RNA-seq sample requirements

Note that the user is responsible 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.

- For standard RNA sample prep, RNA should be at a concentration of 100 ng/µl (min. 50 ng/ul).
- For CEL-Seq2 RNA requirements please see the guidelines in the *CEL-seq2 sample requirements*.
- For low-input RNA samples, please contact us.
- Concentration, 260/280 & 260/230 values should be determined using the NanoDrop. Pure RNA samples should be in the following range: 260/280 ≥ 1.8, 260/230 ≥ 2.0.
- RNA samples should be suspended in DDW or Tris buffer, with no EDTA.
- It is recommended to treat RNA samples with DNase as part of the extraction protocol (for rRNA removal libraries, DNase treatment is mandatory!). Careful consideration should be taken when choosing the DNAse reagent and protocol. Heat inactivation should be avoided as it may result in RNA degradation. RNA clean-up should be performed following DNAse treatment and resuspension with Tris or DDW, to avoid inhibitors carry-over from the inactivation buffer. It is important to make sure there is sufficient RNA remaining following this procedure!
- Please provide 2 aliquots:
    - One of 3 µl for Tapestation analysis (in 0.2ml tube), if required.
    - One of 20 µl for library preparation.
    - RNA samples should be submitted in 1.7 ml Eppendorf tubes (up to 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.
- Deliver samples frozen (dry ice) to assure sample integrity.
- An electronic Sample Submission Form should be filled out and sent by email to linde@technion.ac.il.

Recommendations-

1. Please use a column-based kit (such as Qiagen) for RNA extraction.
2. If needed, re-check the RNA concentration after the initial dilution, to ensure that the final concentration is 50-100 ng/ul.