

Bulk RNAseq service information for FFPE samples

FFPE-seq - 3'end bulk RNAseq method

SCellex FFPE-seq provides a cost-efficient and robust solution for transcriptome profiling of formalin-fixed paraffin-embedded (FFPE) samples. The workflow is specifically optimized for fragmented RNA commonly recovered from FFPE tissues and performs reliably with samples exhibiting DV200 (Distribution Value of RNA fragments > 200 nucleotides) values above 35%.

By introducing unique molecular barcodes early in the workflow, multiple samples can be pooled and processed simultaneously, significantly reducing both hands-on time and overall project costs. This approach enables accurate and reproducible gene expression quantification, making it well suited for large-scale expression profiling studies and retrospective analyses of archived tissue collections. The method utilizes a 3'-end RNA sequencing strategy, capturing transcript ends for efficient and reliable gene-level expression measurement, even from partially degraded RNA. While this approach does not support full-length transcript or isoform analysis, it provides high-quality data for differential gene expression studies. SCellex FFPE-seq is optimized for FFPE samples with DV200 >35%, enabling robust transcriptome analysis from moderately preserved RNA and maximizing the utility of valuable, archived samples.

SCellex provides bulk RNA-seq services for research use only (RUO). Unless otherwise agreed, the service includes RNA extraction, RNA quality assessment, library preparation, and data analysis. Data analysis after sequencing is included to the extent agreed. Sequencing itself is excluded and remains the responsibility of the customer.

Sample preparation

1. Samples should be delivered in **RNase-free 1.5 mL microcentrifuge tubes**.
2. Provide FFPE tissue sections as **1–5 scrolls per sample**, with a section thickness of **5 µm** and a total tissue area of approximately **200–600 mm²** per scroll. Other sample types need to be agreed separately with SCellex.
3. Samples should be labeled properly with individual sample names and placed in a plastic bag indicating the date, SCellex order number, and customer name.
4. A filled sample sheet must be delivered within the samples.
5. Inform SCellex of the **shipment details in advance** (courier, tracking number, expected arrival) so we can arrange sample reception.

If the RNA extracted from a submitted FFPE sample does not meet SCellex's internal quality criteria for library preparation, SCellex will contact the customer to discuss available options before proceeding.

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 of human origin, therefore belonging to the sensitive data category, (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 samples you are sending to avoid any confusion.

Samples can be delivered either by shipment or by drop-off at the SCellex facility. For shipped samples, please provide SCellex with the shipment details in advance (courier, tracking number, and expected arrival date) so that we can arrange receipt of your samples. If you wish to drop off samples at our SCellex facility in Meilahti, Helsinki, please contact us in advance, and we will provide you detailed instructions.

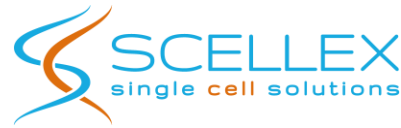
Data processing

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.



Please contact us before submitting an order request if samples are from any other organism than the following: human, mouse, rat.

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 provide 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.