

WHAT IS CLAIMED IS:

1. A composition for amplifying a polynucleotide, the composition comprising:
 - a substrate comprising a first region and a second region;
 - a first plurality of capture primers coupled to the first region of the substrate;
 - a second plurality of capture primers coupled to the second region of the substrate, the capture primers of the second plurality of capture primers being longer than the capture primers of the first plurality of capture primers;
 - a first plurality of orthogonal capture primers coupled to the first region of the substrate; and
 - a second plurality of orthogonal capture primers coupled to the second region of the substrate, the orthogonal capture primers of the second plurality of orthogonal capture primers being shorter than the orthogonal capture primers of the first plurality of orthogonal capture primers.
2. The composition of claim 1, further comprising a fluid comprising target polynucleotides, each of the target polynucleotides comprising a first adapter that is complementary to the orthogonal capture primers of the first and second pluralities of orthogonal capture primers, and a second adapter that is complementary to the capture primers of the first and second pluralities of capture primers;
3. ~~The composition of claim 2, optionally~~ wherein the first adapters of the target polynucleotides are shorter than the orthogonal capture primers of the first plurality of orthogonal capture primers, and wherein the second adapters of the target polynucleotides are shorter than the capture primers of the second plurality of capture primers.
43. The composition of claim 2 ~~or claim 3~~, wherein:
 - the first adapter of a first one of the target polynucleotides is hybridized to a first one of the orthogonal capture primers of the first plurality of orthogonal capture primers, or wherein the second adapter of the first one of the target polynucleotides is hybridized to a first one of the capture primers of the second plurality of capture primers;
5. ~~The composition of claim 4, optionally~~ wherein a duplex formed from the hybridization between the first adapter of the first one of the target polynucleotides and the

first one of the orthogonal capture primers has a melting temperature (T_m) of greater than about 40°C.

~~64.~~ The composition of any one of claims 1 to ~~53~~, further comprising (i) a first amplicon covalently coupled to one of the orthogonal capture primers of the first plurality of orthogonal capture primers, the first amplicon having a second adapter completely hybridized to one of the capture primers of the second plurality of capture primers; and/or

~~7.~~ ~~The composition of any one of claims 1 to 6, further comprising (ii) a second amplicon covalently coupled to one of the capture primers of the first plurality of capture primers, the second amplicon having a first adapter completely hybridized to one of the orthogonal capture primers of the second plurality of orthogonal capture primers.~~

~~85.~~ The composition of any one of claims 1 to ~~74~~, further comprising a third amplicon covalently coupled to one of the orthogonal capture primers of the first plurality of orthogonal capture primers, the third amplicon having a second adapter that is unable to completely hybridize to any of the capture primers of the first plurality of capture primers.

~~96.~~ The composition of claim ~~58~~, wherein the inability of the second adapter to completely hybridize to any of the capture primers of the first plurality of capture primers inhibits amplification of the third amplicon;

~~10.~~ ~~The composition of claim 9, optionally wherein any partial duplex between the second adapter of the third amplicon and any of the capture primers of the first plurality of capture primers has a melting temperature (T_m) of less than about 20°C.~~

~~117.~~ The composition of any one of claims 1 to ~~106~~, wherein:
(i) the composition further comprising comprises a fourth amplicon covalently coupled to one of the capture primers of the second plurality of capture primers, the fourth amplicon having a first adapter that is unable to completely hybridize to any of the orthogonal capture primers of the second plurality of orthogonal capture primers;

~~12. — The composition of claim 11, optionally~~ wherein the inability of the first adapter to completely hybridize to any of the orthogonal capture primers of the second plurality of orthogonal capture primers inhibits amplification of the fourth amplicon-; and/or

~~13. — The composition of any one of claims 1 to 12, wherein (ii)~~ the capture primers of the second plurality of capture primers are P5 capture primers, and wherein the orthogonal capture primers of the first plurality of orthogonal capture primers are P7 capture primers-

~~14. — The composition of claim 13, optionally~~ wherein the capture primers of the first plurality of capture primers are shortened P5 capture primers, and wherein the orthogonal capture primers of the second plurality of orthogonal capture primers are shortened P7 capture primers.

