6. ~~The method of claim 4 or 5, wherein the at least one enzyme to produce an abasic site at the cleavage site comprises uracil DNA glycosylase and an endonuclease selected from DNA glycosylase-lyase Endonuclease VIII or FpG glycosylase.~~
7. ~~The method of claim 1, wherein the first sequencing primer is in solution.~~
8. ~~The method of claim 7, wherein the 5' end of the first sequencing primer is blocked.~~
9. ~~The method of claim 1, wherein the second sequencing primer comprises the 3' end of the cleavage site.~~
10. ~~The method of claim 6, wherein the cleaving with an enzyme results in the 3' end of the cleavage sites terminating with a 3' phosphate, the method further comprising contacting the array with a phosphatase to result in the cleavage sites terminating with a 3' OH.~~
11. ~~The method of claim 9 or 10, wherein the second sequencing reaction comprises use of a DNA polymerase with strand displacing activity.~~
12. ~~The method of claim 1, wherein the second sequencing primer is in solution.~~
13. ~~The method of claim 12, wherein the second sequencing reaction comprises subjecting the cleaved double-stranded amplicons to conditions that remove the unattached first templates,~~

and hybridizing the second sequencing primer to a universal sequence present on the complementary strands.

14. — The method of claim 13, wherein the removal of the unattached first templates comprises contacting the array with an exonuclease comprising a 5' to 3' exonuclease activity.

15. — The method of claim 14, wherein the exonuclease is T7 exonuclease.

16. — The method of claim 1, wherein at least 95% of the amplification sites on the array comprise clonal single stranded amplicons derived from amplification of different single target nucleic acids.

17. — The method of claim 1, wherein the first sequencing reaction determines sequences of regions of the single stranded amplicons, wherein the second sequencing reaction determines sequences of regions of the complementary strands, and wherein the regions of the single stranded amplicons are complementary to the regions of the complementary strands.

18. — The method of claim 1, wherein the first sequencing reaction determines sequences of regions of the single stranded amplicons, wherein the second sequencing reaction determines sequences of regions of the complementary strands, and wherein the regions of the single stranded amplicons are not complementary to the regions of the complementary strands.

19. — The method of claim 1, wherein the nucleotides incorporated during (d) or (f) comprise fully functional nucleotides.

20. — The method of claim 1, wherein the first sequencing reaction comprises sequential addition of a predetermined number of nucleotides to the first sequencing primer.

21. — The method of claim 1, wherein the second sequencing reaction comprises sequential addition of a predetermined number of nucleotides to the second sequencing primer.

22. ~~The method of claim 1, wherein the capture nucleic acids, the first sequencing primer, or the second sequencing primer comprise at least one non-nucleotide chemical moiety, non-naturally occurring nucleotide, or non-naturally occurring backbone linkage.~~
23. ~~The method of claim 1, wherein the single stranded amplicons comprise two indexes.~~
24. ~~The method of claim 23, further comprising sequencing the indexes.~~
25. ~~The method of claim 24, wherein the first index is sequenced after (c).~~
26. ~~The method of claim 24, wherein the second index is sequenced after (f).~~
27. ~~The method of claim 1, wherein the array is produced by a method comprising:~~  
~~—— (a) providing an amplification reagent comprising~~  
~~—— (i) the array of amplification sites,~~  
~~—— (ii) a solution comprising a plurality of different target nucleic acids and a primer,~~  
~~—— wherein each of the amplification sites comprises the plurality of capture nucleic acids capable of hybridizing to a universal sequence present on the different target nucleic acids in the solution,~~  
~~—— wherein the number of the different target nucleic acids in the solution exceeds the number of amplification sites in the array,~~  
~~—— wherein the different target nucleic acids have fluidic access to the plurality of amplification sites, and~~  
~~—— wherein each of the amplification sites comprises a capacity for several nucleic acids in the plurality of different target nucleic acids; and~~  
~~—— (b) reacting the amplification reagent to produce a plurality of amplification sites that each comprise a clonal population of double-stranded amplicons from an individual target nucleic acid from the solution,~~  
~~—— wherein the double stranded amplicons comprise a first strand that is attached at its 5' end to a capture nucleic acid and a second strand that is not attached to the amplification site,~~  
~~—— wherein the reacting comprises simultaneously~~

———(i) transporting the different target nucleic acids to the amplification sites at an average transport rate, and

———(ii) amplifying the target nucleic acids that are at the amplification sites at an average amplification rate, wherein the average amplification rate exceeds the average transport rate.

