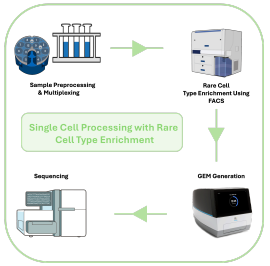
Dec 02, 2025

Sample-Multiplexed, FACS-Preprocessing of PBMCs with Rare Cell Type enrichment Enables Scalable scRNA-seq workflow



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We use this protocol and it's working

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Disclaimer

Abstract

This protocol describes an optimized fluorescence-activated cell sorting (FACS)–based workflow for preprocessing peripheral blood mononuclear cells (PBMCs) prior to single-cell RNA sequencing (scRNA-Seq). It enables selective enrichment of rare immune cell types, such as plasmacytoid dendritic cells (pDCs), while maintaining the overall balance of major PBMC populations. The workflow integrates dead cell removal, targeted enrichment, and antibody-based hashing (sample multiplexing), allowing multiple samples to be processed in parallel with minimal handling time and reduced batch effects. This protocol is designed to minimize cellular stress and technical variability, the protocol supports both fresh and cryopreserved PBMCs from clinical or laboratory sources, improving throughput and reproducibility in studies of immune heterogeneity.

Guidelines

All steps where possible are performed on ice (4°C).
For the first time, it is recommended to do a full test run with non-precious samples.
As the volume used of the hashtag antibody is quite small be sure to pipette directly into the sample and confirm that there is nothing left in the pipette tip.

Protocol materials

- RT Enzyme C 2000085/2000102 - stored in -20°C **10x Genomics**
- Human TruStain FcX™ (Fc Receptor Blocking Solution) **BioLegend Catalog #**422301, 422302
- TotalSeq™-C0253 anti-human Hashtag 3 Antibody **BioLegend Catalog #**394665
- PE anti-human CD123 Antibody **BioLegend Catalog #**306006
- TotalSeq™-C0251 anti-human Hashtag 1 Antibody **BioLegend Catalog #**394661
- TotalSeq™-C0254 anti-human Hashtag 4 Antibody **BioLegend Catalog #**394667
- APC anti-human CD303 (BDCA-2) Antibody **BioLegend Catalog #**354206
- TotalSeq™-C0252 anti-human Hashtag 2 Antibody **BioLegend Catalog #**394663
- poly-dT RT Primer 2000007 - stored in -20°C **10x Genomics**
- Single Cell VDJ 5 Gel Bead 1000264 - stored in -80°C **10x Genomics**
- RT reagent B 2000165 - stored in -20°C **10x Genomics**
- Reducing Agent B 2000087 - stored in -20°C **10x Genomics**
- Trypan Blue **Invitrogen - Thermo Fisher Catalog #**T10282
- SYTOX™ Green Nucleic Acid Stain **Thermo Fisher Scientific Catalog #**S7020
- Fetal Bovine Serum (FBS) **ATCC Catalog #**30-2020
- 1X PBS (Phosphate-buffered saline)
- 10x Vortex Adapter **10x Genomics Catalog #**120251/ 330002
- Chromium Next GEM Secondary Holder **10x Genomics Catalog #**1000142/ 3000332
- 10X Gasket 3000072 **10x Genomics**
- Glycerin (glycerol), 50% (v/v) Aqueous Solution **Ricca Chemical Company Catalog #**3290-32
- Chromium Next GEM Chip K 2000182 **10x Genomics**
- Partitioning Oil 2000190 **10x Genomics**

Safety warnings

Use/Safety training for operation of FACS (laser safety) required.

Ethics statement

For Peripheral Blood Mononuclear Cell (PBMC) isolation from peripheral blood samples, written informed consent was obtained from all participants or their legal guardians. Please refer to the associated article for the complete ethics statement.

