10x Submission Instructions for HTSF 10x Library Preparation

Sample Submission Requirements

Important: By default any new project is set up to use the Next GEM – the newest version of the 10x chips and appropriate reagents and protocols. If you need your samples to be backwards compatible with a previously processed ones please inform the HTSF when setting up the experiment so we can get you the right version of reagents.

1. Single Cell Gene Expression (scRNAseq)

Please contact the HTSF to set up the submission and hand off procedure. Fresh cells must be run as soon as possible after preparation. HTSF will need at least 2 hours for this initial processing so the time of hand-off must be at least two hours before the lab closes.

a. Here is the 10x Genomics recommended general cell preparation protocol – CG000053_CellPrepGuide_RevC.pdf

   This is generic protocol – there are more protocols on 10x webpage - https://support.10xgenomics.com/single-cell-gene-expression/sample-prep.

   - The best protocol for your cells may differ depending on your cell type and source. You can use the one you prefer but please check it gives you the good quality of cells and that they are at least 90% live. The 10x library preparation is expensive and if it fails due to cells dying HTSF will need to charge you for the reagents used.
   - It's best for the cells to be at least 90% alive for the 10x process. If you are unable to achieve that cell sorting might help. HTSF doesn't have cell sorting ability but AACore *(link here*) can do it and they offer 10x Genomics scRNAseq processing too.
   - If you would like to do different cell preparation e.g. nuclei, methanol preservation – please contact HTSF to discuss options.

b. You will need to decide the target number of cells you want to analyze per sample. You will need to supply ~1.6x that number of cells to HTS for processing (eg. For 10 000 cell target you'll need to deliver approximately 16 000 cells).

   Please follow the instructions in this table - 10x scRNAseq Cell input calculator - for volume and concentration of cells for desired target.

   The way this table is to be used:

   - Find your desired target cell number in the top row – e.g. 5000 cells

- Go down the column to find the table cell in the blue background range and look the number in red (number in blue is for HTSF) – e.g 10.3 µl
- Go to the first column showing the final concentration of cells you will need and resuspend your cells to have that concentration – e.g. 800 cells/ µl
  Also multiplying that number by volume above will give you the number of cells required as input – e.g 800 x 10.3 = 8240 cells for 5000 cell target
- Deliver to HTSF 2 aliquots of your cells at calculated concentration at the volume in red – e.g. 2 x 10.3 ul of 800 cells/ul.
  Unless your sample is limited it is preferred to have 2 aliquots in case there is a clog in 10x chip resulting in samples loss (happens in rare cases). This will allow HTSF to repeat the run with the second aliquot (no charge).
- If possible (or if you want HTSF to double check quality of your cells) also deliver and aliquot for QC purpose only. If your sample is limited, you can deliver 10x diluted version of your test aliquots.

If you have any questions about processing please contact HTSF before proceeding.

2. Single Cell ATAC (scATACseq)

Please contact the HTSF to set up the submission and hand off procedure. Prepared nuclei must be run as soon as possible after preparation. HTSF will need at least 2 hours for this initial processing so the time of hand-off must be at least two hours before the lab closes.

Before starting the protocol contact HTSF for the 10x nuclei buffer.

Here is the 10x Genomics recommended nuclei preparation protocol for:

a. Single cells - CG000169_DemonstratedProtocol_NucleiIsolation_ATAC_Sequencing_RevD

   Note: This protocol outlines how to isolate, wash, and count nuclei suspensions for use with the Chromium Single Cell ATAC protocol. Cryopreserved primary cells (PBMCs) and cell lines (GM12878 cells; EL4 cells) were used to develop this protocol. PBMCs were cryopreserved in IMDM + 40% FBS + 15% DMSO. Cell lines were cryopreserved in RPMI + 15% FBS + 5% DMSO. Optimization of some protocol steps (e.g. lysis time, centrifugation speed/time and filtration steps) may be needed based on cell type.

b. Tissue - CG000169_DemonstratedProtocol_NucleiIsolation_ATAC_Sequencing_RevD

   Note: This protocol outlines how to isolate, wash, and count single nuclei from fresh, cryopreserved, and flash frozen mouse brain tissue samples for use with the Chromium Single Cell ATAC Solution. Tissue triturated into a nearly single cell suspension and subsequently frozen in media containing 10% DMSO produced metrics comparable to fresh tissue. High quality data can also be obtained using flash frozen tissue. Optimization of some protocol steps (e.g. homogenization, lysis reagent/time, centrifugation speed/time and filtration steps) may be needed when working with mouse brain tissues from different sources.

Follow the instructions in the protocol for calculating the Nuclei Stock Concentration (nuclei/µl) range required for your required Targeted Nuclei Recovery.

Before submitting first sample please run test to see what your optimal lysis protocol is. The recommendation is to start with >95% live cells (any dead cells could spill out the DNA and lead to high background). After lysis cells should be mostly dead (<5% live) but not completely (0% live) as that may indicate overlysis. Overlysis of the nuclei can lead to them breaking up and releasing the DNA.

If you have any questions about processing please contact HTSF before proceeding.

10x Submissions

1- 48hrs ahead of the time for expected sample delivery to the HTSF, please confirm with HTSF that staff and consumables are available
- M-F notification
- HTSF will confirm the day and time is ok

2- On the day before sample drop off, use TracSeq to submit your samples.
- Note on the manifest fields # of cells or nuclei in the CONCENTRATIONN FIELD
- Add to batch special needs notes: Study is targeting # cells/ nuclei for final assay
- HTSF CS staff will accept submission and send quote email.
- Study should print out confirmed manifest form QUOTE email

3- On day of sample drop off, email customer service group to confirm the submission will move forward or if it has been cancelled
- if it is cancelled, HTSF will update the submission to not expect cells/nuclei will arrive
- Customer Service will notify staff that submission was cancelled.
- If moving forward, confirm a participant ID you need noted on the submission.

