CPGR | CENTRE FOR PROTEOMIC & GENOMIC RESEARCH

Quality Control and Quality Assurance of Illumina Sequencing Libraries

QC of individual libraries

Following preparation of sequencing libraries, each individual library undergoes two QC steps:

1) quantification using the Qubit DNA HS Assay Kit (ThermoFisher Scientific)
2) the library fragment size distribution is determined using a suitable Bioanalyzer or Tape Station assay (Agilent)

QC of pooled libraries

Prior to loading a sequencing flow cell, the individual libraries are pooled in an equimolar concentration.

The concentration and fragment size distribution of the final pooled library is confirmed using:

1) qPCR-based quantification of adapter-ligated fragments using the Kapa Library Quantification Kit (Roche)
2) the pooled library fragment size distribution is confirmed using a suitable Bioanalyzer High Sensitivity DNA Chip Kit or Tape Station assay (Agilent)

Sequencing control

A PhiX positive control (Illumina) is spiked into the final pooled library in order to evaluate the efficiency of the sequencing reaction.

Sequencing Quality Metrics

After the sequencing run, a summary of the Quality Metrics of the run is obtained using the Sequence Analysis Viewer program (Illumina). Cluster density, clusters passing filter, reads passing filter, data output and the percentage reads with an average Q-score ≥ 30 are assessed and compared to Illumina's guidelines (a Q-score ≥ 30 is equivalent to a ≤ 0.1% probability that an incorrect base has been called by the sequencer).

Sequencing Output

CPGR will provide all FASTQ sequencing files via Basespace download. This is accompanied by an Analytical report outlining all the methods used and the QC results obtained.