~~158. The composition of any one of claims 1 to 147, wherein:~~
~~(i)~~ the capture primers of the first plurality of capture primers are at least 5 bases shorter than the capture primers of the second plurality of capture primers, and wherein the orthogonal capture primers of the second plurality of orthogonal capture primers are at least 5 bases shorter than the orthogonal capture primers of the first plurality of orthogonal capture primers-; and/or

~~16. — The composition of any one of claims 1 to 15, wherein (ii)~~ the capture primers of the first plurality of capture primers are approximately the same length as the orthogonal capture primers of the second plurality of capture primers-; and/or

~~17. — The composition of any one of claims 1 to 16, wherein (iii)~~ the capture primers of the second plurality of capture primers are approximately the same length as the orthogonal capture primers of the first plurality of capture primers-; and/or

~~18. — The composition of any one of claims 1 to 17, wherein (iv)~~ the first region of the substrate is adjacent to the second region of the substrate-; and/or

~~19. — The composition of any one of claims 1 to 18, wherein (v)~~ the first region of the substrate surrounds the second region of the substrate-

~~20. The composition of any one of claims 1 to 18, wherein~~ the second region of the substrate surrounds the first region of the substrate.

~~219.~~ A composition for amplifying a polynucleotide, the composition comprising:
a substrate comprising a first region and a second region;
a first plurality of capture primers coupled to the first region of the substrate;
a first plurality of orthogonal capture primers coupled to the first region of the substrate;
a second plurality of capture primers coupled to the second region of the substrate;
a second plurality of orthogonal capture primers coupled to the second region of the substrate;
a first plurality of removable blocking groups coupled to the capture primers of the second plurality of capture primers; and
a second plurality of removable blocking groups coupled to the orthogonal capture primers of the second plurality of capture primers.

~~2210.~~ The composition of claim ~~219~~, further comprising a fluid comprising target polynucleotides, each of the target polynucleotides comprising a first adapter that is complementary to the orthogonal capture primers of the first and second pluralities of orthogonal capture primers, and a second adapter that is complementary to the capture primers of the first and second pluralities of capture primers;

~~23. The composition of claim 22, optionally~~ wherein the first adapters of the target polynucleotides are about the same length as the capture primers of the first and second pluralities of capture primers, and wherein the second adapters of the target polynucleotides are about the same length as the orthogonal capture primers of the second plurality of orthogonal capture primers.

~~2411.~~ The composition of ~~claim 22 or claim 2310~~, wherein the first adapter of a first one of the target polynucleotides is hybridized to one of the orthogonal capture primers of the first plurality of orthogonal capture primers, or wherein the second adapter of the first one of the target polynucleotides is hybridized to one of the capture primers of the first plurality of capture primers.

~~25. The composition of claim 24,~~

~~optionally~~ wherein a duplex formed from the hybridization between the second adapter of the first one of the target polynucleotides and the one of the capture primers has a melting temperature (T_m) of greater than about 40°C, or wherein a duplex formed from the hybridization between the first adapter of the first one of the target polynucleotides and the one of the orthogonal capture primers has a melting temperature (T_m) of greater than about 40°C.

~~26~~12. The composition of ~~any one of claims 22 to 25~~10 or claim 11, wherein the first adapter of a second one of the target polynucleotides is hybridized to one of the orthogonal capture primers of the second plurality of orthogonal capture primers, or wherein the second adapter of the second one of the target polynucleotides is hybridized to one of the capture primers of the second plurality of capture primers;

~~27. The composition of claim 26,~~ ~~optionally~~ wherein the removable blocking group coupled to the one of the capture primers inhibits amplification of the second one of the target polynucleotides, or wherein the removable blocking group coupled to the one of the orthogonal capture primers inhibits amplification of the second one of the target polynucleotides.

