CEL-Seq2 sample requirements

Bulk CEL-Seq2 is a high-throughput low-input 3'-mRNA-seq method. The protocol involves barcoding of samples by reverse transcription using an oligo dT primer, pooling of samples and subsequent molecular reactions for linear amplification and preparation for Illumina sequencing. For references see *Hashimshony, T., Senderovich, N., Avital, G. et al. CEL-Seq2: sensitive highly-multiplexed single-cell RNA-Seq. Genome Biol 17, 77 (2016).*

It is important to note that because of sample-pooling early in the CEL-seq protocol, some samples may be inadequately represented in the subsequent sequencing data. This may result in excluding these samples (approximately 10%) from downstream analysis. For this reason, we strongly recommend performing additional biological replicates for each experimental group and randomizing the order of submitted samples. By doing so, and subsequently removing outlier replicates, enough replicates can be retained to ensure a robust dataset for statistically meaningful analyses.

Please read carefully and follow the instructions below for RNA submission:

- Measure RNA concentration with Qubit. The samples should be diluted to a concentration of 10 ng/µl and submitted with at least 20 ul. If Qubit is not available and samples are measured with Nanodrop, please provide diluted samples at 20 ng/ul. Please be advised that Nandrop measurements <20 ng/ul are not reliable.
  For samples outside the recommended concentration and/or volume, please contact us.
- TapeStation should be performed to test RNA integrity (recommended RIN>5).
- Samples should be submitted in PCR strips only (preferably with separately attached caps) as follows:
  - Samples should be randomly distributed in the PCR strip. The samples will be prepared according to the order submitted by the researcher.
  - Write the sample number (not name) on the side and on the top of the tube. 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). Space and special characters aren't not acceptable in the sample name (use _ instead of space).
    Ensure that the number on the tube is identical to the serial number of the sample in the electronic Sample Submission Form.
- Fill out the RNA Submission Form and send it together with TapeStation results to linde@technion.ac.il.

Recommendations-

It is recommended to use column-based kit (such as Qiagen) for RNA extraction.