WHAT IS CLAIMED

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.

2) A microarray comprising:

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

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

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 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;

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.

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 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.

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 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.

6) 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.

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

a) producing a layer on a substrate, wherein the substrate comprises at least one well, a surface surrounding the well and an inner well surface, wherein the well has a diameter of about 1 μm or more and wherein the layer at least partially covers the inner well surface.

b) depositing at least one first capture primer pair and at least one second capture primer pair in the layer, wherein the primer density of the at least one first capture primer pair is higher than the primer density of the at least second primer pair;

c) 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 the second primer, and

d) performing a KEA to produce a monoclonal population of amplicons from the single target polynucleotide hybridized to the second primer inside the well, thereby amplifying the single target polynucleotide.

8) A method for modifying an immobilized capture primer comprising:

a) contacting a substrate comprising a plurality of immobilized capture primers with a plurality of template nucleic acids under conditions sufficient for hybridization to produce one or more immobilized template nucleic acids,

wherein the plurality of immobilized capture primers comprises a first plurality of primers comprising a 5'-terminal universal capture region Y and a second plurality of primers comprising a 3'-terminal universal capture region Z, and

wherein each template nucleic acid is flanked by 5'-terminal and a 3'-terminal universal capture regions Y or Z and comprises one or more restriction sites and the target-specific capture region between the 5'-terminal universal capture region and the one or more restriction sites or between the 3'-terminal universal capture region and the one or more restriction sites, and

b) extending one or more immobilized capture primers to produce one or more immobilized extension products complementary to the one or more template nucleic acid.

9) A method for modifying an immobilized capture primer comprising:

a) contacting a substrate comprising a plurality of immobilized capture primers with a plurality of template nucleic acids under conditions sufficient for hybridization to produce one or more immobilized template nucleic acid,

wherein the plurality of immobilized capture primers comprises a first plurality of primers comprising a 3'-terminal Illumina® P5 primer nucleotide sequence and a second plurality of primers comprising a 3'-terminal Illumina® P7 primer nucleotide sequence, and

wherein each template nucleic acid is flanked by a 3'-terminal complementary Illumina® P5' primer nucleotide sequence and a 5'-terminal complementary Illumina® P7' primer nucleotide sequence, and comprises two SapI restriction sites, a spacer region between the SapI restriction sites, and a target-specific capture region between the 3'terminal complementary Illumina® P5' primer nucleotide sequence and the SapI restriction sites; and

b) extending one or more immobilized capture primers to produce one or more immobilized extension products complementary to the one or more template nucleic acids.

c) amplifying the one or more immobilized extension products by bridge amplification or KEA to produce one or more monoclonal clusters of immobilized double-stranded template nucleic acids;

d) contacting the one or more monoclonal cluster of immobilized double-stranded template nucleic acids with SapI to cut the two restriction sites in a plurality of immobilized double-stranded template nucleic acids to produce a plurality of immobilized double-stranded chimeric capture primers comprising the Illumina® P5 primer nucleotide sequence and the target-specific capture region and a plurality of immobilized double-stranded regenerated universal capture primers comprising the Illumina® P7 primer nucleotide sequence, and

e) optionally, contacting the plurality of immobilized double-stranded chimeric capture primers and immobilized double-stranded regenerated universal capture primers with a 5'-3' dsDNA-exonuclease to produce a plurality of

immobilized single-stranded chimeric capture primers and a plurality of immobilized single-stranded regenerated universal capture primers.

10) A method for modifying an immobilized capture primer comprising:

a) contacting a substrate comprising a plurality of immobilized capture primers with a plurality of template nucleic acids under conditions sufficient for hybridization to produce one or more immobilized template nucleic acids,

wherein the plurality of immobilized capture primers comprises a first plurality of primers comprising a 3'-terminal universal capture region Y and a second plurality of primers comprising a 3'-terminal universal capture region Z, and

wherein each template nucleic acid is flanked by a 5'-terminal and a 3'-terminal universal capture region Y or Z and comprises one or more restriction sites and a target-specific capture region between the one or more restriction sites and the 3'-terminal universal capture region;

b) extending one or more immobilized capture primer to produce one or more immobilized extension products complementary to the one or more template nucleic acids;

c) amplifying the one or more immobilized extension product by PCR to produce one or more monoclonal clusters of immobilized double-stranded template nucleic acids;

d) contacting the one or more monoclonal clusters of immobilized double-stranded template nucleic acids with a restriction enzyme to cut the one or more restriction sites in a plurality of the immobilized double-stranded template nucleic acids to produce a plurality of immobilized double-stranded chimeric capture primers comprising the universal capture region Z and the target-specific capture region and a plurality of immobilized double-stranded regenerated universal capture primers comprising the universal capture region Y.