28.—— The method of claim 27, further comprising subjecting the double stranded amplicons to conditions that remove the second strand that is not attached to the amplification site.

29.—— The method of claim 28, wherein the conditions that remove the second strand comprise a denaturant.

30.—— The method of claim 29, wherein the denaturant comprises formamide.

31.—— The method of claim 27, wherein the solution comprises a molecular crowding agent.

32.—— The method of claim 27, wherein the primer hybridizes to a universal sequence present on each single stranded amplicon.

33.—— A composition comprising an array of amplification sites,

———wherein amplification sites comprise a plurality of clonal double stranded amplicons,

———wherein each double stranded amplicon comprises a first strand attached to the surface of the amplification site by the 5' end and comprising a break in the backbone, and a second strand that is not attached to the surface of the amplification site and comprises nucleotides that are complementary to and hybridized to nucleotides of the first strand,

———wherein the break in the backbone of the first strand is flanked on both sides by complementary nucleotides of the second strand.

34.—— The composition of claim 33, wherein at least 95% of the amplification sites on the array comprise clonal single stranded amplicons.

35. — ~~The composition of claim 33, wherein the break in the backbone comprises a break in first strand comprises at least one missing a phosphodiester bond.~~

36. — ~~The composition of claim 33, wherein the first strand comprises non-naturally occurring backbone linkage.~~

37. — ~~The composition of claim 36, wherein the break in the backbone comprises a break in first strand comprises at least one missing non-naturally occurring backbone linkage.~~

38. — ~~The composition of claim 33, wherein break in the backbone comprises at least one abasic site.~~

39. — ~~The composition of claim 33, wherein the second strand is complementary to less than all nucleotides of the first strand~~

40. — ~~The composition of claim 33, wherein the 5' end of the second strand comprises a 5' block.~~

41. — ~~The composition of claim 33, wherein the break in the backbone is located from 5 to 50 nucleotides from the attached 5' end.~~

42. — ~~The composition of claim 33, wherein the first strand comprises at least 5 nucleotides located 5' of the break that are hybridized to the second strand.~~

43. — ~~A compound, composition, or method including one or more features described herein.~~

~~AMENDED CLAIMS~~~~received by the International Bureau on 05 April 2020 (05.04.2020)~~

- ~~1. A method for pairwise sequencing of first and second regions of a target nucleic acid, wherein the first and second regions are in complementary strands of the target nucleic acid, the method comprising:
  - ~~(a) providing an array comprising a plurality of amplification sites, wherein the amplification sites comprise (i) a plurality of capture nucleic acids, and (ii) a plurality of clonal single-stranded amplicons, wherein each single-stranded amplicon is attached at its 5' end to a capture nucleic acid wherein the capture nucleic acids comprise a cleavage site,~~
  - ~~(b) hybridizing a first sequencing primer to a universal sequence present on each single-stranded amplicon,~~
  - ~~(c) carrying out a first sequencing reaction by sequential addition of nucleotides to the first sequencing primer using the single-stranded amplicon as a first template to determine the sequence of a first region and to produce a complementary strand of the first region,~~
  - ~~(d) extending the complementary strand of the first region to form a double-stranded amplicon that comprises the first sequencing primer, nucleotides incorporated during the sequencing reaction, and nucleotides incorporated during the extending, wherein the complementary strand of the first region is not attached to the surface of the amplification site,~~
  - ~~(e) cleaving the capture nucleic acid attached to the single-stranded amplicons, wherein the cleavage converts the single-stranded amplicons into (i) shortened capture nucleic acids and (ii) unattached first templates that are not attached at the 5' end to the capture nucleic acid,~~
  - ~~(f) carrying out a second sequencing reaction by sequential addition of nucleotides to a second sequencing primer hybridized to the complementary strand and using the complementary strand as a second template to determine the sequence of a second region, wherein the second sequencing primer is in solution, and wherein the second sequencing reaction comprises subjecting the cleaved double-stranded amplicons to conditions that remove the unattached first templates, and hybridizing the second sequencing primer to a universal sequence present on the complementary strands.~~~~

