

What is claimed is:

1. A kit, comprising:
a flow cell, including:
a substrate; and
5 first and second capture primers attached to the substrate; and
a genotyping probe fluid, including:
a liquid carrier; and
a genotyping oligonucleotide in the liquid carrier, the genotyping
oligonucleotide including:
10 a first primer sequence;
a probe sequence representative of a target genotyping
locus;
a restriction endonuclease site; and
a second primer sequence that is at least partially
15 complementary to the second capture primer.
2. The kit as defined in claim 1, wherein the genotyping probe fluid
includes a plurality of genotyping oligonucleotides, and wherein each
genotyping oligonucleotide includes a different probe sequence than each
20 other genotyping oligonucleotide.
3. The kit as defined in one of claims 1 or 2, wherein the second
capture primer includes a cleavage site.
- 25 4. The kit as defined in claim 3, wherein the restriction endonuclease
site is sensitive to a restriction endonuclease selected from the group
consisting of a 4 base cutter restriction endonuclease, a 5 base cutter
restriction endonuclease, and a 6 base cutter restriction endonuclease.
- 30 5. The kit as defined in one of claims 1 or 2, wherein the second
capture primer further includes a second restriction endonuclease site,

wherein the second restriction endonuclease site is complementary to the restriction endonuclease site of the genotyping oligonucleotide.

5 6. The kit as defined in claim 5, wherein the restriction endonuclease site and the second restriction endonuclease site are sensitive to a Type IIS methyl sensitive restriction endonuclease.

10 7. The kit as defined in one of claims 1 through 6, wherein the genotyping oligonucleotide further comprises an index sequence portion and a priming site portion between the first primer sequence and the probe sequence.

15 8. The kit as defined in one of claims 1 through 7, wherein:
the substrate has depressions separated by interstitial regions; and
the first and second capture primers are attached within each of the depressions.

 9. A method, comprising:
introducing a genotyping probe fluid to a flow cell including first and
20 second capture primers, the genotyping probe fluid including a plurality of genotyping oligonucleotides, each of the genotyping oligonucleotides including:
a first primer sequence;
a probe sequence representative of a respective target
25 genotyping locus;
a restriction endonuclease site; and
a second primer sequence that is at least partially complementary to the second capture primer;
whereby a respective genotyping oligonucleotide reacts to produce respective
30 clonal populations of amplicons from the respective genotyping oligonucleotide;
linearizing the amplicons to produce probe templates;

sequencing at least one probe identifying section of the probe templates to identify each of the probe sequences;
removing at least respective nascent strands from the probe templates, whereby a 3' OH group at an end of the probe templates is exposed;
5 hybridizing respective samples to the probe templates; and
performing respective genotyping reactions of the samples at the exposed 3' OH groups.

10 10. The method as defined in claim 9, wherein linearizing the amplicons involves:
cleaving the amplicons attached to the second capture primers at respective cleavage sites of the second capture primers; and
denaturing cleaved portions of the amplicons attached to the second capture primers to produce the probe templates.

15 11. The method as defined in claim 10, wherein sequencing the at least one probe identifying section of the probe templates involves performing a base extension reaction along the at least one probe identifying section using the second capture primer as a sequencing primer.

20 12. The method as defined in claim 11, wherein removing at least respective nascent strands from the probe templates involves:
digesting the restriction endonuclease sites; and
denaturing a remaining nascent strand from the probe templates.

25 13. The method as defined in claim 9, wherein each of the second capture primers further includes a second restriction endonuclease site, wherein the second restriction endonuclease site is complementary to the restriction endonuclease site of the genotyping oligonucleotide, and wherein
30 linearizing the amplicons involves:
digesting a restriction endonuclease portion of the amplicons to leave the second capture primers; and

denaturing a remaining portion of the amplicons to produce single stranded probe templates including the first capture primers and the at least one probe identifying section.

5 14. The method as defined in claim 13, wherein sequencing the at least one probe identifying section portion of the single stranded probe templates involves:

 introducing a sequencing primer; and
 performing a base extension reaction along the at least one probe
10 identifying section of each of the single stranded probe templates.

 15. The method as defined in claim 14, wherein removing at least respective nascent strands from the single stranded probe templates involves denaturing the nascent strands from the at least one probe identifying section.

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 16. The method as defined in one of claims 13 through 15, further comprising blocking the second capture primers prior to sequencing along the at least one probe identifying section of each of the single stranded probe templates.

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 17. The method as defined in one of claims 9 through 16, further comprising correlating each identified probe sequence with a respective clonal population of amplicons.

25 18. The method as defined in one of claims 9 through 17, wherein prior to hybridizing the respective samples to the probe templates, the method further comprising preparing the respective samples by:

 contacting a single stranded genomic DNA template with a low processivity polymerase, a plurality of primers, and free nucleotides, thereby
30 generating complementary fragments of the single stranded genomic DNA template; and

displacing the complementary fragments from the single stranded genomic DNA template, thereby generating at least some of the respective samples.

5 19. The method as defined in claim 18, further comprising denaturing a double stranded genomic deoxyribonucleic acid (DNA), thereby generating the single stranded genomic DNA template.

10 20. The method as defined in claim 18, further comprising repeating both the contacting and the displacing a predetermined number of cycles to generate additional complementary fragments in each of the predetermined number of cycles.

15 21. The method as defined in claim 18, wherein the low processivity polymerase is selected from the group consisting of T4 DNA polymerase, T7 DNA polymerase, Taq polymerase, Stoffel Fragment, Klenow Fragment, Bsu DNA polymerase, Bst DNA polymerase, and an engineered polymerase.

20 22. The method as defined in one of claims 9 through 17, wherein prior to hybridizing the respective samples to the probe templates, the method further comprising preparing the respective samples by:

 contacting a single stranded genomic DNA template with a polymerase, a plurality of primers, and a mixture of free nucleotides including natural nucleotides and dideoxythymidine triphosphate, thereby generating
25 truncated complementary fragments of the single stranded genomic DNA template; and

 displacing the truncated complementary fragments from the single stranded genomic DNA template, thereby generating at least some of the respective samples.

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 23. The method as defined in claim 22, wherein the natural nucleotides include deoxyadenine triphosphate, deoxythymine triphosphate,

deoxyguanine triphosphate, and deoxycytosine triphosphate, and wherein the mixture of free nucleotides includes a ratio of deoxythymine triphosphate to dideoxythymidine triphosphate ranging from about 10:5 to about 10:0.01.

5 24. The method as defined in claim 22, further comprising denaturing a double stranded genomic deoxyribonucleic acid (DNA), thereby generating the single stranded genomic DNA template.

10 25. The method as defined in claim 22, further comprising repeating both the contacting and the displacing a predetermined number of cycles to generate additional truncated complementary fragments in each of the predetermined number of cycles.

15 26. The method as defined in claim 22, wherein the polymerase is selected from the group consisting of T4 DNA polymerase, T7 DNA polymerase, Taq polymerase, Stoffel Fragment, Klenow Fragment, Bsu DNA polymerase, Bst DNA polymerase, and an engineered polymerase.