CLAIMS

1) A microarray comprising:

a) a substrate comprising at least one well, a surface surrounding the well and an inner well surface;

b) a first layer covering the inner well surface and comprising at least one first capture primer pair; and

c) a second layer covering the first layer and the surface surrounding the well, wherein the first capture primer in the first layer is present and functional after the second layer has been provided.

2) A method for amplifying a nucleic acid, comprising:

a) producing a first layer on a substrate, wherein the substrate comprises at least one well, a surface surrounding the well and an inner well surface, wherein the first layer covers the inner well surface;

b) depositing at least one first capture primer pair in the first layer;

c) producing a second layer on the substrate covering the first layer and the surface surrounding the well wherein the first capture primer in the first layer is present and functional after the second layer has been provided;

d) contacting a sample comprising a plurality of target polynucleotides with the substrate under conditions sufficient for a target polynucleotide to hybridize with a capture primer of the at least one first capture primer pair, and

e) performing a first kinetic exclusion assay (KEA) to produce a clonal population of amplicons from the target polynucleotide inside the well, thereby amplifying the target polynucleotide.

3) A method for amplifying a nucleic acid, comprising:

a) producing a first layer on a substrate, wherein the substrate comprises at least one well, a surface surrounding the well and an inner well surface, wherein the first layer at least partially covers the inner well surface;

b) depositing at least one first capture primer pair in the first layer, wherein the first capture primer pair comprises a plurality of first capture primers

comprising a 3' portion comprising an Illumina® P5 primer nucleotide sequence and a plurality of second capture primers comprising a 3' portion comprising an Illumina® P7 primer nucleotide sequence;

c) producing a second layer on the substrate covering the first layer and the surface surrounding the well;

d) depositing at least one second capture primer pair in the second layer, wherein the second capture primer pair is 3' phosphate-terminated and comprises a plurality of first capture primers comprising a 3' portion comprising an Illumina® P5 primer nucleotide sequence and a plurality of second capture primers comprising a 3' portion comprising an Illumina® P7 primer nucleotide sequence;

e) contacting a sample comprising a plurality of target polynucleotides with the substrate under conditions sufficient for a single target polynucleotide per well to hybridize with a primer of the at least one first capture primer pair, wherein the target polynucleotides are flanked by complementary universal primer regions each comprising a complementary Illumina® P5' primer nucleotide sequence or a complementary Illumina® P7' primer nucleotide sequence;

f) performing a first KEA to produce a monoclonal population of amplicons from the single target polynucleotide inside the at least one well, thereby amplifying the target polynucleotide;

g) contacting the substrate with a T4-kinase to deblock the primers of the second primer pair, and

h) performing bridge amplification or a second KEA to enlarge the monoclonal population of amplicons of the single target polynucleotide beyond the well.

4) A method for amplifying a nucleic acid, comprising:

a) producing a first layer on a substrate, wherein the substrate comprises at least one well, a surface surrounding the well and an inner well surface, wherein the first layer at least partially covers the inner well surface;

b) depositing at least one first capture primer pair in the first layer, wherein the first capture primer pair comprises a plurality of at least one first capture primers comprising a 3' portion comprising an Illumina® P5 primer nucleotide

sequence and an Illumina® SBS3 primer nucleotide sequence and a plurality of at least one second capture primers comprising a 3' portion comprising an Illumina® P7 primer nucleotide sequence and an Illumina® SBS8 primer nucleotide sequence;

c) producing a second layer on the substrate covering the first layer and the surface surrounding the well;

d) depositing at least one second capture primer pair in the second layer, wherein the at least one second capture primer pair comprises a plurality of first capture primers comprising a 3' portion comprising an Illumina® P5 primer nucleotide sequence and a plurality of second capture primers comprising an 3' portion comprising an Illumina® P7 nucleotide sequence;

e) contacting a sample comprising a plurality of target polynucleotides with the substrate under conditions sufficient for a single target polynucleotide per well to hybridize with a primer of the at least one first capture primer pair, wherein the plurality of target polynucleotides are flanked by a complementary SBS each comprising a complementary Illumina® SBS3' primer nucleotide sequence or a complementary Illumina® SBS8' nucleotide sequence, and

f) performing a KEA for an extended time to produce a monoclonal population of amplicons from the single target polynucleotide inside and outside the at least one well, thereby amplifying the single target polynucleotide inside the well and enlarging the monoclonal population of target polynucleotides beyond the at least one well.