Day 1

12h 45m

1

2m

Preparation of materials - Pre-FACS

Note

Keep the samples on **dry ice** until all reagents and materials are in place and ready.
Unless a specific temperature is indicated, all steps should proceed with cooling/on ice.
Prepare a water bath - set to 37°C

1.1 Prepare fresh FACS Buffer. For 50 mL, add 2.5 ml FBS to 47.5ml PBS. 2m

Fetal Bovine Serum (FBS) **ATCC Catalog #**30-2020

1X PBS (Phosphate-buffered saline)

Note

For 4 samples, 80 mL of FACS Buffer should be sufficient. Keep on ice

1.2 Place (number of samples)x2 15ml conical tubes on ice. One conical tube will be used to contain the real sample (***primary*** conical ***tube***) and the other conical tube will be used as calibration controls (***secondary*** conical ***tube***) for the FACS protocol in later steps. Label the tubes. 2m

1.3 After preparing the FACS Buffer, thaw the samples quickly in a 37°C water bath. Be cautious, as the labeling can come off; marking the tops of the tubes may be a more reliable option. 3m

Note

The freezing medium contains DMSO, which is toxic to cells at room temperature; therefore, these steps should be performed as quickly as possible.

1.4 Thawed samples should be quickly transferred into pre-chilled 15ml conical tube (primary conical tube) on ice.

1.5 Wash the cryo-tube (sample container) with approximately 2 ml of FACS Buffer. 2m

1.6 Transfer the 2 ml of FACS Buffer from the cryo-tube into a 15 ml conical tube. 2m

1.7 Top-up the conical tube with FACS Buffer until the total volume reaches 15 ml, then invert the tube 5 times to mix. 1m

1.8 550 rcf, 4°C, 00:05:00 , 5 to 8 minutes 5m

Centrifuge the samples. For our purposes, we used a centrifuge with cooling and with adaptors suitable for holding 15 ml conical tubes.

Equipment	
Megafuge™ 8R	NAME
Centrifuge	TYPE
Thermo Fisher Scientific	BRAND
75007210	SKU

1.9 After centrifugation, **carefully** decant the solution from each sample tube to the secondary conical tube (prepared in step 1.2), **keeping the pellet** (primary conical tube) **undisturbed**. 15m

Note

FOR CALIBRATION CONTROLS (secondary conical tube) ONLY!
Centrifuge the conical tube:
880 rcf, 4°C, 00:05:00 , between 880 and 950 rcf
Decant excess fluid (supernatant)
Wash/resuspend the base of one of the secondary conical tubes with 1 ml FACS Buffer and transfer the whole volume to the next secondary conical tube and wash/resuspend. Repeat for all secondary conical tubes in order to combine into a pool.

Half of the volume of this pool will be directly transferred to a FACS tube through a cell strainer to use in FACS calibration as a "no fluorescent staining" control.

The other half of the volume can be transferred to a 1.5 ml eppendorf tube and centrifuged again:
880 rcf, 4°C, 00:05:00 , between 880 and 950 rcf
Pipette-off the supernatant, leaving approximately 50 µL and then handle the same as the real samples with blocking (without the hashtags) and antibody staining (see step 1.16 and 1.17) resulting in a "fluorescent-stained" control

Keep on ice.

1.10 Resuspend each pellet (**primary conical tube**) with 1 ml FACS Buffer 2m

Note

This step is helpful to remove as much of the DMSO as possible without drastically affecting the cells or their number.

1.11 Load a 96-well plate with 10µL
Trypan Blue **Invitrogen - Thermo Fisher Catalog #**T10282 to each well corresponding a sample. 1m

Note

Can use PCR tubes instead of 96-well plate.

Add 10µL of each sample (**primary conical tube**) to 10µL Trypan blue and mix well

Note

Counting Cells with Countess using default setting or a specific (custom) profile:
- For counting the cells, add 10 µL of each sample to the corresponding well/tube containing Trypan Blue and mix by pipetting (can use plate or tubes as described in step 1.11).
- Pipette 10 µL of the mixture into a Countess cell counting chamber slide. Follow User guide instructions and recommendations.