~~28~~13. The composition of any one of claims ~~21-9~~to 2712, wherein:

(i) the composition further comprising comprises an amplicon covalently coupled to one of the orthogonal capture primers of the first plurality of orthogonal capture primers, the amplicon having a second adapter completely hybridized to one of the capture primers of the first plurality of capture primers; and/or

~~29. The composition of any one of claims 21 to 28,~~ (ii) the composition further comprising comprises an amplicon covalently coupled to one of the capture primers of the first plurality of capture primers, the amplicon having a first adapter completely hybridized to one of the orthogonal capture primers of the first plurality of orthogonal capture primers; and/or

~~30. The composition of any one of claims 21 to 29,~~ wherein (iii) the capture primers of the first and second pluralities of capture primers are P5 capture primers, and wherein the

orthogonal capture primers of the first and second pluralities of orthogonal capture primers are P7 capture primers; and/or:

~~31. The composition of any one of claims 21 to 30,~~
~~wherein (iv)~~ the capture primers of the first plurality of capture primers are approximately the same length as the capture primers of the second plurality of capture primers; and/or

~~32. The composition of any one of claims 21 to 31, wherein (v)~~ the capture primers of the second plurality of capture primers are approximately the same length as the orthogonal capture primers of the first plurality of orthogonal capture primers; and/or

~~33. The composition of any one of claims 21 to 32, wherein (vi)~~ the first region of the substrate is adjacent to the second region of the substrate; and /or

~~34. The composition of any one of claims 21 to 33, wherein (vii)~~ the first region of the substrate surrounds the second region of the substrate-

~~35. The composition of any one of claims 21 to 34, wherein or~~ the second region of the substrate surrounds the first region of the substrate.

~~36~~14. A method for amplifying a polynucleotide, the method comprising:

contacting a composition with a fluid, wherein the composition comprises:

a substrate comprising a first region and a second region;

a first plurality of capture primers coupled to the first region of the substrate;

a second plurality of capture primers coupled to the second region of the substrate, the capture primers of the second plurality of capture primers being longer than the capture primers of the first plurality of capture primers;

a first plurality of orthogonal capture primers coupled to the first region of the substrate; and

a second plurality of orthogonal capture primers coupled to the second region of the substrate, the orthogonal capture primers of the second plurality of orthogonal capture primers being shorter than the orthogonal capture primers of the first plurality of orthogonal capture primers, and

wherein the fluid comprises:

target polynucleotides, each of the target polynucleotides comprising a first adapter that is complementary to the orthogonal capture primers of the first and second pluralities of orthogonal capture primers, and a second adapter that is complementary to the capture primers of the first and second pluralities of capture primers;

hybridizing the first adapter of a first one of the target polynucleotides to one of the orthogonal capture primers of the first or second plurality of orthogonal capture primers, or hybridizing the second adapter of the first one of the target polynucleotides to one of the capture primers of the first or second plurality of capture primers; and then

amplifying the first one of the target polynucleotides, the amplifying comprising generating an amplicon of the first one of the target polynucleotides.

3715. The method of claim 3614, wherein:

(i) the first adapters of the target polynucleotides are shorter than the orthogonal capture primers of the first plurality of orthogonal capture primers, and wherein the second adapters of the target polynucleotides are shorter than the capture primers of the second plurality of capture primers; and/or

38. ~~The method of claim 37 or claim 38, wherein~~ (ii) a duplex formed from the hybridization between the first adapter of the first one of the target polynucleotides and the first one of the orthogonal capture primers has a melting temperature (T_m) of greater than about 40°C; and/or

39. ~~The method of any one of claims 36 to 38, wherein~~ (iii) the amplicon is hybridized to the first one of the target polynucleotides; optionally wherein the method

40. ~~The method of claim 39, further comprising~~ comprises dehybridizing the first one of the target polynucleotides, and then amplifying the amplicon; and/or

