Bulk RNAseq service information

3’end bulk RNAseq method

SCellex method offers acost-efficient solution for RNAseq profiling making it ideal for projects with a large number of samples. By introducing unique molecular barcodes early in the workflow, many samples can be pooled and processed together, dramatically reducing both time and costs. This approach ensures reliable measurement of gene expression levels, enabling clear comparisons between experimental conditions. While optimized for efficiency, it is important to note that the 3’ end methods are not optimal for full length transcriptome or isoform analyses. Instead, it provides robust data for expression profiling studies. Technically, this is achieved through a 3'-end based barcoding strategy, focusing only on the ends of transcripts to deliver precise and cost-effective results.

SCellex provides bulk RNAseq services **for research purposes only**. The services are not intended for clinical diagnosis or therapeutic use. Please note that the SCellex service includes library preparation and data analysis after sequencing, to the extent agreed. Sequencing is excluded and is the responsibility of the customer.

Sample preparation

- Submit your total RNA samples in a **96-well plate format** (not in individual tubes).
- Prepare your RNA samples in **RNase-free water** to ensure stability and compatibility with our workflow.
- All samples must be **diluted to the same concentration 20 ng/µl** and minimum volume of 10 **µl** before submission, lower concentrations need to be agreed separately with SCellex.
- Ensure your RNA is of **good integrity (RIN/RQN > 8),** and if available, please include QC data from a Bioanalyzer, Tapestation, or similar instrument.
- Verify RNA concentration using **Qubit (or another fluorescence-based method)** for accuracy.
- **DNA contamination must be less than 10%** in all samples.
- Arrange the samples in the plate in **row orientation (A1, A2, A3, …)**, in the same order as listed in the sample sheet.
- Label the plate clearly with the **date, and SCellex order number**.
- Seal the plate securely with an **appropriate cover or sealing film** to prevent leakage or evaporation during transport.
- Ship the samples on **dry ice** and include enough dry ice to cover possible transport delays.
- Inform SCellex of the **shipment details in advance** (courier, tracking number, expected arrival) so we can arrange timely sample reception.

Sample types

SCellex method is species agnostic. However, please note that the method relies on a poly(A)-based capture system, so only RNA molecules with a poly(A) tail can be used. Species that lack polyadenylated mRNA are not compatible with this workflow. Please inform if the samples are human origin (excluding established human cell lines) when placing the order and they will be handled accordingly.

Sample delivery

Before sending your samples, please complete the order form. Do not send any samples until your order has been accepted and you have received a SCellex order number. Make sure the SCellex order number is clearly marked on the plate you are sending to avoid any confusion.

Samples can be delivered either by dropping them off at SCellex facility or by sending them as a dry-ice shipment. Drop-off is available on weekdays from 9:00 to 16:00, please let us know in advance. Upon arrival, please contact the security guard at the building reception or call SCellex at (+358449813762) so we can receive your sample. If you have building access, SCellex is located in the F Wing on the P-floor (room F113) — please ring the doorbell. For shipments, ensure that enough dry ice is included to maintain RNA stability. Please provide SCellex with the shipment details in advance (courier, tracking number, expected arrival) so we can arrange timely reception of your samples.

Data processing

As part of SCellex standard service, SCellex typically provides the following file types as service results (only for non-human and established human cell lines):

- **DGE (Digital Gene Expression matrix) –** A processed table of gene-level counts representing expression levels across your dataset.
- **Sample-barcode list (tab-delimited) –** A simple table linking each sample ID to its barcode/index sequence, used for identifying and demultiplexing samples.

FASTQ – Raw sequencing reads with base calls and quality scores, will be provided by the sequencing facility where the sequencing was performed. SCellex pipeline is used to demultiplex the barcodes, so the original FASTQ files contain all samples combined. **SCellex does not provide demultiplexed FASTQ files.**

For human derived samples SCellex provides further instructions on how the academic customer can upload and analyze the sensitive raw data using e.g. CSC SD Desktop.

Data storage

SCellex will store customer files (DGE and sample-barcode list) for one month (30 days) from the date of sending the customer the notification of data release. **Customers should download and securely store these files during the 30-day period.** Customers will additionally receive FASTQ files from the sequencing facility where the sequencing was performed. Customers should keep in mind that many journals require submission of the original data (FASTQ) along with the gene expression matrix (DGE) when publishing research. To submit these data to a public repository, customers will also need the sample-barcode list. For more information, please refer to the "Data Publishing" section.

Please note that SCellex is not responsible for long-term storage of the raw FASTQ files.

Further data analysis

The DGE (Digital Gene Expression) matrix provides gene-level counts for all customer samples and can be used for downstream RNA-seq analysis, such as differential expression, clustering, and visualization. Commonly used software for these analyses includes DESeq2 and edgeR among others. If you require guidance or assistance with data analysis, please indicate this when completing the order form. SCellex team will be happy to provide this additional service.

Data publishing

Many scientific journals require that RNA-seq studies include publication of the raw sequencing data to ensure reproducibility. Typically, this involves depositing the original FASTQ files, along with associated metadata such as the sample-barcode list and processed data like the DGE matrix, into a public repository (e.g., GEO or SRA). Once submitted, the repository provides an accession number that can be cited in your manuscript, allowing other researchers to access and reanalyze the data.

Please note that it is the researcher's responsibility to submit the data to the chosen public repository. The sequencing core and SCellex team provides the necessary files (FASTQ, DGE matrix, and sample-barcode list), but ensuring the data is correctly uploaded, complete, and accompanied by the required metadata is up to the user. Be sure to obtain and include the repository accession number when citing the data in your manuscript.