5) A method for amplifying a nucleic acid, comprising:

a) producing a first layer on a substrate, wherein the substrate comprises at least one well, a surface surrounding the well, and an inner well surface, wherein the first layer at least partially covers the inner well surface;

b) depositing at least one first capture primer pair in the first layer, wherein the first primer pair comprises a plurality of first capture primers comprising a 3' portion comprising an Illumina® P5 primer nucleotide sequence and a plurality of second capture primers comprising a 3' portion comprising an Illumina® P7 primer nucleotide sequence;

c) producing a second layer on the substrate covering the first layer and the surface surrounding the well;

d) contacting a sample comprising a plurality of target polynucleotides with the substrate under conditions sufficient for a single target polynucleotide per well to hybridize with a primer of the at least one first capture primer pair, wherein the plurality of polynucleotides are flanked by complementary universal primer regions each comprising a complementary Illumina® P5' primer nucleotide sequence or a complementary Illumina® P7' primer nucleotide sequence;

e) performing a first KEA to produce a monoclonal population of amplicons from the single target polynucleotide inside the at least one well, thereby amplifying the target polynucleotide;

f) depositing at least one second capture primer pair in the second layer, wherein the at least one second capture primer pair comprises a plurality of first capture primers comprising a 3' portion comprising an Illumina® P5 primer nucleotide sequence and a plurality of second capture primers comprising a 3' portion comprising an Illumina® P7 primer nucleotide sequence, and

g) performing bridge amplification or a second KEA to enlarge the monoclonal population of amplicons of the single target polynucleotide.

6) The microarray of claim 1 or the method of claim 2 wherein at least one of

(i) the first layer does not cover the surface surrounding the well,

(ii) the diameter of the well is less than about 1μm,

(iii) the first layer comprises a polymer coating, optionally wherein the polymer coating comprises poly(N-(5 azidoacetamidylpentyl)acrylamide-co-acrylamide (PAZAM).

7) The method of claim 2, wherein the sample comprising the plurality of target polynucleotides is contacted with the substrate under conditions sufficient for a single target polynucleotide per well to hybridize with a capture primer of the at least one first capture primer pair, optionally

wherein the first KEA produces a monoclonal population of amplicons from a single target polynucleotide hybridized with a capture primer in the at least one well, optionally

wherein the at least one well is a plurality of wells and wherein a monoclonal population of amplicons is produced from a single target polynucleotide in two or more wells of the plurality of wells, optionally

wherein a monoclonal population of amplicons is produced from
(i) the same single target polynucleotide in the two or more wells of the plurality of wells, or
(ii) two or more single target polynucleotides in the two or more wells of the plurality of wells.

8. The microarray of claim 1, wherein the at least one well is a plurality of wells.

9. The microarray of claim 8, wherein the wells are spaced at a pitch of about 700 nm.

10. The microarray of claim 1, wherein the diameter of the well is between about 30 nm and about 900 nm, or is between about 100 nm and about 400 nm, or is about 400 nm.

11. The microarray of claim 1, wherein the second layer comprises a polymer coating.

12. The microarray of claim 11, wherein the polymer coating comprises PAZAM or silane-free acrylamide (SFA).

13. The microarray of claim 1, wherein the at least one first capture primer pair is a plurality of first capture primer pairs.

14. The microarray of claim 1, wherein the primers of the at least one first capture primer pair comprise a universal capture region,

optionally wherein the first primer of the at least one first capture primer pair comprises an Illumina® P5 primer nucleotide sequence and the second primer of the at least one first capture primer pair comprises an Illumina® P7 primer nucleotide sequence,

optionally wherein the primers of the at least one first capture primer pair further comprises a sequencing primer binding site (SBS),

optionally wherein the first primer of the at least one first capture primer pairs comprises an Illumina® P5 primer nucleotide sequence and an Illumina® SBS3 primer nucleotide sequence, and the second primer of the at least one first capture primer pair comprises an Illumina® P7 primer nucleotide sequence and an Illumina® SBS8 primer nucleotide sequence.

