What is the Illumina method of DNA sequencing?

Illumina sequencing has been used to sequence many genomes and has enabled the comparison of DNA sequences to improve understanding of health and disease.

Illumina sequencing generates many millions of highly accurate reads making it much faster and cheaper than other available sequencing? methods.

How does Illumina DNA sequencing work?

1. The first step in this sequencing technique is to break up the DNA? into more manageable fragments of around 200 to 600 base pairs? .

2. Short sequences of DNA called adaptors? , are attached to the DNA fragments.

3. The DNA fragments attached to adaptors are then made single stranded. This is done by incubating the fragments with sodium hydroxide.

4. Once prepared, the DNA fragments are washed across the flowcell. The complementary DNA binds to primers? on the surface of the flowcell and DNA that doesn't attach is washed away.

5. The DNA attached to the flowcell is then replicated to form small clusters of DNA with the same sequence. When sequenced, each cluster of DNA molecules will emit a signal that is strong enough to be detected by a camera.

6. Unlabelled nucleotide bases[?] and DNA polymerase[?] are then added to lengthen and join the strands of DNA attached to the flowcell. This creates 'bridges' of double-stranded DNA between the primers on the flowcell surface.

7. The double-stranded DNA is then broken down into single-stranded DNA using heat, leaving several million dense clusters of identical DNA sequences.

8. Primers and fluorescently[?] -labelled terminators (terminators are a version of nucleotide base – A, C, G or T – that stop DNA synthesis) are added to the flowcell.

9. The primer attaches to the DNA being sequenced.

10. The DNA polymerase then binds to the primer and adds the first fluorescently-labelled terminator to the new DNA strand. Once a base has been added no more bases can be added to the strand of DNA until the terminator base is cut from the DNA.

11. Lasers are passed over the flowcell to activate the fluorescent label on the nucleotide base. This fluorescence is detected by a camera and recorded on a computer. Each of the terminator bases (A, C, G and T) give off a different colour.

12. The fluorescently-labelled terminator group is then removed from the first base and the next fluorescently-labelled terminator base can be added alongside. And so the process continues until millions of clusters have been sequenced.

13. The DNA sequence is analysed base-by-base during Illumina sequencing, making it a highly accurate method. The sequence generated can then be aligned to a reference sequence, this looks for matches or changes in the sequenced DNA.

Illumina sequencing machines in the sequencing centre at the Sanger Institute in 2009.
Image credit: Genome Research Limited