4- Study prepares the cells/ nuclei

5- Study calls lab to confirm they are on the way with the cells/ nuclei.
- 2 aliquots per cell/ nuclei type (on wet ice)
- bring the confirmed manifest with samples
- CS staff does drop off and check in while study is on their way

6- Study arrives at GSB 1153 for sample drop off.
- Customer service rep labels tubes and takes them to library group for Preparation.
- 10x staff will prepare libraries
- HTSF does not recheck cell number or viability
- QAQC for libraries will be sent upon completion

Cell Suspension Volume Calculator Table

(for step 1.2 of Chromium Next GEM Single Cell 3' v3.1 protocol)

Volume of Cell Suspension Stock per reaction (µl) | Volume of Nuclease-free Water per reaction (µl)

Cell Stock Concentration (Cells/µl)	Targeted Cell Recovery										
	500	1000	2000	3000	4000	5000	6000	7000	8000	9000	10000
100	8.3 35.0	16.5 26.7	33.0 10.2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
200	4.1 39.1	8.3 35.0	16.5 26.7	24.8 18.5	33.0 10.2	41.3 2.0	n/a	n/a	n/a	n/a	n/a
300	2.8 40.5	5.5 37.7	11.0 32.2	16.5 26.7	22.0 21.2	27.5 15.7	33.0 10.2	38.5 4.7	n/a	n/a	n/a
400	2.1 41.1	4.1 39.1	8.3 35.0	12.4 30.8	16.5 26.7	20.6 22.6	24.8 18.5	28.9 14.3	33.0 10.2	37.1 6.1	41.3 2.0
500	1.7 41.6	3.3 39.9	6.6 36.6	9.9 33.3	13.2 30.0	16.5 26.7	19.8 23.4	23.1 20.1	26.4 16.8	29.7 13.5	33.0 10.2
600	1.4 41.8	2.8 40.5	5.5 37.7	8.3 35.0	11.0 32.2	13.8 29.5	16.5 26.7	19.3 24.0	22.0 21.2	24.8 18.5	27.5 15.7
700	1.2 42.0	2.4 40.8	4.7 38.5	7.1 36.1	9.4 33.8	11.8 31.4	14.1 29.1	16.5 26.7	18.9 24.3	21.2 22.0	23.6 19.6
800	1.0 42.2	2.1 41.1	4.1 39.1	6.2 37.0	8.3 35.0	10.3 32.9	12.4 30.8	14.4 28.8	16.5 26.7	18.6 24.6	20.6 22.6
900	0.9 42.3	1.8 41.4	3.7 39.5	5.5 37.7	7.3 35.9	9.2 34.0	11.0 32.2	12.8 30.4	14.7 28.5	16.5 26.7	18.3 24.9
1000	0.8 42.4	1.7 41.6	3.3 39.9	5.0 38.3	6.6 36.6	8.3 35.0	9.9 33.3	11.6 31.7	13.2 30.0	14.9 28.4	16.5 26.7
1100	0.8 42.5	1.5 41.7	3.0 40.2	4.5 38.7	6.0 37.2	7.5 35.7	9.0 34.2	10.5 32.7	12.0 31.2	13.5 29.7	15.0 28.2
1200	0.7 42.5	1.4 41.8	2.8 40.5	4.1 39.1	5.5 37.7	6.9 36.3	8.3 35.0	9.6 33.6	11.0 32.2	12.4 30.8	13.8 29.5
1300	0.6 42.6	1.3 41.9	2.5 40.7	3.8 39.4	5.1 38.1	6.3 36.9	7.6 35.6	8.9 34.3	10.2 33.0	11.4 31.8	12.7 30.5
1400	0.6 42.6	1.2 42.0	2.4 40.8	3.5 39.7	4.7 38.5	5.9 37.3	7.1 36.1	8.3 35.0	9.4 33.8	10.6 32.6	11.8 31.4
1500	0.6 42.7	1.1 42.1	2.2 41.0	3.3 39.9	4.4 38.8	5.5 37.7	6.6 36.6	7.7 35.5	8.8 34.4	9.9 33.3	11.0 32.2
1600	0.5 42.7	1.0 42.2	2.1 41.1	3.1 40.1	4.1 39.1	5.2 38.0	6.2 37.0	7.2 36.0	8.3 35.0	9.3 33.9	10.3 32.9
1700	0.5 42.7	1.0 42.2	1.9 41.3	2.9 40.3	3.9 39.3	4.9 38.3	5.8 37.4	6.8 36.4	7.8 35.4	8.7 34.5	9.7 33.5
1800	0.5 42.7	0.9 42.3	1.8 41.4	2.8 40.5	3.7 39.5	4.6 38.6	5.5 37.7	6.4 36.8	7.3 35.9	8.3 35.0	9.2 34.0
1900	0.4 42.8	0.9 42.3	1.7 41.5	2.6 40.6	3.5 39.7	4.3 38.9	5.2 38.0	6.1 37.1	6.9 36.3	7.8 35.4	8.7 34.5
2000	0.4 42.8	0.8 42.4	1.7 41.6	2.5 40.7	3.3 39.9	4.1 39.1	5.0 38.3	5.8 37.4	6.6 36.6	7.4 35.8	8.3 35.0

Grey boxes: Volumes that would exceed the allowable water volume in each reaction
Yellow boxes: Indicate a low transfer volume that may result in higher cell load variability
Blue boxes: Optimal range of cell stock concentration to maximize the likelihood of achieving the desired cell recovery target