

How to sequence using Illumina technology

Alt-text Figure 4 - Schematic illustration of Illumina sequencing protocol steps 1-6

Printed in the image: 1) Prepare genomic DNA Sample: randomly fragmented genomic DNA and ligate adapters to both ends of the fragments. 2) Attach DNA to the surface: bind single-stranded fragments randomly to the inside surface of the flow cell channels. 3) Bridge amplification: add unlabeled nucleotides and enzymes to initiate solid-phase bridge amplification. 4) Fragments become double-stranded: the enzyme incorporates nucleotides to build double-stranded bridges on the solid-phase substrate. 5) Denature the double-stranded molecules: denaturation leaves single-stranded templates anchored to the substrate. 6) Complete amplification: several million dense clusters of double-stranded DNA are generated in each channel of the flow cell.

Alt-text Figure 5 - Schematic illustration of Illumina sequencing protocol steps 7-12

Printed in the image: 7) Determine the first base: the first sequencing cycle begins by adding four labelled reversible terminators, primers, and DNA polymerase. 8) Image first base: after laser excitation, the emitted fluorescence from each cluster is captured and the first base is identified. 9) Determine the second base: the next cycle repeats the incorporation of four labelled reversible terminators, primers, and DNA polymerase. 10) Image second chemistry cycle: after laser excitation, the image is captured as before, and the identity of the second base is recorded. 11) Sequencing over multiple chemistry cycles: the sequencing cycles are represented to determine the sequence of bases in a fragment, one base at a time. 12) Align data: the data are aligned and compared to a reference, and sequencing differences are identified.