41. ~~The method of any one of claims 36 to 40,~~ (iv) the method further comprising comprises covalently coupling a first amplicon to one of the orthogonal capture primers of the first plurality of orthogonal capture primers, and completely hybridizing a second adapter of the first amplicon to one of the capture primers of the second plurality of capture primers; and/or

~~42. The method of any one of claims 36 to 41, (v) the method further comprising~~
comprises covalently coupling a second amplicon to one of the capture primers of the first plurality of capture primers, and completely hybridizing a first adapter of the second amplicon to one of the orthogonal capture primers of the second plurality of orthogonal capture primers; and/or

~~43. The method of any one of claims 36 to 42, (vi) the method further comprising~~
comprises covalently coupling a third amplicon to one of the orthogonal capture primers of the first plurality of orthogonal capture primers, and being unable to completely hybridize a second adapter of the third amplicon to any of the capture primers of the first plurality of capture primers.

~~44. The method of claim 43,; optionally~~ wherein the inability of the second adapter to completely hybridize to any of the capture primers of the first plurality of capture primers inhibits amplification of the third amplicon; and/or

~~45. The method of claim 36 to 44, wherein (vii)~~ any partial duplex between the second adapter of the third amplicon and any of the capture primers of the first plurality of capture primers has a melting temperature (T_m) of less than about 20°C; and/or

~~46. The method of any one of claims 36 to 45, (viii) the method further comprising~~
comprises covalently coupling a fourth amplicon to one of the capture primers of the second plurality of capture primers, and being unable to completely hybridize a first adapter of the fourth amplicon to any of the orthogonal capture primers of the second plurality of orthogonal capture primers; optionally

~~47. The method of claim 46, wherein the inability of the first adapter to completely~~
hybridize to any of the orthogonal capture primers of the second plurality of orthogonal capture primers inhibits amplification of the fourth amplicon.

~~48~~16. The method of ~~any one of claims 36-14 to or claim 47~~15, wherein the capture primers of the second plurality of capture primers are P5 capture primers, and wherein the orthogonal capture primers of the first plurality of orthogonal capture primers are P7 capture primers-

~~49. The method of claim 48, optionally~~ wherein the capture primers of the first plurality of capture primers are shortened P5 capture primers, and wherein the orthogonal capture primers of the second plurality of orthogonal capture primers are shortened P7 capture primers.

~~50~~17. The method of any one of claims ~~36-14 to 49~~16, wherein:

(i) the capture primers of the first plurality of capture primers are at least 5 bases shorter than the capture primers of the second plurality of capture primers, and wherein the orthogonal capture primers of the second plurality of orthogonal capture primers are at least 5 bases shorter than the orthogonal capture primers of the first plurality of orthogonal capture primers-; and/or

~~51. The method of any one of claims 36 to 50, wherein (ii)~~ the capture primers of the first plurality of capture primers are approximately the same length as the orthogonal capture primers of the second plurality of capture primers-; and/or

~~52. The method of any one of claims 36 to 51, wherein (iii)~~ the capture primers of the second plurality of capture primers are approximately the same length as the orthogonal capture primers of the first plurality of capture primers-; and/or

~~53. The method of any one of claims 36 to 52, (iv) wherein~~ the first region of the substrate is adjacent to the second region of the substrate; and/or-

~~54. The method of any one of claims 36 to 53, (v) wherein~~ the first region of the substrate surrounds the second region of the substrate-

~~55. The method of any one of claims 36 to 53, wherein or~~ the second region of the substrate surrounds the first region of the substrate.

