

CLAIMS

1. A method for carrying out a strand resynthesis extension reaction during pairwise sequencing, wherein a strand resynthesis extension reaction is carried out between a first sequencing read and a second sequencing read, and wherein said strand resynthesis extension reaction extends one or more immobilised primers to copy a first template strand to generate a second immobilised template strand; characterised in that the strand resynthesis extension reaction is carried out using a non-thermostable strand displacement polymerase at a temperature of less than 55 °C, wherein said first template strands are a cluster, and wherein the same non-thermostable strand displacement polymerase is used during initial cluster generation and the strand resynthesis extension reaction.
2. The method of claim 1, wherein the non-thermostable polymerase has an optimum incubation temperature and/or optimum activity temperature below 55 °C, preferably below 50°C, below 45°C, below 40°C, below 39°C, at or below 38°C, around 38°C or around 37°C; particularly preferably around 38°C.
3. The method of any preceding claim, wherein the extension reaction is carried out at a temperature of less than 55 °C, preferably less than 50°C, preferably less than 40°C, preferably 38°C +/- 2°C, preferably 38°C +/- 1°C, preferably 38°C.
4. The method of any preceding claim, wherein the non-thermostable polymerase is Bsu, phi29, Klenow, and DNA Polymerase I (E. coli), preferably Bsu or a functional fragment thereof.
5. The method of any preceding claim, wherein the strand resynthesis extension reaction is repeated through multiple cycles of extension and denaturation, wherein preferably, the method comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more cycles; preferably 2-5 cycles, 2-4 cycles, 3-5 cycles, 3 cycles or 4 cycles; most preferably 3 cycles.
6. The method of any preceding claim, wherein each cycle may last for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, or 30 minutes, wherein preferably the method comprises the following cycles of extension and denaturation: 3x5mins; 3x30mins; 12x2mins; 6x4mins; 2x12mins or 3x5mins; preferably 12x2mins.
7. The method of any preceding claim, wherein denaturation is carried out substantially at the temperature of the extension reaction, wherein preferably denaturation is carried out using acidic nucleic acid denaturants such as acetic acid, HCl, or nitric acid; basic nucleic acid

denaturants such as NaOH; or other nucleic acid denaturants such as DMSO, formamide, betaine, guanidine, sodium salicylate, propylene glycol or urea; preferably formamide and NaOH, particularly preferably formamide.

8. The method according to any preceding claim, wherein the strand resynthesis extension reaction is carried out at a lower temperature than the sequencing read steps.

9. The method according to any preceding claim, wherein said cluster was initially generated by bridge amplification.

10. The method according to any preceding claim, wherein at least one of the immobilised primers is blocked at the 3' end, and the block is removed prior to the strand resynthesis extension reaction, wherein preferably the block is a phosphate group and is treated with a phosphatase to remove the block.

11. The method according to any preceding claim, further comprising a step of treating with a restriction enzyme prior to the strand resynthesis extension reaction to shorten the immobilised primer and release a free 3' hydroxyl for extension.

12. The method according to any preceding claim, wherein the immobilised primer is extended prior to the strand resynthesis extension reaction, wherein preferably the immobilised primer is extended by hybridisation of a non-immobilised complementary sequence with a 5'-overhang, and the immobilised primer is extended to copy the overhang.

13. A method for pairwise sequencing of first and second regions of a target double-stranded polynucleotide, wherein said first and second regions are in the same target double-stranded polynucleotide, said pairwise sequencing including the steps as disclosed in any one of claims 1 to 12.

14. Use of the method of claim 13 for obtaining two linked or paired reads of sequencing information from each of the double-stranded template on a clustered array.

15. A method of improving the data quality of a sequencing reaction, comprising carrying out an extension reaction as claimed in any one of claims 1 to 12.