Technion Genomics Center

Technion Israel Institute of Technology

Oxford Nanopore sample requirements

Please note that Nanopore platform is especially sensitive to DNA sample quality. Low molecular weight, incorrectly quantified and/or contaminated DNA (*e.g.,* salt, EDTA, protein, organic solvents) can have a significant impact on downstream processes and ultimately, your sequencing runs. It is the user's responsibility for the results in case the samples submitted do not match the specifications below or do not meet the criteria of the chosen lab procedure.

- DNA submitted to Nanopore sequencing should meet the following criteria:
  - **Purity as measured using Nanodrop – OD 260/280 of 1.8 and OD 260/230 of 2.0–2.2.** Chemical impurities such as detergents, denaturants, chelating agents, and high concentrations of salts should be avoided as these may affect the efficiency of enzymatic steps.
  - **Average fragment size**, as measured by gel-electrophoresis or TapeStation analysis, >30 kb.
  - **Input mass, as measured by Qubit ≥1 ug** (it is recommended to bring 2 ug DNA, if possible). For non-high molecular weight DNA (*e.g.* amplicons), please submit 200 fmol.
  - Sample volume 20-50 ul.
- Most of the RNA should be removed by RNase digestion. *Please be aware that certain RNase treatments can lead to the digestion of DNA, as well as RNA*.
- When handling high molecular weight DNA samples, avoid vortexing the samples or pipetting up and down, as this will cause shearing, which will limit the fragment sizes available to the nanopore.
- DNA samples should be suspended in DDW or Tris buffer, with no EDTA.
- DNA samples should be submitted as follows:
  - in 1.7 ml Eppendorf tubes (<12 samples) or in PCR strip/96 well plate with an aluminum seal (>12 samples).
  - Tubes must be clearly marked with a serial number on the top and the side of the tube (not sample name), corresponding to the sample's number in the Sample Information Form. In the matching electronic sample submission form, write the number and a meaningful name (this name will be used as the sample name in your fastq files and subsequent data analysis). Ensure that the number on the tube is identical to the serial number of the sample in the electronic Sample Submission Form.
  - Arrange samples in the correct order in a box. We cannot accept samples in plastic bags or randomly placed on ice. Mark the box with your name and date.
- An electronic Sample Submission Form should be filled out and sent by email to linde@technion.ac.il.
- For **Amplicon-seq**, please perform amplicon validation prior to sending your samples. Amplicon should be a single fragment at the expected length. There is no size-selection during library prep and all fragments in the sample will be sequenced (which may cause adverse effects on the sequencing yield).

Recommendations

DNA extraction methods that are based on spin columns are preferred.