11) A method for modifying an immobilized capture primer comprising:

a) contacting a plurality of universal capture primers immobilized on a substrate with a plurality of template nucleic acids under conditions

sufficient for hybridization to produce one or more immobilized template nucleic acids,

wherein the plurality of universal capture primers comprises a first plurality of primers comprising a 3'-terminal universal capture region Y and a second plurality of primers comprising a 3'-terminal universal capture region Z,

wherein each template nucleic acid comprises a 5'-terminal universal capture region Y, a 3'-terminal universal capture region Z, a target-specific capture region, a restriction site between the 5'-terminal universal capture region Y and the target-specific capture region, and a SBS between the 3'-terminal universal capture region Z and the target-specific capture portion;

b) extending one or more universal capture primers to produce one or more immobilized extension products complementary to the one or more immobilized template nucleic acids;

c) amplifying the one or more immobilized extension products by bridge amplification or KEA to produce one or more monoclonal amplicons of immobilized extension products;

d) contacting the one or more monoclonal clusters of immobilized extension products with a restriction enzyme to produce a plurality of immobilized chimeric capture primers comprising a universal capture region Z and the target-specific capture region and a plurality of immobilized regenerated universal capture primers comprising a universal capture region Y and a partial restriction site.

12) A method for modifying an immobilized capture primer comprising:

a) contacting a plurality of universal capture primers immobilized on a substrate with a plurality of template nucleic acids under conditions sufficient for hybridization to produce one or more immobilized template nucleic acid,

wherein the plurality of universal capture primers comprises a first plurality of primers comprising a 3'-terminal universal capture region Y and a first pre-determined cleavage site and a second plurality of primers comprising a 3'-terminal universal capture region Z and a 5'-portion comprising a second pre-determined cleavage site,

wherein each template nucleic acid comprises a 5'-terminal universal capture region Y, a 3'-terminal universal capture region Z, a target-specific capture region, a restriction site between the 5'-terminal universal capture region Y and the target-specific capture region, and a SBS between the 3'-terminal universal capture region Z and the target-specific capture region;

b) extending one or more universal capture primers to produce one or more immobilized extension products complementary to the one or more template nucleic acid;

c) amplifying the one or more immobilized extension products by bridge amplification or KEA to produce one or more monoclonal amplicons of immobilized extension products;

d) contacting the one or more monoclonal amplicons of immobilized extension products with a restriction enzyme to produce a plurality of immobilized chimeric capture primers comprising the universal capture region Z and the target-specific capture region and a plurality of immobilized regenerated universal capture primers comprising the universal capture region Y and a partial restriction site

e) removing the partial restriction site from the plurality of immobilized regenerated universal capture primers through cleavage at the first pre-determined cleavage site.

13) A method for modifying an immobilized capture primer comprising:

a) contacting a plurality of universal capture primers immobilized on a substrate with a plurality of template nucleic acids under conditions sufficient for hybridization to produce one or more immobilized template nucleic acids,

wherein the plurality of universal capture primers comprises a first plurality of primers comprising a 3'-terminal universal capture region Y and a second plurality of primers comprising a 3'-terminal universal capture region Z,

wherein each template nucleic acid comprises a 5'-terminal universal capture region Y, a 3'-terminal universal capture region Z, a central portion comprising a first and a second restriction site and a spacer region between the first and the second restriction site, and a target-specific region between the central portion and the 3'-terminal universal capture region Z;

b) extending one or more universal capture primer of the plurality of universal capture primers to produce one or more immobilized extension product complementary to the one or more template nucleic acids;

c) amplifying the one or more immobilized extension products by bridge amplification or KEA to produce one or more monoclonal amplicons of immobilized extension products, and

d) contacting the one or more monoclonal amplicons of immobilized extension products with a restriction enzyme to produce a plurality of immobilized chimeric capture primers comprising a universal capture region Z and a target-specific capture region and a plurality of immobilized regenerated universal capture primers comprising a universal capture region Y.

14) A method for modifying an immobilized capture primer comprising:

a) contacting a plurality of universal capture primers immobilized on a substrate with a plurality of template nucleic acids under conditions sufficient for hybridization to produce one or more immobilized template nucleic acids, wherein the plurality of the universal capture primers comprises first plurality of primers comprising a 3'-terminal universal capture region Y and a second plurality of primers comprising a 3'-terminal universal capture region Z,

wherein each template nucleic acid comprises a 5'-terminal universal capture region Y, a 3'-terminal universal capture region Z, a target-specific capture region and a restriction site between the 5'-terminal universal capture region Y and the target-specific capture region;

b) extending one or more universal capture primers of the plurality of universal capture primers to produce one or more immobilized extension product complementary to the one or more template nucleic acids;

c) amplifying the one or more immobilized extension products by bridge amplification or KEA to produce one or more monoclonal amplicons of immobilized extension products;