~~56~~18. A method for amplifying a polynucleotide, the method comprising:

contacting a composition with a fluid, wherein the composition comprises:

- a substrate comprising a first region and a second region;
- a first plurality of capture primers coupled to the first region of the substrate;
- a first plurality of orthogonal capture primers coupled to the first region of the substrate;
- a second plurality of capture primers coupled to the second region of the substrate;
- a second plurality of orthogonal capture primers coupled to the second region of the substrate;
- a first plurality of removable blocking groups coupled to the capture primers of the second plurality of capture primers; and
- a second plurality of removable blocking groups coupled to the orthogonal capture primers of the second plurality of capture primers, and

wherein the fluid comprises target polynucleotides, each of the target polynucleotides comprising a first adapter that is complementary to the orthogonal capture primers of the first and second pluralities of orthogonal capture primers, and a second adapter that is complementary to the capture primers of the first and second pluralities of capture primers;

hybridizing the first adapter of a first one of the target polynucleotides to a first one of the orthogonal capture primers of the first plurality of orthogonal capture primers,

or

hybridizing the second adapter of the first one of the target polynucleotides to a first one of the capture primers of the first plurality of capture primers;

amplifying the first one of the target polynucleotides, the amplifying comprising generating a first amplicon of the first one of the target polynucleotides in the first region of the substrate;

removing the first and second pluralities of removable blocking groups; and then

further amplifying the first one of the target polynucleotides, the amplifying comprising generating additional amplicons of the first one of the target polynucleotides in the second region of the substrate.

5719. The method of claim ~~56~~18, wherein:

(i) the first adapters of the target polynucleotides are about the same length as the orthogonal capture primers of the first and second pluralities of orthogonal capture primers, and wherein

the second adapters of the target polynucleotides are about the same length as the capture primers of the first and second pluralities of capture primers; and/or

~~58. The method of claim 56 or claim 57, wherein (ii) a duplex formed from the hybridization between the second adapter of the first one of the target polynucleotides and the one of the capture primers has a melting temperature (T_m) of greater than about 40°C, or wherein a duplex formed from the hybridization between the first adapter of the first one of the target polynucleotides and the one of the orthogonal capture primers has a melting temperature (T_m) of greater than about 40°C;~~ and/or

~~59. The method of any one of 56 to 58, wherein (iii) the first adapter of a second one of the target polynucleotides is hybridized to one of the orthogonal capture primers of the second plurality of orthogonal capture primers, or wherein the second adapter of the second one of the target polynucleotides is hybridized to one of the capture primers of the second plurality of capture primers;~~ optionally

~~60. The method of claim 59, wherein the removable blocking group coupled to the one of the capture primers inhibits amplification of the second one of the target polynucleotides, or wherein the removable blocking group coupled to the one of the orthogonal capture primers inhibits amplification of the second one of the target polynucleotides.~~

~~61~~20. The method of ~~any one of claims 56 to 60~~18 or claim 19, wherein:

(i) the method further comprises~~ing~~ covalently coupling an amplicon to one of the orthogonal capture primers of the first plurality of orthogonal capture primers, the amplicon having a second adapter completely hybridized to one of the capture primers of the first plurality of capture primers; and/or

~~62. The method of any one of claims 56 to 61,~~ (ii) the method further comprises~~ing~~ covalently coupling an amplicon to one of the capture primers of the first plurality of capture primers, the amplicon having a first adapter completely hybridized to one of the orthogonal capture primers of the first plurality of orthogonal capture primers; and/or

~~63. The method of any one of claims 56 to 62, wherein (iii) the capture primers of the first and second pluralities of capture primers are P5 capture primers, and wherein the orthogonal~~

capture primers of the first and second pluralities of orthogonal capture primers are P7 capture primers; and/or

64. ~~The method of any one of claims 56 to 63, wherein~~(iv) the capture primers of the first plurality of capture primers are approximately the same length as the capture primers of the second plurality of capture primers; and/or

65. ~~The method of any one of claims 56 to 64, wherein~~(v) the capture primers of the second plurality of capture primers are approximately the same length as the orthogonal capture primers of the first plurality of orthogonal capture primers; and/or

66. ~~The method of any one of claims 56 to 65, wherein~~(vi) the first region of the substrate is adjacent to the second region of the substrate; and/or

67. ~~The method of any one of claims 56 to 66, wherein~~(vii) the first region of the substrate surrounds the second region of the substrate;

68. ~~The method of any one of claims 56 to 66, wherein~~or the second region of the substrate surrounds the first region of the substrate.