

## Single Cell RNA Seq 3' Gene Expression Sample Submission Form

### Sample information and client requirement

PI: \_\_\_\_\_ PI E-mail: \_\_\_\_\_

Telephone: \_\_\_\_\_ Samples submitted by: \_\_\_\_\_

Submitter E-mail: \_\_\_\_\_ Submitter Signature: \_\_\_\_\_

Institution/Company Name: \_\_\_\_\_

1) Genome:  Human (GRCh38)  Mouse (mm10)

Custom (transgene, viral genome, alternative genome build, etc.)

If custom, please indicate what is needed: \_\_\_\_\_

\*For reference genes that are not human or mouse, please send us the reference genome for evaluation

2) Cell type: \_\_\_\_\_  Cell Culture  Tissue Prep

3) Single cell freezing buffer: \_\_\_\_\_

4) Centrifuge speed (rpm) and time: \_\_\_\_\_

5) FACS performed:  Yes  No

6) Number of target cells recovery: \_\_\_\_\_

\* Maximum target cell recovery is 10,000cells

7) Number of samples: \_\_\_\_\_ Name of samples: \_\_\_\_\_

\*If number of samples > 8, please send us samples names in an excel sheet

Sample name must begin with alphabet and in maximum of 12 characters. Naming in alphanumeric and underscore are acceptable. Non-standard names will be amended following this naming requirements, comma can be used to separate sample names.

8) How long have cells been frozen for and freezing conditions: \_\_\_\_\_

e.g. 6 months in liquid nitrogen

9) Number of vials per sample: \_\_\_\_\_

10) Cell Size Range (um): \_\_\_\_\_

11) Viability on ice after thawing at 0, 10, 30 and 60 min: \_\_\_\_\_

12) How long are samples stable in -80°C: \_\_\_\_\_

13) Is this a joint submission with another group?  Yes  No

If Yes, please list other group's PI: \_\_\_\_\_

14) Please provide us with the following details before submitting samples

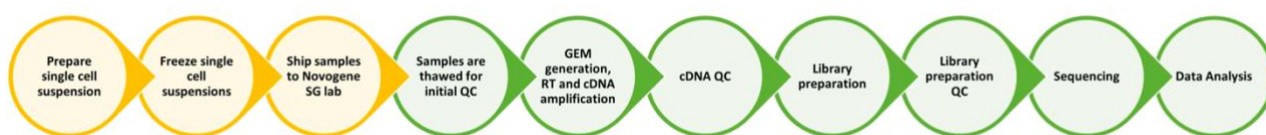
Cell count and viability: \_\_\_\_\_

Single cell suspension light microscope image with scale bar

## Sample Requirements

- Cell viability should be >85% before freezing
- Freeze more than 1,000,000 cells per vial and submit at least two (2) 1.5 mL cryovials if possible. If not, please contact your Technical Specialist for further support
- Sample must be clear from debris, clean background before freezing. Please provide images of your single cell suspensions before freezing with scale bar
- Cell size to be <30  $\mu\text{m}$

## Workflow



Yellow circles to be completed by customer

## Single Cell Sequencing Recommendations

- Different tissue or sample type may require different dissociation protocol or handling. Please consult the 10x Genomics sample prep recommendations (<https://support.10xgenomics.com/single-cell-gene-expression/sample-prep>) to ensure that the protocol/ cell types are compatible with single cell sequencing. For new or unusual tissue/cell types, check relevant publications for compatibility with single cell sequencing. You may consider a test run with reduced targeted cell number.
- Please refer to the demonstrated 10x protocols for freezing single cell suspensions. All cells will be washed with and resuspended in PBS + 0.04% BSA. If the customer intends to wash and resuspend cells in a different buffer, please contact our technical specialist.
- Single cell GEM generation makes use of microfluidics system with narrow channels. It is essential that cells are in single cell suspension with no debris or aggregates. It is recommended to pass the cell suspension through a 40 $\mu\text{m}$  strainer at least twice prior to freezing for single cell sequencing to avoid chip clogging.
- A minimum viability of 70% is recommended for single cell sequencing. A lower viability may result in reduced cell recovery or difficulty in interpreting the result.
- After freezing, store 1.5 mL cryovials in liquid N<sub>2</sub> for at least one (1) week before shipping to Novogene SG lab. Please arrange shipment for samples on dry ice and provide us with the tracking number for tracking.

# Service User Agreement

Please read and agree to the following disclaimer before using our Chromium Single Cell 3' Gene Expression service.

1. If light microscope images of the single cell suspension prior to freezing do not meet the requirements (insufficient cell concentration or total cell count, low viability (<70%), clumping cells, poor cell morphology or debris), NovogeneAIT reserves the right to reject the samples. In this case, the customer will need to re-prepare the sample.
2. Before proceeding with the service, we will confirm the quality (cell count and viability) of the samples (QC). Please note that there may be variations to the final count and viability compared to manual counting. For sensitive cell types, we aim to minimise time between thawing and GEM generation to reduce viability loss. If specific handling is required for your samples (viability decreases dramatically on ice after thawing), we will bypass initial cell QC to maximise cell viability if viability before freezing is more than 85%.
3. Sample viability is vital for processing. If sample viability is measured to be less than 70% at the initial QC step, the samples will not be processed. You will be contacted by our Technical specialist/Product manager to ask if you are willing to proceed with the scRNA seq experiment. If only one (1) vial is submitted, NovogeneAIT reserves the right to stop the experiment and charges will apply (USD 80 per sample). Please note that the TAT will increase by one (1) week if the customer chooses to proceed with the second (back- up) vial.
4. If preliminary results obtained through our services do not meet NovogeneAIT's standard requirements, final data output, data quality and data analysis results cannot be guaranteed.
5. If the results obtained through our services do not meet the customer's expectations or the customer stops the experiment prior to completion, please note that there will be a charge for parts of the experiment that has been performed.

The following charges will apply should the service be stopped at the following steps:

- a. GEM Generation: 70% of 10x Single Cell 3'GeneExpression library service
- b. cDNA Amplification and Library Preparation: 100% of 10x Single Cell 3'GeneExpression library service

6. Please note that samples will NOT be returned after the service is complete. Samples and data will be deleted from our systems three (3) months after delivery, with an email being sent out ten (10) days prior to deletion.
7. All analytical services are used for research purposes. If the result of this service is used for purposes other than research, NovogeneAIT is not responsible for any loss or damage caused thereby.

Date

Signature

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