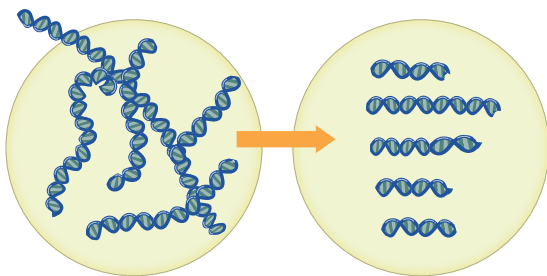


ILLUMINA SEQUENCING TECHNOLOGY

The Illumina approach relies on attaching fragmented genomic DNA prepared in a sample library to a planar, optically transparent surface on a flow cell. These templates are sequenced using a four-color DNA sequencing-by-synthesis technology that employs reversible terminators with removable fluorescence. This highly parallel approach can generate close to 400 billion bases (Gigabases) with high accuracy, with 1.3 billion reads per flow cell run as paired-end 150 basepair reads.

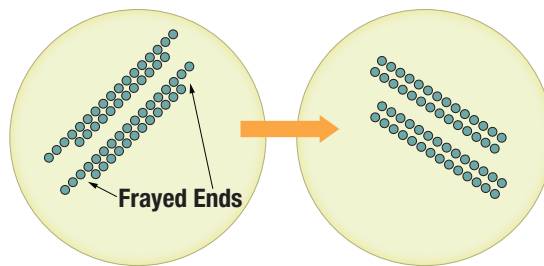
Labeled nucleotides are incorporated at each cycle and high sensitivity fluorescence detection is achieved using laser excitation and total internal reflection optics. Images are compiled and processed to produce base sequences for each DNA template. Applications are de novo sequencing where there is no reference available and resequencing, where short sequence reads are aligned against a reference. The genetic differences on the sequences are called using a specially developed data pipeline.

1 SONICATION



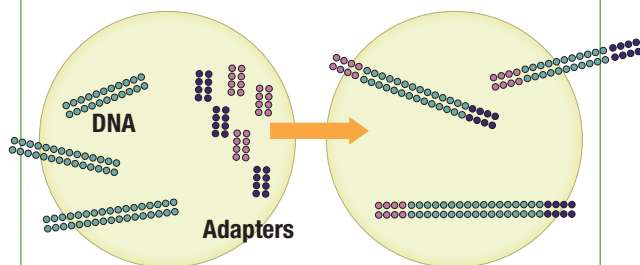
Genomic DNA is fragmented into 100-500 base pair fragments by sonication to create a library.

2 FRAGMENT END REPAIR



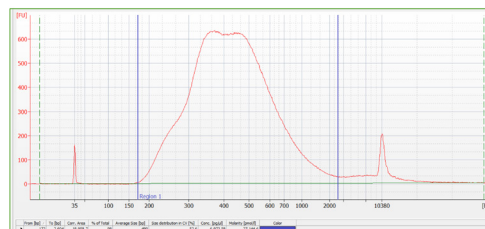
Sonication creates frayed DNA ends which must be blunted or repaired.

3 A-TAILING AND ADAPTER LIGATION



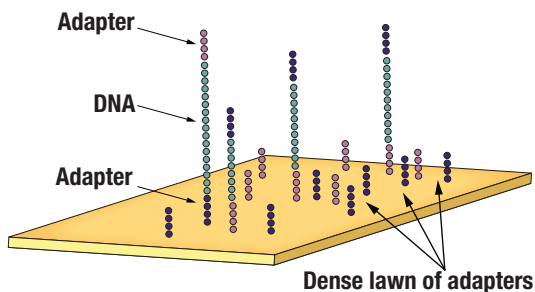
Adapters are ligated to each end of the A-tailed DNA fragment.

4 QC CHECK



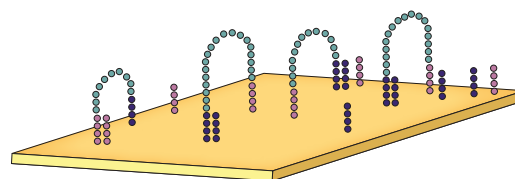
The electropherogram shows the size and concentration of the final library. This library size also confirms the ligation of adapters.

5 CBOT CLUSTER GENERATION SYSTEM



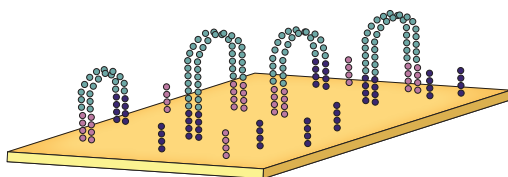
Sodium hydroxide creates single-stranded DNA. Randomly bind these single-stranded DNA to the top and bottom of each channel in the flow cell.

6 BRIDGE FORMATION



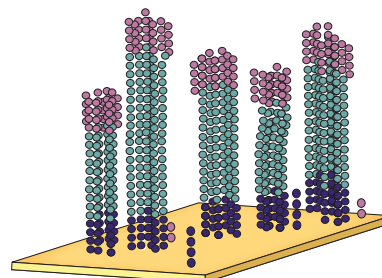
Free DNA end binds to complimentary primer to form a bridge.

7 BRIDGE AMPLIFICATION



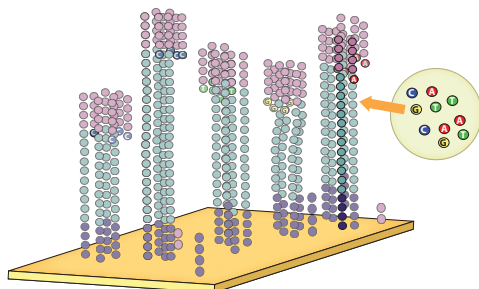
Add unlabeled nucleotides and enzyme to initiate solid-phase bridge amplification. Fragments become double-stranded DNA bridges. Thirty-five (35) cycles of amplification create clusters of identical DNA fragments.

8 FINISHED FLOWCELL



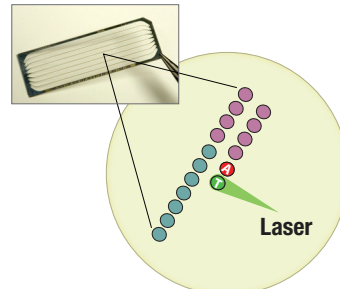
By completion of amplification, several million dense clusters of single-stranded DNA have been generated in each channel of the flow cell with a sequencing primer attached.

9 DNA SEQUENCING



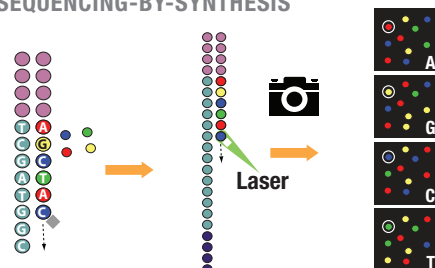
To initiate the first sequencing cycle and determine the first base, all four labeled reversible terminators and DNA polymerase enzyme are first added. Only one base can incorporate at a time.

10 BASE CALLING



Lasers excite the fluorescent tags and the images are captured via CCD camera. The identity of the first base in each cluster is recorded, and then the fluorescent tag is removed.

11 SEQUENCING-BY-SYNTHESIS



In the first cycle, the first base is incorporated. Its identity is determined by the signal given off and then recorded. In subsequent cycles, the process of adding sequencing reagents, removing unincorporated bases and capturing the signal of the next base to identify is repeated.

12 DUAL FLOW CELLS



Once the top surface of the flow cell channel has been scanned, the imaging step is repeated on the bottom surface.