Single cell profiling assays using the 10x Genomics platform require prior consultation and coordination with the core.

General:
- Please use the link below to find pricing information and general instruction instructions on how to submit an online service request with our core [http://epicore.med.cornell.edu/services.php](http://epicore.med.cornell.edu/services.php).
- **If you are a new single-cell-user**, prior to submitting a request and booking a time for your experiment you need to schedule an appointment to discuss your experiment with the core director. Please email us at epigenomicscore@med.cornell.edu.
- **If you are an experienced user**, you can submit a Single Cell Profiling request as indicated below:
  - We suggest you bring up to 4 samples at a time on your 1st experiment.
  - Turn-around time is 6-8 weeks from sample submission to uploading the sequencing data to our server.
  - **This is a multi-step service, please do not send emails to individual core personnel or use the Epigenomics core email address.** Any changes or questions regarding your service, should be informed to by using the single cell coordination email epicore-sc-coord@med.cornell.edu and confirmed by adding a comment in your iLab request.

**To submit a Single Cell Profiling request:**
- Login to your Agilent CrossLab account and select the Epigenomics core facility [https://wcmc.corefacilities.org/service_center/show_external/2922](https://wcmc.corefacilities.org/service_center/show_external/2922).
- Initiate a request for Single Cell Sequencing; select Single Cell Profiling, fill in the questionnaire and submit the request.
- Make a note of the Agilent CrossLab ID.
- **Select the "Scheduling" tab on the top left. A new window will open**
- Click the "View Schedule" option on the right-hand side (Make sure you select the schedule labeled "10x Chromium Single Cell Scheduling")

*We are accepting samples Mon through Thursday from 11am to 3:30PM, make a reservation at least ONE week in advance, and the core will edit it if necessary.*
- Click and drag in order to select the day and time frame that you would like to drop off your samples, which will initiate a pop-up window.
- On the pop-up window, under General, find the Event Notes tab.
- In the Event Notes tab, please enter your Agilent CrossLab service ID, single cell profiling category (Gene Expression, Immune Profiling), and total sample numbers.
- Disregard the rest of the tabs and scroll down to Save Reservation.

**Single Cell Drop off:**
- At the scheduled day and time bring the samples to the Epicore: 1300 York Ave A-430
Single Cell Gene Expression and Immune Profiling Submissions

- Give us a call at 212-746-7719 to let us know you are bringing the samples, if you are late calls us as well
- Bring cells **on ice, >80% viability; ~50,000 cells at ~800 cell/µl (ie ~60µl)**
  - Optimal Cell Concentration is 700-1,200 cells/µl
  - If the concentration is lower than above, resuspend in less volume
- Transfer your samples to the ice bucket provided in A-430, on the bench close to the sink.
- Please keep your social distance while we QC your sample. Only ONE person can come to the drop-off the sample
- We will quantitate the cells using the Invitrogen Cell Countess (10µl) before proceeding, and reserve the right to reject cells if they are below standards
- If your cells are heterogenous or otherwise difficult to count, please QC in your lab and bring them ready for processing.

**Single Cell preparation:**
Given the variety of cells and sample types, sample preparation needs to be optimized by you for your experiment. 10x Genomics has a number of demonstrated protocols available in their website that you can use as a guideline

[https://support.10xgenomics.com/single-cell-gene-expression/sample-prep](https://support.10xgenomics.com/single-cell-gene-expression/sample-prep)

- Regardless of the protocol you use, you need to provide **single cell suspensions**, free of debris and aggregates.
- Aggregated cells, as well as large debris will clog the instrument. Clogging results in **complete or partial cell loss** -depending on the number of aggregates- this means poor quality data or no data.
- Cells need to be at >-80% viability, lesser viabilities can be processed but this will affect performance. We use the total number of cells to calculate cell target recovery, thus with lower cell viability you will be able to target less cells.
- Either **FACS sort** or use a **cell strainer** to remove clumps or debris.
- Treat the cells **gently** to minimize cell lysis. If the cells lyse, the mRNA will contaminate other GEMSs, which will result in higher noise during data analysis.
- You can wash the cells with a P1000 Wide Orifice pipette tip, **do not cut the tip off** a regular tip with a blade, plastic particles will clog the instrument.
- You can submit the cells resuspend in PBS + non-acetylated BSA (0.04-2%). BSA is added to prevent cell losses and/or aggregation.
- If the **cells will lyse in PBS**, the following alternative buffers and media have been tested by 10x Genomics
  - Alternative Buffer: no influence on performance
    - Dulbecco’s Phosphate-Buffered Saline (DPBS)
    - Hank’s Balanced Salt Solution (HBSS)
  - Alternative Media: minimal reduction to no loss in performance
    - Eagle’s Minimum Essential Medium (EMEM) + 10% FBS
    - Dulbecco’s Modified Eagle Medium (DMEM) + 10% FBS
    - Iscove’s Modified Eagle Medium (IMEM) + 10% FBS
    - Roswell Park Memorial Institute (RPMI) + 10% FBS
    - Ham’s F12 + 10% FBS
    - 1:1 DMEM/F12 + 10% FBS
    - M199
Single Cell Gene Expression and Immune Profiling Submissions

