

CLAIMS

1. A method of preparing a template for a nucleic acid sequencing reaction, the method comprising:

5 a) providing a solid support, wherein said solid support comprises a plurality of nucleic acid primers immobilised thereon;

10 b) contacting a nucleic acid library preparation with the solid support, the library preparation comprising a plurality of single stranded fragments of template nucleic acid to be sequenced, the template nucleic acid fragments further comprising one or more adaptor nucleic acid sequences which hybridise to one or more of the nucleic acid primers, and the library preparation having a nucleic acid concentration of 400 pM or less;

c) allowing single stranded fragments of template nucleic acid to bind to said nucleic acid primers, thereby immobilising said single stranded fragments on said solid support; and

15 d) repeating steps b) and c) at least three further times, wherein repetition of the contacting step takes place with the same library preparation as used in step (b);

to thereby provide a solid support having single stranded fragments of template nucleic acid immobilised thereon, wherein the method does not comprise an amplification step until steps b) and c) have been repeated at least three times.

20 2. The method of claim 1, wherein the method is a method of preparing a template for a nucleic acid sequencing reaction, the method comprising:

25 a) providing a flow cell comprising a solid support having a plurality of nanowells formed thereon, each nanowell comprising a plurality of nucleic acid primers immobilised on the solid support, and the nanowells being formed in a patterned array having a pitch of 500 nm or less;

b) contacting a nucleic acid library preparation with the flow cell, the library preparation comprising a plurality of single stranded fragments of template nucleic acid to be sequenced, the template nucleic acid fragments further comprising one or more adaptor nucleic acid sequences which hybridise to one or more of the nucleic acid

primers, and the library preparation having a nucleic acid concentration of 400 pM or less;

5 c) allowing single stranded fragments of template nucleic acid to bind to said nucleic acid primers, thereby immobilising said single stranded fragments in the nanowells; and

d) repeating steps b) and c) at least three further times, wherein repetition of the contacting step takes place with the same library preparation as used in step (b);

10 to thereby provide a flow cell having single stranded fragments of template nucleic acid immobilised in nanowells, wherein the method does not comprise an amplification step until steps b) and c) have been repeated at least three times.

3. The method of claim 1 or claim 2 wherein an effective nucleic acid concentration calculated as number of cycles of step (c) times library nucleic acid concentration is at least 800 pM.

15 4. The method of any preceding claim wherein the library preparation has a nucleic acid concentration of 250 pM or less.

5. The method of any preceding claim comprising denaturing a library preparation comprising double stranded fragments of template nucleic acid to be sequenced, to obtain the single stranded fragments of template nucleic acid to be sequenced used in step (b).

20 6. The method of any preceding claim further comprising amplifying the immobilised single stranded fragments of nucleic acid, to thereby generate multiple copies of the fragments.

7. The method of any preceding claim wherein the solid support is glass.

25 8. The method of claim 1, or claims 3 to 7 when dependent on claim 1, wherein said solid support is on a flowcell, wherein said solid support has a plurality of nanowells formed thereon, each nanowell comprising a plurality of nucleic acid primers immobilised on the solid support, and the nanowells being formed in a patterned array.

9. The method of claim 8 wherein the pitch of the nanowell array is 500 nm or less.

30 10. The method of any one of claims 2 to 9 wherein the pitch of the nanowell array is 350 nm or less.

11. The method of claim 1, or claims 3 to 7 when dependent on claim 1, wherein the solid support is a microbead.

12. The method of any preceding claim further comprising sequencing the immobilised single stranded fragments of nucleic acid.