Bulk RNAseq service information

3’end bulk RNAseq method

The SCellex method offers a cost-effective solution for RNA-seq 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 3' end methods are not suitable for full-length transcriptome or isoform analyses. Instead, the method provides robust data for gene expression profiling studies. Technically, this is achieved through a 3' end-based barcoding strategy that focuses only on the ends of transcripts, delivering precise and cost-effective results.

SCellex provides bulk RNA-seq 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 have a **minimum volume of 10 µl** before submission. Lower concentrations must be agreed upon separately with SCellex.
- Ensure that your RNA is of **good integrity (RIN/RQN > 8)** and, if available, include QC data from a Bioanalyzer, TapeStation, or a similar instrument.
- Verify RNA concentration using **Qubit (or another fluorescence-based method)** for accurate quantification.
- **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**, including enough dry ice to cover possible transport delays.
- Inform SCellex of the **shipment details in advance** (courier, tracking number, expected arrival) so that we can arrange timely sample reception.

Sample types

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

RNA Quality Check

SCellex can perform RNA quality control (QC) analysis of isolated RNA samples prior to library preparation. This service provides an assessment of RNA integrity and allows verification of sample quality before sequencing.

For RNA QC, please provide a **separate 96-well plate** containing the RNA samples intended for quality assessment. The plate must be clearly labeled with the **project number** and the designation **"QC"**.

RNA samples submitted for QC should meet the following requirements:

- RNA concentration: **5 ng/µl**
- Minimum sample volume: **10 µl**
- Samples must be arranged in the same order as listed in the sample sheet.

The same requirements regarding **shipment on dry ice**, **secure plate sealing**, and **sample handling and labeling** apply to QC samples as to samples submitted for library preparation.

Following the analysis, SCellex will provide the customer with an **RNA QC report**, including the **RIN (RNA Integrity Number) values** for all analyzed samples.

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 the SCellex facility or by sending them as a dry-ice shipment. Drop-off is available on weekdays from 9:00 to 16:00; please notify us in advance. Upon arrival, please contact the security guard at the building reception or call SCellex at

(+358449813762) so we can receive your samples. 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 that we can arrange timely receipt of your samples.

Data processing

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

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

FASTQ files containing raw sequencing reads with base calls and quality scores are provided by the sequencing facility where the sequencing is performed. The SCellex pipeline is used to demultiplex sample barcodes; therefore, 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 academic customers can upload and analyze sensitive raw data using platforms such as CSC SD Desktop.

Data storage

SCellex will store customer files (DGE and sample-barcode list) for one month (30 days) from the date the customer is notified that the data are available. **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, edgeR, and others.

If you require guidance or assistance with data analysis, please indicate this when completing the order form. The 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 such as 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 facility and the SCellex team provide the necessary files (FASTQ, DGE matrix, and sample-barcode list), but ensuring that the data are uploaded correctly, complete, and accompanied by the required metadata is the responsibility of the user. Be sure to obtain and include the repository accession number when citing the data in your manuscript.