- ~~2. The method of claim 1, wherein the cleavage site permits enzymatic, chemical, or photochemical cleavage.~~
- ~~3. The method of claim 2, wherein cleavage site is a site for cleavage with a nicking endonuclease.~~
- ~~4. The method of claim 2, wherein the cleaving comprises contacting the array with a composition comprising at least one enzyme to produce an abasic site at the cleavage site, wherein cleavage occurs at the cleavage site.~~
- ~~5. The method of claim 4, wherein the capture nucleic acids comprise a uracil base or an 8-oxo-guanine base.~~
- ~~6. The method of claim 4 or 5, wherein the at least one enzyme to produce an abasic site at the cleavage site comprises uracil DNA glycosylase and an endonuclease selected from DNA glycosylase lyase Endonuclease VIII or FpG glycosylase.~~
- ~~7. The method of claim 1, wherein the first sequencing primer is in solution.~~
- ~~8. The method of claim 7, wherein the 5' end of the first sequencing primer is blocked.~~
- ~~9. The method of claim 1, wherein the second sequencing primer comprises the 3' end of the cleavage site.~~
- ~~10. The method of claim 6, wherein the cleaving with an enzyme results in the 3' end of the cleavage sites terminating with a 3' phosphate, the method further comprising contacting the array with a phosphatase to result in the cleavage sites terminating with a 3'-OH.~~
- ~~11. The method of claim 9 or 10, wherein the second sequencing reaction comprises use of a DNA polymerase with strand displacing activity.~~
- ~~12-13. Cancelled~~

~~14. The method of claim 13, wherein the removal of the unattached first templates comprises contacting the array with an exonuclease comprising a 5' to 3' exonuclease activity.~~

~~15. The method of claim 14, wherein the exonuclease is T7 exonuclease.~~

~~16. The method of claim 1, wherein at least 95% of the amplification sites on the array comprise clonal single stranded amplicons derived from amplification of different single target nucleic acids.~~

~~17. The method of claim 1, wherein the first sequencing reaction determines sequences of regions of the single stranded amplicons, wherein the second sequencing reaction determines sequences of regions of the complementary strands, and wherein the regions of the single stranded amplicons are complementary to the regions of the complementary strands.~~

~~18. The method of claim 1, wherein the first sequencing reaction determines sequences of regions of the single stranded amplicons, wherein the second sequencing reaction determines sequences of regions of the complementary strands, and wherein the regions of the single stranded amplicons are not complementary to the regions of the complementary strands.~~

~~19. The method of claim 1, wherein the nucleotides incorporated during (d) or (f) comprise fully functional nucleotides.~~

~~20. The method of claim 1, wherein the first sequencing reaction comprises sequential addition of a predetermined number of nucleotides to the first sequencing primer.~~

~~21. The method of claim 1, wherein the second sequencing reaction comprises sequential addition of a predetermined number of nucleotides to the second sequencing primer.~~

~~22. The method of claim 1, wherein the capture nucleic acids, the first sequencing primer, or the second sequencing primer comprise at least one non-nucleotide chemical moiety, non-naturally occurring nucleotide, or non-naturally occurring backbone linkage.~~

~~23. The method of claim 1, wherein the single stranded amplicons comprise two indexes.~~

