Instructions for bulk MARS-seq sample submission

Bulk MARS-seq 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 (Jaitin et al., Science 2014; Keren-Shaul et al., Nature Protocols, 2019).

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

1. **Measure RNA concentration** with Qubit or NanoDrop. Fill in the results in the RNA Submission Form. Please note that NanoDrop is reliable only if RNA concentration is >20ng/μl (if lower – use Qubit). In case there is no information on the RNA, please specify cell information in the comments (number of cells taken for RNA purification, and cell type).
2. It is advised to **run a TapeStation** for at least some of the samples, in order to test **RNA integrity** (recommended RIN>8). Note that RIN score can be determined for total RNA, but not for mRNA.
3. **Dilute** each sample **to 10 ng/μl**.
4. We ask for at least **20 μl total RNA at a concentration of 10 ng/μl** (if you have less material, please contact us).
5. If possible, it is advised to place samples in a **96-well PCR plate**, as follows:
    - Arrange samples in the 96-well plate **by columns** (sample 1 in position 1A, sample 2 in 1B, samples 3 in 1C etc.).
    - **Seal** plate tightly with an **aluminum seal** (only! Other seals peel off at -80°c).
    - **Spin** the plate, and quickly **freeze it at -80°c**.
    - Fill in the plate map.
    - Bring samples to the Genomics Unit while plate is placed on **dry ice.**
6. Otherwise, submit samples in **1.7ml low-bind Eppendorf tubes** as follows:
    - **Write the sample number** (not name) on the side and on the top of the tube. In the matching Excel sample submission form, write the number + a meaningful name (this name will be used through data analysis). For example, on the tube write '1', '2', '3'; on the Excel write '1-WT1', '2-WT2', '3-KO1'. Ensure that the number on the tube is identical to the serial number of the sample in the Excel Sample Submission Form. Use **EtOH-resistant marker** to mark the tubes.
    - **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. Use EtOH-resistant marker to mark the box.
7. **Fill out the RNA Submission Form** and send it together with TapeStation results to INCPM.samples@weizmann.ac.il
8. Call the genomics lab (08-9345168) to **coordinate date** and time for sample submission.

General note - assessment of RNA quality:

When submitting purified RNA, sample quality can be assessed by concentration and RIN score. Sample quality is also tested during QC1 of the bulk MARS-seq protocol, but only for common species such as human / mouse / rat etc. (as it is based on qPCR of house-keeping genes, and we have validated primers only for common species). In other cases we can only assess library quality at the end of the protocol. If you have a large project of a non-common species and wishes to discuss generation of qPCR primers contact us.