Sample requirements, sequencing and data Analysis

Custom prepared libraries

Libraries must be compatible with the sequencing reagents provided by Illumina.

Libraries must be provided in at least 40 µL with a concentration of minimum 4 nM in TE or EB supplemented with 0.1% Tween.

DNA - Whole genome sequencing

Sample requirements for the preparation of whole genome libraries starting from genomic DNA:

- At least 2 µg of DNA (RNase treated)
- OD 260/280 nm ratio of approximately 1.8
- Concentration of 12 ng/uL or above in 10 mM Tris-Cl, pH 8.5 (no EDTA)
- Gel electrophoresis or equivalent to verify that sample is high molecular weight
- DNA library preparation performs most optimally on intact DNA but can be performed on partially degraded RNA, such as obtained from formalin-fixed, paraffin-embedded (FFPE) samples. However, the quality of FFPE DNA can be highly variable due to the tissue-fixation procedure, age of the sample, storage conditions, de-fixation process, etc. Therefore, we cannot guarantee success with every FFPE DNA sample.

DNA - Targeted sequencing

If only specific regions of the genome are of interest, targeted DNA sequencing is the feasible choice. By using in-solution probe based targeting methods it is possible to select and enrich these regions of interest before sequencing. Targeting of all exons is offered as 'on the shelf' whole Exome (Twist Bioscience). For smaller regions of interest, MOMA offers targeted sequencing of several smaller gene panels: https://moma.dk/list-of-genetic-analyses/current-genepanels.

Sample requirements for Exome sequencing and panel targeted sequencing are the same as for whole genome sequencing.

RNA sequencing

RNA-Seq libraries are prepared using the KAPA RNA HyperPrep kits (Roche) providing strand-specific, multiplexed and paired-end libraries. The input RNA is isolated from total purified RNA using either poly(dA) selection (mRNA capture, Roche) or rRNA depletion (RiboErase, Roche).

Sample requirements for the preparation of RNA-Seq libraries starting from total purified RNA:

- At least 500 ng of RNA (DNase treated)
- OD 260/280 nm ratio of approximately 2.0
- Concentration of 40 ng/uL or above in 10 mM Tris-Cl, pH 8.5 (no EDTA)
- Gel electrophoresis or equivalent to verify the size profile of the total-RNA
- For poly(dA) selection, the RNA must have an RNA Integrity Number (RIN) value greater than 8 as estimated using the Agilent Technologies 2100 Bioanalyzer, or 4200 Tapestation. Lower RIN values can be accepted if Ribo-Zero is to be applied.
- RNA-Seq performs most optimally on intact RNA but can be performed on partially degraded RNA, such as obtained from formalin-fixed, paraffin-embedded (FFPE) samples. However, the quality of FFPE RNA can be highly variable due to the tissue-fixation procedure, age of the sample, storage conditions, de-fixation process, etc. Therefore, we cannot guarantee success with every FFPE RNA sample.
- For highly degraded RNA samples, RNA-seq library preparation can be attempted using a 3' mRNA-seq library kit (Quantseq, Lexogen), which generates Illumina compatible libraries of sequences close to the 3' end of polyadenylated RNA.

Sequencing

Sequencing is conducted using Illumina Novaseq 6000 instruments. A number of different run types are available, see Illumina.com for the most recent information.

Data analysis

Data is provided as demultiplexed fastq files with adjacent QC files. The data is to be downloaded using a provided link and should be checked for consistency upon arrival. The data will be deleted 30 days after delivery.