- ~~24. The method of claim 23, further comprising sequencing the indexes.~~
- ~~25. The method of claim 24, wherein the first index is sequenced after (c).~~
- ~~26. The method of claim 24, wherein the second index is sequenced after (f).~~
- ~~27. The method of claim 1, wherein the array is produced by a method comprising:~~
- ~~(a) providing an amplification reagent comprising
    - ~~(i) the array of amplification sites,~~
    - ~~(ii) a solution comprising a plurality of different target nucleic acids and a primer,~~~~
  - ~~wherein each of the amplification sites comprises the plurality of capture nucleic acids capable of hybridizing to a universal sequence present on the different target nucleic acids in the solution,~~
  - ~~wherein the number of the different target nucleic acids in the solution exceeds the number of amplification sites in the array,~~
  - ~~wherein the different target nucleic acids have fluidic access to the plurality of amplification sites, and~~
  - ~~wherein each of the amplification sites comprises a capacity for several nucleic acids in the plurality of different target nucleic acids; and~~
  - ~~(b) reacting the amplification reagent to produce a plurality of amplification sites that each comprise a clonal population of double-stranded amplicons from an individual target nucleic acid from the solution,~~
  - ~~wherein the double-stranded amplicons comprise a first strand that is attached at its 5' end to a capture nucleic acid and a second strand that is not attached to the amplification site,~~
  - ~~wherein the reacting comprises simultaneously~~
    - ~~(i) transporting the different target nucleic acids to the amplification sites at an average transport rate, and~~
    - ~~(ii) amplifying the target nucleic acids that are at the amplification sites at an average amplification rate, wherein the average amplification rate exceeds the average transport rate.~~

~~28. The method of claim 27, further comprising subjecting the double stranded amplicons to conditions that remove the second strand that is not attached to the amplification site.~~

~~29. The method of claim 28, wherein the conditions that remove the second strand comprise a denaturant.~~

~~30. The method of claim 29, wherein the denaturant comprises formamide.~~

~~31. The method of claim 27, wherein the solution comprises a molecular crowding agent.~~

~~32. The method of claim 27, wherein the primer hybridizes to a universal sequence present on each single stranded amplicon.~~

~~33. A composition comprising an array of amplification sites,  
wherein amplification sites comprise a plurality of clonal double stranded amplicons,  
wherein each double stranded amplicon comprises a first strand attached to the surface of the amplification site by the 5' end and comprising a break in the backbone, and a second strand that is not attached to the surface of the amplification site and comprises nucleotides that are complementary to and hybridized to nucleotides of the first strand,  
wherein the break in the backbone of the first strand is flanked on both sides by complementary nucleotides of the second strand, and  
wherein the break in the backbone is located from 5 to 50 nucleotides from the attached 5' end.~~

~~34. The composition of claim 33, wherein at least 95% of the amplification sites on the array comprise clonal single stranded amplicons.~~

~~35. The composition of claim 33, wherein the break in the backbone comprises a break in first strand comprises at least one missing a phosphodiester bond.~~

~~36. The composition of claim 33, wherein the first strand comprises non-naturally occurring backbone linkage.~~

~~37. The composition of claim 36, wherein the break in the backbone comprises a break in first strand comprises at least one missing non-naturally occurring backbone linkage.~~

~~38. The composition of claim 33, wherein break in the backbone comprises at least one abasic site.~~

~~39. The composition of claim 33, wherein the second strand is complementary to less than all nucleotides of the first strand~~

~~40. The composition of claim 33, wherein the 5' end of the second strand comprises a 5' block.~~

~~41. Cancelled~~

~~42. The composition of claim 33, wherein the first strand comprises at least 5 nucleotides located 5' of the break that are hybridized to the second strand.~~

~~43. Cancelled~~

~~44. A composition comprising an array of amplification sites,~~

~~wherein amplification sites comprise a plurality of clonal double-stranded amplicons,~~

~~wherein each double-stranded amplicon comprises a first strand attached to the surface of the amplification site by the 5' end and comprising a break in the backbone, and a second strand that is not attached to the surface of the amplification site and comprises nucleotides that are complementary to and hybridized to nucleotides of the first strand,~~

