Guidelines for the use of the Chromium 10X Controller

The Chromium 10X Controller is intended to be a multi-user piece of equipment, open to all MBL resident scientists, Whitman Scientists, researchers from the University of Chicago, and the various educational programs run at the MBL. The instrument was purchased using funds from the Echeverri and Rosenthal Labs, N. Patel, and an equipment grant from the Neuroscience Institute at the University of Chicago. The MBL is providing access to the 10X Chromium controller for scientists to carry out their own experiments; it is not a single cell service facility. It is important to note that sample preparation prior to the use of the controller can be complex, and protocols often must be custom tailored to different organisms/tissues. In addition, the output from the controller must be converted into a library and then sequenced on an Illumina platform. The MBL does not provide training or troubleshooting on these workflows. Nor does it provide reagents or disposables for the instrument. It is expected that all users are fully trained before using the instrument. To ensure upkeep and maintenance of the instrument we are charging a Project based user fee of $750 for six months (Price is subject to change).

Guidelines for its use are as follows:

1. Users must sign up for time on the machine through the PPMS system at the MBL. If you do not have a PPMS account, set up one here:

https://ppms.us/mbl/areq/?pf=2

2. When signing up for time on the PPMS system, you will be asked to provide a short description of your project and include the names of all personnel who will be using the machine. Before starting, you are encouraged to discuss your project with one of our current 10X Genomics resident scientists listed below per year of responsibility.

2. Users are only allowed to use validated reagents and chips purchased from 10X genomics in the unit.

3. Users must provide all their own reagents and disposables when using the machine. This includes kits and chips from 10X Genomics, as well as pipette tips, microfuge tubes etc...

4. The bench adjacent to the unit is intended for sample preparations. It must be thoroughly cleaned and left organized after use. No other space in the lab is open for general use.

5. Key card access to Rowe 322 will only be granted after the User fee is paid. Please contact Josh Rosenthal for access to Rowe 322 (contact: jrosenthal@mbl.edu)

6. Space in Rowe 322 is only intended for GEM preparation using the Chromium 10X Controller (i.e. step 1 in the time line below). All subsequent steps in library preparation (PCR amplifications etc..) must be performed in the researcher's own laboratory.

7.Prior to the first use the 10X instrument, all users must first watch this training:

https://www.10xgenomics.com/videos/ivskc1in27?autoplay=true

8. Failure to comply with the user guidelines will result in removal of access to use the instrument.

MBL Resident Scientists User Group

Contact Person by Year:

2023: Karen Echeverri and Josh Rosenthal
(kecheverri@mbl.edu, rosenthal.joshua@gmail.com)

2024: Andrew Gillis and Zak Swartz
(agillis@mbl.edu, zswartz@mbl.edu)

2025: Carrie Albertin and Kate Rawlinson
(calbertin@mbl.edu, krawlinson@mbl.edu)

Important Information for Single Cell Experiments.

The MBL is providing access to the 10X Chromium controller for scientists to carry out their own experiments; it is not a single cell service facility.

If you are new to single cell experiments here are some quick guidelines and links that may be helpful as you plan your experiments. The average cost of reagents to prep 4 samples is $10,000.

1. The first key step is having established a good protocol for isolating single cells from your organism/tissue of interest. Helpful guidelines for this can be found here:

   https://www.10xgenomics.com/support/single-cell-gene-expression/documentation/steps/sample-prep/single-cell-protocols-cell-preparation-guide

2. The single cell protocol requires many steps with multiple kits, user guidelines can be found here:
   https://www.10xgenomics.com/support/user-guides/single-cell-gene-expression?menu[productNames]=Single%20Cell%20Gene%20Expression

3. 10X Genomics provides detailed protocol and videos for each step of the process online:
   https://www.10xgenomics.com/support

Overview of the timeline from preparing single cell to having libraries ready to sequence:

Day	Steps		Timing	Stop & Store
2 h	**Cell Preparation**			
	Dependent on Cell Type		~1-1.5 h	
	Step 1 – GEM Generation & Barcoding			
4 h	1.1	Prepare Reaction Mix	20 min	
	1.2	Load Chromium Next GEM Chip G	10 min	
	1.3	Run the Chromium Controller	18 min	
	1.4	Transfer GEMs	3 min	
	1.5	GEM-RT Incubation	55 min	STOP 4°C ≤72 h or –20°C ≤1 week
	Step 2 – Post GEM-RT Cleanup & cDNA Amplification			
6 h	2.1	Post GEM RT-Cleanup – Dynabead	45 min	
	2.2	cDNA Amplification	40 min	STOP 4°C ≤72 h or –20°C ≤1 week
	2.3	cDNA Cleanup – SPRIselect	20 min	STOP 4°C ≤72 h –20°C ≤4 weeks
	2.4	cDNA QC & Quantification	50 min	
	Step 3 – 3' Gene Expression Library Construction			
8 h	3.1	Fragmentation, End Repair & A-tailing	50 min	
	3.2	Post Fragmentation, End Repair & A-tailing Double Sided Size Selection – SPRIselect	30 min	
	3.3	Adaptor Ligation	25 min	
	3.4	Post Ligation Cleanup- SPRIselect	20 min	
	3.5	Sample Index PCR	40 min	STOP 4°C ≤72 h
	3.6	Post Sample Index PCR Double Sided Size Selection- SPRIselect	30 min	STOP 4°C ≤72 h or -20°C long term
	3.7	Post Library Construction QC	50 min	