d) contacting the one or more monoclonal amplicons of immobilized extension products with a restriction enzyme to produce a plurality of double-stranded immobilized chimeric capture primers comprising a universal

capture region Z and a target-specific capture region and a plurality of double-stranded immobilized regenerated universal capture primers comprising a universal capture region Y and a single-stranded partial restriction site;

e) denaturing the plurality of double-stranded immobilized chimeric capture primers and the plurality of double-stranded immobilized regenerated universal capture primers to produce a plurality of single-stranded immobilized chimeric capture primers and a plurality of single-stranded immobilized regenerated universal capture primers;

f) hybridizing reverse complementary oligonucleotide to the plurality of single-stranded immobilized chimeric capture primers and the plurality single-stranded immobilized regenerated universal capture primers to form double-stranded universal capture regions and double-stranded target-specific regions, and

g) contacting the surface with exonuclease I to remove the single-stranded partial restriction site from the plurality of double-stranded immobilized regenerated universal capture primers.

15) A method for modifying an immobilized capture primer comprising:

a) contacting a plurality of universal capture primers immobilized on a substrate with a plurality of template nucleic acids under conditions sufficient for hybridization to produce one or more immobilized template nucleic acids,

wherein the plurality of universal capture primers comprises a first plurality of primers comprising a 3'-terminal universal capture region Y, and a second plurality of primers comprising a 3'-terminal universal capture region Z and a third plurality of primers comprising a 3'-terminal region X and a 5' portion comprising a pre-determined cleavage site,

wherein each template nucleic acid comprises a 5'-terminal region X, a 3'-terminal universal capture region Z, a target-specific capture region, and a restriction site between the region X and the target-specific capture region;

b) extending one or more universal capture primers to produce one or more immobilized extension products complementary to the one or more template nucleic acids;

c) amplifying the one or more immobilized extensions products by bridge amplification or KEA to produce one or more monoclonal amplicons of immobilized extension products;

d) contacting the one or more monoclonal amplicons of immobilized extension products with a restriction enzyme to produce a plurality of immobilized chimeric capture primers comprising a universal capture region Z and a target-specific capture region and a plurality of immobilized regenerated universal capture primers comprising a region X and a partial restriction site, and

e) removing the plurality of immobilized regenerated capture primers comprising the region X from the substrate through cleavage at the pre-determined cleavage site.

16) A method for modifying an immobilized capture primer comprising:

a) contacting a substrate comprising a plurality of immobilized capture primers with a plurality of different seed nucleic acids under conditions sufficient for hybridization to produce a plurality of different immobilized seed nucleic acids;

b) extending two or more of the immobilized capture primers to produce a plurality of different immobilized extension products complementary to two or more of the plurality of different immobilized seed nucleic acids;

c) activating one immobilized extension product of the plurality of different immobilized extension products, to form an activated capture primer, and

d) optionally, amplifying the activated capture primer to produce a monoclonal cluster of immobilized modified capture primers.

17) The microarray of claim 1, or 2 or method of claim 3 wherein at least one of

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

(ii) the first layer at least partially covers the inner well surface,

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

(iv) wherein the first layer comprises a polymer coating, optionally

wherein the polymer coating comprises poly(N-(5 azidoacetamidylpentyl)acrylamide-co-acrylamide (PAZAM).

18) The method of claim 3, 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.

19) The method of claim 7, wherein the plurality of target polynucleotides are flanked by complementary SBSs each comprising a complementary Illumina® SBS3' primer nucleotide sequence or a complementary Illumina® SBS8' primer nucleotide sequence, optionally

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

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

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

wherein the at least one second capture primer pair comprises a plurality of first capture primers comprising an Illumina® P5 primer nucleotide sequence, or a fragment thereof, and an Illumina® SBS3 primer nucleotide sequence, or a fragment thereof, and a second plurality of capture primers comprising an Illumina® P7 primer nucleotide sequence, or a fragment thereof, and an Illumina® SBS8 primer nucleotide sequence, or a fragment thereof.

20) The method of claim 8, wherein the universal capture region Y comprises an Illumina® P5 primer nucleotide sequence and the universal capture region Z comprises an Illumina® P7 primer nucleotide sequence, optionally

wherein the 5'-terminal universal capture region of each template nucleic acid comprises a complementary Illumina® P5' primer nucleotide sequence and the 3'-terminal universal capture region of each template nucleic acid comprises a complementary Illumina® P7' primer nucleotide sequence, optionally

wherein the 5'-terminal universal capture region of each template nucleic acid comprises a complementary Illumina® P3' primer nucleotide sequence and the 3'-terminal universal capture region of each template nucleic acid comprises a complementary Illumina® P7' primer nucleotide sequence, optionally

wherein the target-specific capture region of each template nucleic acid is between the 3'-terminal universal capture region and the one or more restriction sites.