~~wherein the break in the backbone of the first strand is flanked on both sides by complementary nucleotides of the second strand, and~~

~~wherein the first strand comprises at least 5 nucleotides located 5' of the break that are hybridized to the second strand.~~

~~45. The composition of claim 44, wherein at least 95% of the amplification sites on the array comprise clonal single-stranded amplicons.~~

~~46. The composition of claim 44, wherein the break in the backbone comprises a break in first strand comprises at least one missing a phosphodiester bond.~~

~~47. The composition of claim 44, wherein the first strand comprises non-naturally occurring backbone linkage.~~

~~48. The composition of claim 47, wherein the break in the backbone comprises a break in first strand comprises at least one missing non-naturally occurring backbone linkage.~~

~~49. The composition of claim 44, wherein break in the backbone comprises at least one abasic site.~~

~~50. The composition of claim 44, wherein the second strand is complementary to less than all nucleotides of the first strand~~

~~51. The composition of claim 44, wherein the 5' end of the second strand comprises a 5' block.~~

~~52. The composition of claim 44, wherein the break in the backbone is located from 5 to 50 nucleotides from the attached 5' end.~~

## CLAIMS

1. A method for pairwise sequencing of first and second regions of a target nucleic acid, wherein the first and second regions are in complementary strands of the target nucleic acid, the method comprising:

\_\_\_\_\_ (a) providing an array comprising a plurality of amplification sites,  
\_\_\_\_\_ wherein the amplification sites comprise (i) a plurality of capture nucleic acids, and (ii) a plurality of clonal single-stranded amplicons,

\_\_\_\_\_ wherein each single-stranded amplicon is attached at its 5' end to a capture nucleic acid,  
\_\_\_\_\_ wherein the capture nucleic acids comprise a cleavage site;

\_\_\_\_\_ (b) hybridizing a first sequencing primer to a universal sequence present on each single-stranded amplicon;

\_\_\_\_\_ (c) carrying out a first sequencing reaction by sequential addition of nucleotides to the first sequencing primer using the single-stranded amplicon as a first template to determine the sequence of a first region and to produce a complementary strand of the first region;

\_\_\_\_\_ (d) extending the complementary strand of the first region to form a double-stranded amplicon that comprises the first sequencing primer, nucleotides incorporated during the sequencing reaction, and nucleotides incorporated during the extending;

\_\_\_\_\_ (e) cleaving the capture nucleic acids attached to the single-stranded amplicons,  
\_\_\_\_\_ wherein the cleavage converts the single-stranded amplicons into (i) shortened capture nucleic acids and (ii) unattached first templates that are not attached at the 5' end to the capture nucleic acids; and

\_\_\_\_\_ (f) carrying out a second sequencing reaction by sequential addition of nucleotides to a second sequencing primer hybridized to the complementary strand and using the complementary strand as a second template to determine the sequence of a second region, wherein the second sequencing primer is in solution, wherein the complementary strand is not attached to the surface of the amplification site, and wherein the second sequencing reaction comprises subjecting the cleaved double-stranded amplicons to conditions that remove the unattached first templates, and hybridizing the second sequencing primer to a universal sequence present on the complementary strands.

2. The method of claim 1, wherein the cleavage site permits enzymatic, chemical, or photochemical cleavage.
3. The method of claim 2, wherein the cleavage site is a site for cleavage with a nicking endonuclease.
4. The method of claim 2, wherein the cleaving comprises contacting the array with a composition comprising at least one enzyme to produce an abasic site at the cleavage site, wherein cleavage occurs at the cleavage site.
5. The method of claim 4, wherein the capture nucleic acids comprise a uracil base or an 8-oxo-guanine base.
6. The method of claim 4, wherein the at least one enzyme to produce an abasic site at the cleavage site comprises uracil DNA glycosylase and an endonuclease selected from DNA glycosylase-lyase Endonuclease VIII or FpG glycosylase.
7. The method of claim 1, wherein the first sequencing primer is in solution.
8. The method of claim 7, wherein the 5' end of the first sequencing primer is blocked.
9. The method of claim 1, wherein the second sequencing primer comprises the 3' end of the cleavage site.
10. The method of claim 6, wherein the cleaving with an enzyme results in the 3' end of the cleavage sites terminating with a 3'-phosphate, the method further comprising contacting the array with a phosphatase to result in the cleavage sites terminating with a 3'-OH.
11. The method of claim 9, wherein the second sequencing reaction comprises use of a DNA polymerase with strand displacing activity.