15. The microarray of claim 1, wherein the second layer comprises at least one second capture primer pair,

optionally wherein the at least one second capture primer pair is a plurality of second capture primer pairs,

optionally wherein the primers of the at least one second capture primer pair are blocked at the 3'-end,

optionally wherein the primers of the at least one second capture primer pair are 3'-phosphate-terminated,

optionally wherein the 3'-phosphate terminated primers of the at least one second capture primer pair comprise a universal capture region,

optionally wherein the first primer of the at least one second capture primer pair comprises an Illumina® P5 primer nucleotide sequence and the second primer of the at least one second capture primer pair comprises an Illumina® P7 primer nucleotide sequence.

16. The microarray of claim 15, wherein the primers of the at least one second capture primer pair are not blocked at the 3'-end,

optionally wherein the primers of the at least one second capture primer pair comprise a universal capture region,

optionally wherein the first primer of the at least one second capture primer pair comprises an Illumina® P5 primer nucleotide sequence and the second primer of the at least one second capture primer pair comprises an Illumina® P7 primer nucleotide sequence.

17. The microarray of claim 1, wherein the at least first capture primer is a plurality of capture primers, and wherein a plurality of capture primers of the plurality of first capture primer pairs each are attached to a target polynucleotide,

optionally wherein the plurality of target polynucleotides form a monoclonal population of target polynucleotides in the at least one well,

optionally wherein the at least one well comprises a plurality of wells and wherein two or more wells of the plurality of wells comprise a monoclonal population of target polynucleotides,

optionally wherein the two or more wells of the plurality of wells comprise a monoclonal population of the same target polynucleotide or the two or more wells of the plurality of wells comprise a monoclonal population of two or more different target polynucleotides.

18. The microarray of claim 1, wherein the second layer comprises a second capture primer pair, the at least one first capture primer pair is a plurality of first capture primer pairs, and the at least one second capture primer pair is a plurality of second capture primer pairs, wherein a plurality of primers of the plurality of first capture primer pairs and the plurality of second capture primer pairs is attached to a plurality of target polynucleotide,

optionally wherein the plurality of target polynucleotides form a monoclonal population of target polynucleotides in the at least one well,

optionally wherein the at least one well is a plurality of wells and wherein two or more wells of the plurality of wells comprise a monoclonal population of target polynucleotides,

optionally wherein the two or more wells of the plurality of wells comprise a monoclonal population of the same target polynucleotide or the two or more wells of the plurality of wells comprise a monoclonal population of two or more different target polynucleotides.

19. The method of claim 2:

(i) wherein the at least one first capture primer pair is a plurality of first capture primer pairs, or

(ii) further comprising depositing at least one second capture primer pair in the second layer,

optionally wherein the at least one second capture primer pair is a plurality of second capture primer pairs,

optionally wherein the at least one second capture primer pair is deposited prior to performing the first KEA,

optionally wherein the primers of the at least one second capture primer pair are blocked at the 3'-end, optionally wherein the 3'-blocked primers comprise a universal capture region,

optionally further comprising deblocking the primers of the at least one second capture primer pair after performing the first KEA, optionally wherein the primers of the at least one second capture primer pair are deblocked using T4-kinase, or

(iii) wherein the primers of the at least one first capture primer pair comprise a universal capture region,

optionally wherein the first capture primer of the at least one first capture primer pair comprises an Illumina® P5 primer nucleotide sequence and the second capture primer of the at least one first capture primer pair comprises an Illumina® P7 primer nucleotide sequence, or

(iv) wherein the plurality of target polynucleotides are flanked by one or more complementary universal capture regions, or

(v) further comprising performing bridge amplification or a second KEA to enlarge the clonal population of target polynucleotide amplicons,

optionally wherein the primers of the first capture primer pair further comprise a SBS, optionally wherein the first primer of the at least one first primer pair comprises an Illumina® P5 primer nucleotide sequence and an Illumina® SBS3 primer nucleotide sequence and the second primer of the at least one first primer pair comprises an Illumina® P7 primer nucleotide sequence and an Illumina® SBS8 primer nucleotide sequence, optionally wherein the plurality of target polynucleotides are flanked by one or more complementary SBSs.