- After QC the cells, we use the 10x Genomics cell suspension volume calculator table to bring the cells to the appropriate number to achieve the desired targeted cell recovery (see table on page 5).

Single Cell Profiling information:

- 10x Genomics has very comprehensive support pages, please make time to pore over them [https://support.10xgenomics.com/](https://support.10xgenomics.com/).
- We accept samples for Gene Expression and for Immune Profiling.
- If you want to add Feature Barcoding Technology, which allows measurements of cell surface proteins.

You are responsible for purchasing the barcoded antibodies and or/dextramers and tagging the cells prior to bringing the samples to the core.

- Information related to Gene Expression with Feature Barcoding technology can be found here ([Feature Barcode](https://www.biolegend.com/totalseq)). For a detailed pdf, and information on where to obtain the antibodies, download the Product Sheet from this link ([Cellular Diversity](https://www.biolegend.com/totalseq)).

- Information related to Immune Profiling with Feature Barcoding can be found here ([Immune Profiling FB](https://www.biolegend.com/totalseq)). For a detailed pdf, and information on where to obtain the antibodies and/or dextramers, download the Product Sheet from this link ([Multiomic Immune Profiling](https://www.biolegend.com/totalseq)).
  - Compatible Partner Product: Immudex dCODE Dextramers.

- CITE-seq ([Cellular Indexing of Transcriptomics and Epitopes by Sequencing](https://www.biolegend.com/totalseq)) is the method developed by the NYGC that employs the barcoded antibodies technology. These antibodies are the same antibodies mentioned in the above links.

For the iLab sample submission you need to know the following:

- **Targeted Cell Recovery**: number of cells you wish to analyze. This number needs to be determined empirically depending on your scientific question and should be discussed with your PI and bioinformatician. The instrument can encapsulate from 500-10,000 cells (up to 30µM diameter) in a pre-determined volume as per the cell suspension volume calculator tables provided in this guide.
  - Note: Recovering the desired number of cells depends on the viability and accurate quantification of your cells.
Single Cell Gene Expression and Immune Profiling Submissions

- **Sequencing Depth:** 10x Genomics recommendations for v3.1 or Immune Profiling libraries is a pair end 28-10-10-90 sequencing flow cell.
  - For **Gene Expression:** To obtain about 50% sequencing saturation, a minimum of 25,000 read pairs per recovered cell are required. Thus, the number of sequenced reads your experiment will require will depend on the number of samples processed and the estimated number of cells to be recovered (**read depth & cell number**).
  - For **V(D)J** libraries: A minimum of 5,000 read pairs per recovered cell (**sequencing requirements**).
  - For **Feature barcoding** libraries: A minimum of 5,000 read pairs per recovered cell (**v3.1, Immune Profiling**).

- **Data Processing:** The sequencing data will be demultiplexed and post-processed using the 10x Genomics Cell Ranger pipeline [https://support.10xgenomics.com/single-cell-gene-expression/software/pipelines/latest/what-is-cell-ranger](https://support.10xgenomics.com/single-cell-gene-expression/software/pipelines/latest/what-is-cell-ranger)

**Targeted Gene Expression:**
10x Genomics has four enrichment panels of about 1,000 genes (**panel selection**) designed to enrich libraries for relevant genes (**CG000349**).
If you wish to use any of these panels when planning please note:
- One target enrichment reaction can be used for one single cell gene expression library or to pool up to eight single cell gene expression libraries (**CG000345**).
- Recommended sequencing depth is 2,000 reads per single cell.
<table>
<thead>
<tr>
<th>Cell Stock Concentration (Cells/μl)</th>
<th>Targeted Cell Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>500</td>
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<tr>
<td>100</td>
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</tr>
<tr>
<td>2000</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Columns explained as follows:
- **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 (500-10,000 cells)
- **Purple boxes**: Refer to Cell Multiplexing (for 3’v3.1 only), see Cell Multiplexing guide if interested