12. The method of claim 1, wherein the removal of the unattached first templates comprises contacting the array with an exonuclease comprising a 5' to 3' exonuclease activity.
13. The method of claim 12, wherein the exonuclease is T7 exonuclease.
14. The method of claim 1, wherein at least 95% of the amplification sites on the array comprise clonal single-stranded amplicons, wherein the amplicons of each amplification site are derived from amplification of a different single target nucleic acid.
15. The method of claim 1, wherein the first sequencing reaction determines sequences of regions of the single-stranded amplicons, wherein the second sequencing reaction determines sequences of regions of the complementary strands, and wherein the regions of the single-stranded amplicons are complementary to the regions of the complementary strands.
16. The method of claim 1, wherein the first sequencing reaction determines sequences of regions of the single-stranded amplicons, wherein the second sequencing reaction determines sequences of regions of the complementary strands, and wherein the regions of the single-stranded amplicons are not complementary to the regions of the complementary strands.
17. The method of claim 1, wherein the nucleotides incorporated during (d) or (f) comprise fully functional nucleotides.
18. The method of claim 1, wherein the first sequencing reaction comprises sequential addition of a predetermined number of nucleotides to the first sequencing primer.
19. The method of claim 1, wherein the second sequencing reaction comprises sequential addition of a predetermined number of nucleotides to the second sequencing primer.

20. The method of claim 1, wherein the capture nucleic acids, the first sequencing primer, or the second sequencing primer comprise at least one non-nucleotide chemical moiety, non-naturally occurring nucleotide, or non-naturally occurring backbone linkage.
21. The method of claim 1, wherein the single-stranded amplicons comprise a first index and a second index.
22. The method of claim 21, further comprising sequencing the indexes.
23. The method of claim 22, wherein the first index is sequenced after (c).
24. The method of claim 22, wherein the second index is sequenced after (f).
25. The method of claim 1, wherein the array is produced by a method comprising:  
(a) providing an amplification reagent comprising  
(i) the array of amplification sites,  
(ii) a solution comprising a plurality of different target nucleic acids and a primer,  
wherein each of the amplification sites comprises the plurality of capture nucleic acids capable of hybridizing to a universal sequence present on the different target nucleic acids in the solution,  
wherein the number of the different target nucleic acids in the solution exceeds the number of amplification sites in the array,  
wherein the different target nucleic acids have fluidic access to the plurality of amplification sites, and  
wherein each of the amplification sites comprises a capacity for several nucleic acids in the plurality of different target nucleic acids; and  
(b) reacting the amplification reagent to produce a plurality of amplification sites that each comprise a clonal population of double-stranded amplicons from an individual target nucleic acid from the solution,

wherein the double-stranded amplicons comprise a first strand that is attached at its 5' end to a capture nucleic acid and a second strand that is not attached to the amplification site,

wherein the reacting comprises simultaneously

(i) transporting the different target nucleic acids to the amplification sites at an average transport rate, and

(ii) amplifying the target nucleic acids that are at the amplification sites at an average amplification rate, wherein the average amplification rate exceeds the average transport rate.

26. The method of claim 25, further comprising subjecting the double-stranded amplicons to conditions that remove the second strand that is not attached to the amplification site.

27. The method of claim 26, wherein the conditions that remove the second strand comprise a denaturant.

28. The method of claim 27, wherein the denaturant comprises formamide.

29. The method of claim 25, wherein the solution comprises a molecular crowding agent.

30. The method of claim 25, wherein the primer hybridizes to a universal sequence present on each single-stranded amplicon.