Next-generation sequencing (NGS) library preparation (Illumina)

Next-generation sequencing (NGS) allow high-throughput sequencing of whole genomes or their large portions. One of the approaches is so-called genome skimming which is a low-pass sequencing (i.e., with very low average coverage) of the unmodified genomic library. The most common NGS technology is Illumina which require to prepare a specific library prior to sequencing. The following steps are necessary to prepare and sequence the samples:

1. DNA sonication (fragmentation) using ultrasonicator – a specific instrument is required to shear the genomic DNA into pieces under specifically defined conditions. The success of sonication is checked on agarose gel.
2. Library preparation using NEBNext Ultra II DNA Library Prep Kit for Illumina – the sequencing library is prepared from sheared DNA using this kit following the instructions of manufacturer. Optionally (if we want to multiplex several samples prior to sequencing) the barcodes are introduce using NEBNext Multiplex Oligos (single- or dual labelling).
3. Measuring NGS library concentration using Qubit and sending to core facility for sequencing.

1. DNA Shearing with M220 Focused-ultrasonicator (Covaris)

The M220 Focused-ultrasonicator is based on Covaris Adaptive Focused Acoustics (AFA) technology.

Turn on the instrument – The switch is located at its back next to the power cord, turn on the computer. Double click on the SonoLab icon to start the software.

Filling the water bath – Fill the wash bottle with AFA-grade water, slide the blue cover on the nozzle up. Open the safety cover of the instrument, place the tube holder insert into the water bath housing. Place the tip of the wash bottle in the water sense aperture (in the holder) and fill the water bath by squeezing the wash bottle until the water reaches the top surface of the tube holder. Approximately 15 ml of water will be required.

Loading the sample – Lift the sliding weight and rotate it into the loading position. Take the specific sonicator tube (micro TUBE-AFA Fiber Screw-Cap Case-50µl) and pipette 50 µl of genomic DNA into it. Place it to the insert (the yellow part) and together place it to the holder (the blue part). Drop the sliding weight to the running position on top of the sample tube. Close the safety cover.

Processing the sample – Select an existing method in SonoLab. Use, e.g., these parameters: Peak Incident Power: 50W, Duty Factor: 20%, Cycles per Burst: 200, Treatment Time: 45 sec,

Temperature: 20 °C. Click on “Run” to start the sonication. After the process is finished, remove the sample tube.

Checking sonication results on the gel – prepare 0.8 agarose gel and load 2 µl of sonicated sample mixed with 1 µl loading dye. Load 0.7 µl of 1kb ladder in the first well and 0.4 µl of 100kb ladder in the last well. The DNA should be fragmented to the pieces of desired length (i.e., 400-600 bp). If the majority of fragments is too long sonication has to be repeated.

2. Library preparation using NEBNext Ultra II DNA Library Prep Kit for Illumina

For all the steps follow the most recent version of the NEBNext Ultra II protocol (cat. nr. E7645L). The library preparation consists of following steps:

End repair and A-tailing – this correct the sonicated fragments to fully double-stranded DNA and adds A overhangs. End Repair Reaction Buffer and End Prep Enzyme Mix is mixed together with the sonicated sample and incubated for 30 mins at 20 °C and additional 30 mins at 65 °C in thermocycler.

Adaptor ligation – this ligates Illumina-specific adaptors to the fragments. Ligase Master Mix, NEBNext Adaptor for Illumina and Ligation Enhancer is mixed with the repaired sample and incubated for 15 mins at 20 °C. Then, USER enzyme is added and the sample incubated at 37 °C for additional 15 mins.

Sample clean-up – the sample is purified using QIAquick PCR Purification Kit following the protocol. The sample is eluted with 32 µl ddH_2O.

Size selection – the desired fragment lengths are selected either by cutting from the gel (and purified) or using Pippin Prep instrument.

Final concentration measurement – the DNA concentration is precisely measured using fluorometry with Qubit instrument.

3. Measuring DNA concentration using Qubit

- work in gloves to avoid tube wall contamination
- use specific 0.5 ml thin wall tubes from Axygen
- mix 199 µl of Ready-to-use 1× working solution µl of DNA (vortexed, centrifuged)
- vortex briefly and let stand for 2mins before measuring
- to prepare standards for calibration – mix 190 µl of buffer and 1 µl of Standard 1 (or Standard 2), vortex briefly, let stand for 2 mins and measure