Transcript: Next-Generation Sequencing Workflow

The Illumina sequencing system enables a broad array of applications in genomics, transcriptomics and epigenomics

In this short video we will go through all the steps of a next generation sequencing (NGS) workflow

Before starting the next generations sequencing workflow, we need to isolate and purify the nucleic acid. NGS workflows require a QC step: Quantification and assessment of the purity of the nucleic acid.

The first step in NGS workflow is the library preparation. This step is crucial for the success oof the sequencing. Sequencing libraries are prepared by fragmenting the DNA and adding specialised adapters to both ends. These adapters contain complementary sequences that allow the DNA fragments to bind the flow cell. Fragments can then be amplified and purified.

In order to save resources, multiple libraries can be pooled together and sequenced in the same run, this process is called multiplexing. During the adapters ligation, unique sequences or “barcodes” are added to each library. Barcodes are used to distinguish between the libraries during data analysis.

After quantifying each library using a Qubit and Tapestation, we pool them to equimolar quantity (4nM)

Libraries need to undergo a denaturation step to allow the binding of single stand DNA to the flow cell.

To allow denaturation, we mix libraries with a solution 0.2nM NaOH

Complete denaturation of dsDNA to ssDNA occurs in 5 minutes

… 5 minutes later…

Denatured libraries are diluted in the sequencing buffer HT1 to an intermediate concentration (20pM)

Vortex and spin the sample

Dilute the denatured library to the final concentration of 8pM

The second step in NGS workflow is the sequencing. During this step the libraries are loaded onto a flow cell and placed on the sequencer. Clusters of DNA fragments are amplified in a process called cluster generation, resulting in millions of copies of ssDNA.

In a process called sequencing by synthesis (SBS) chemically modified nucleotides bind to the DNA template strand through natural complementarity. Each nucleotide contains a fluorescent tag and a reversible terminator that blocks the incorporation of the next base. The fluorescent signal indicates which nucleotide has been added and the terminator is cleaved so that the next base can bind.

After reading the forward DNA strand, the reads are washed away, and the process repeats for the reverse strand. This method is called paired-end sequencing.

The flow cell is rinsed in distilled water first

And rinsed one more time using ethanol and then it is dried thoroughly

Load the library in the new cartridge

Select the user account to start the sequencer

Replace the old flow cell with the new one just rinsed

Load the sequencing buffer

Remove the old cartridge

Load the new cartridge containing the library

Review the run settings

…and start the sequencer.

The last step in NGS workflow is the data analysis. After sequencing, the software identifies nucleotides, in a process called “base calling”, and the accuracy of those base calls. Sequencing data can be imported in a standard analysis tool or we can set up our own pipeline.

It is also possible to use data analysis apps to analyse NGS data without bioinformatic training or additional lab staff. These apps provide sequence alignment, variant calling, data visualization or interpretation.