Claims

1) 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 3'-terminal universal capture regions complementary to Y or Z, each template nucleic acid comprises one or more restriction sites, and each template nucleic acid comprises a 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.

2) 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 primer nucleotide sequence comprising SEQ ID NO: 1 and a second plurality of primers comprising a 3'-terminal primer nucleotide sequence comprising SEQ ID NO: 2, and

wherein each template nucleic acid is flanked by a 3'-terminal complementary primer nucleotide sequence comprising SEQ ID NO: 3 and a 5'-terminal complementary primer nucleotide sequence comprising SEQ ID NO: 4, 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 primer nucleotide sequence comprising SEQ ID NO: 3 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 kinetic exclusion assay (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 primer nucleotide sequence comprising SEQ ID NO: 1 and the target-specific capture region and a plurality of immobilized double-stranded regenerated universal capture primers comprising the primer nucleotide sequence comprising SEQ ID NO: 2, 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.

3) 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 complementary to 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.

4) 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 complementary to Y, a 3'-terminal universal capture region complementary to Z, a target-specific capture region, a restriction site between the 5'-terminal universal capture region complementary to Y and the target-specific capture region, and a sequencing primer binding site (SBS) between the 3'-terminal universal capture region complementary to 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.

5) 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 complementary to Y, a 3'-terminal universal capture region complementary to Z, a target-specific capture region, a restriction site between the 5'-terminal universal capture region complementary to Y and the target-specific capture region, and a SBS between the 3'-terminal universal capture region complementary to 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.

6) 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 complementary to Y, a 3'-terminal universal capture region complementary to 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 complementary to 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.

7) 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 complementary to Y, a 3'-terminal universal capture region complementary to Z, a target-specific capture region and a restriction site between the 5'-terminal universal capture region complementary to 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.

8) 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 complementary to X, a 3'-terminal universal capture region complementary to Z, a target-specific capture region, and a restriction site between the region complementary to 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.

9) The method of claim 1, wherein the universal capture region Y comprises a primer nucleotide sequence comprising SEQ ID NO: 1 and the universal capture region Z comprises a primer nucleotide sequence comprising SEQ ID NO: 2, optionally

wherein the 5'-terminal universal capture region of each template nucleic acid comprises a complementary primer nucleotide sequence comprising SEQ ID NO: 3 and the 3'-terminal universal capture region of each template nucleic acid comprises a complementary primer nucleotide sequence comprising SEQ ID NO: 4, 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.