

What is claimed is:

Claim 1. A genotyping probe fluid, comprising:

a liquid carrier; and

5 a genotyping oligonucleotide in the liquid carrier, the genotyping oligonucleotide including:

a first primer sequence;

an index sequence portion directly attached to the first primer sequence;

10 a priming site portion directly attached to the index sequence portion;

a probe sequence directly attached to the priming site portion, the probe sequence being representative of a target genotyping locus having a nucleobase of interest, wherein a last base of the probe sequence is

15 representative of a nucleobase of the target genotyping locus that is positioned directly adjacent to the nucleobase of interest;

a restriction endonuclease site; and

a second primer sequence that is at least partially complementary to a flow cell capture primer,

20 wherein one of:

the restriction endonuclease site is positioned between the probe sequence and the second primer sequence; or

the probe sequence is directly attached to the second primer sequence and the restriction endonuclease site is integrated into the second primer sequence at a cleavage distance from the last base of the probe sequence.

Claim 2. The genotyping probe fluid as defined in claim 1, further comprising a plurality of genotyping oligonucleotides in the liquid carrier, wherein
30 each genotyping oligonucleotide includes a different probe sequence than each other genotyping oligonucleotide.

Claim 3. The genotyping probe fluid as defined in one of claims 1 or 2, wherein the restriction endonuclease site is sensitive to a restriction
35 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.

Claim 4. A kit, comprising:

- 5 a flow cell, including:
 - a substrate; and
 - first and second capture primers attached to the substrate, the second capture primer having a restriction endonuclease site;
- a genotyping probe fluid, including:
 - 10 a liquid carrier; and
 - a genotyping oligonucleotide in the liquid carrier, the genotyping oligonucleotide including:
 - a first primer sequence;
 - an index sequence portion directly attached to the first primer
 - 15 sequence;
 - a priming site portion directly attached to the index sequence portion;
 - a probe sequence directly attached to the priming site portion, the probe sequence being representative of a target genotyping locus having a
 - 20 nucleobase of interest, wherein a last base of the probe sequence is representative of a nucleobase of the target genotyping locus that is positioned directly adjacent to the nucleobase of interest;
 - a second restriction endonuclease site that is complementary to the restriction endonuclease site of the second capture primer; and
 - 25 a second primer sequence that is at least partially complementary to the second capture primer,
- wherein one of:
 - the restriction endonuclease site is positioned between the probe sequence and the second primer sequence; or
 - 30 the probe sequence is directly attached to the second primer sequence and the restriction endonuclease site is integrated into the second primer sequence at a cleavage distance from the last base of the probe sequence; and
- genotyping library preparation components, including:
 - 35 a polymerase;

a plurality of random primers, and
free nucleotides.

Claim 5. The kit as defined in claim 4, wherein:

5 the polymerase is a high processivity polymerase 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; and

10 the free nucleotides include a mixture of natural nucleotides and
dideoxythymidine triphosphate (ddTTP).

Claim 6. The kit as defined in claim 4, wherein:

15 the polymerase is a low processivity polymerase selected from the group
consisting of T4 DNA polymerase, T7 DNA polymerase, Taq polymerase, Stoffel
Fragment, Klenow Fragment, Bsu DNA polymerase, Bst DNA polymerase,
monomeric E. coli Pol III or E. coli Pol I, and an engineered polymerase; and

the free nucleotides include deoxyadenine triphosphate, deoxythymine
triphosphate, deoxyguanine triphosphate, and deoxycytosine triphosphate.

20 Claim 7. The kit as defined in any one of claims 4 to 6, wherein the
plurality of random primers is a population of random primers synthesised to
include a higher content of guanine and/or cytosine nucleotides compared to
adenine and thymidine nucleotides.

25 Claim 8. The kit as defined in any one of claims 4 to 7, wherein the
restriction endonuclease site and the second restriction endonuclease site are
sensitive to a Type IIS methyl sensitive restriction endonuclease.

Claim 9. A method, comprising:

30 introducing a genotyping probe fluid as defined in claim 4 to a flow cell as
defined in claim 4, the genotyping probe fluid including a plurality of genotyping
oligonucleotides as defined in claim 4; whereby a respective genotyping
oligonucleotide reacts to produce respective clonal populations of amplicons from
the respective genotyping oligonucleotide;

35 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.

Claim 10. The method as defined in claim 9, wherein, prior to hybridizing
10 the respective samples to the probe templates, the method further comprises 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 truncated
15 complementary fragments of the single stranded genomic DNA template; and
displacing the truncated complementary fragments from the single stranded genomic DNA template.

Claim 11. The method as defined in claim 10, wherein the natural
20 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.

25 Claim 12. The method as defined in claim 10 or 11, further comprising denaturing a double stranded genomic deoxyribonucleic acid (DNA), thereby generating the single stranded genomic DNA template.

Claim 13. The method as defined in any one of claims 10 to 12, further
30 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.

Claim 14. The method as defined in any one of claims 10 to 13, wherein
35 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.