1.12 Measure and record "initial" cell concentration and viabilities post-thaw using a cell counter. For our purposes we used an automated cell counter. 2m

Equipment

Countess™ II automated cell counter	NAME
Thermo Fisher Scientific	BRAND
AMQAX1000	SKU

1.13 Centrifuge the samples (**primary conical tube**) again 550 rcf, 4°C, 00:05:00 10m

1.14 While centrifugation continues, prepare blocking reagent mix: 1m

Human TruStain FcX™ (Fc Receptor Blocking Solution) **BioLegend Catalog #**422301, 422302

	Reagent	Volume per sample	Volume (4x + 10%)
	FACS Buffer	45	198
	Human TruStain FcX (Fc Receptor Blocking Solution)	5	22

Blocking Agent Mix

1.15 Remove the supernatant by pipetting, leaving approximately 50 µL of the supernatant for each sample (**primary conical tube**). 1m

1.16 Add 50 µL **blocking agent mix** to each sample (**primary conical tube**)**+** "fluorescent-stained" control (**secondary conical tube**). Resuspend each sample pellet by pipetting. **Incubate** 10m on ice. 10m

1.17 While incubating, prepare **antibody master mix on ice, pipette mix throughly**: 1m
(Red)
PE anti-human CD123 Antibody **BioLegend Catalog #**306006
(Blue)
APC anti-human CD303 (BDCA-2) Antibody **BioLegend Catalog #**354206
(Hashtags)
TotalSeq™-C0251 anti-human Hashtag 1 Antibody **BioLegend Catalog #**394661
TotalSeq™-C0252 anti-human Hashtag 2 Antibody **BioLegend Catalog #**394663
TotalSeq™-C0253 anti-human Hashtag 3 Antibody **BioLegend Catalog #**394665
TotalSeq™-C0254 anti-human Hashtag 4 Antibody **BioLegend Catalog #**394667

50 µL FACS Buffer
0.5 µL Antibody 1 (RED)
0.5 µL Antibody 2 (BLUE)
1.5 µL Hashtag*number of samples

	Reagent	Volume per sample (µL)	4x + 10% (µL)
	FACS Buffer	50	220
	Antibody 1 (red)	0.5	2.2
	Antibody 2 (blue)	0.5	2.2

Antibody Master Mix

Note

Do not add the hashtags into master mix, hashtags are required to be unique to each sample.

Note

The antibody volume is based on having an approximate/expected total number of cells per sample (roughly 0.5 µL is used for cell numbers in range of 10^5 while 1 µL can be used for 10^6)

	Reagent	Volume per sample (µL)
	Sample	~50
	Hashtag	1.5
	Antibody Master Mix	48.5
	total	~100

Component volume per sample

1.18 Incubate each sample (**primary conical tube**) with hashtags and antibody mix at 4°C (on ice) for 20m. 20m

1.19 Incubate the "fluorescent-stained" control (**secondary conical tube**) with Antibody master mix only (no hashtags) for 20m at 4°C (on ice). 20m

2 *Measure cell concentration and viability prior to FACS*

2.1 After incubation, add FACS Buffer to **each sample + "fluorescent-stained" control** (secondary conical tube) to be at least 10 mL volume and mix by inverting. 1m

Note

This step washes/removes the excess hashtags and antibody dyes.

2.2 550 rcf, 4°C, 00:05:00 , 650 to 800 RCF and 5 to 8 minutes 5m

After its done, remove supernatant by pouring **carefully.**

2.3 Resuspend each **sample pellet + "fluorescent-stained" control pellet** (secondary conical tube) in 0.5-1 mL FACS Buffer. **Keep "fluorescent-stained" control on ice.** 2m

Check and record the cell viability and concentration of each **sample pellet** using the Countess cell counter. This gives us the post-stain viability and cell concentration to use in the pooling calculations.

Note

Counting Cells with Countess using default setting or a specific (custom) profile:
- For counting the cells, add 10 µL of each sample to the corresponding well/tube containing Trypan Blue and mix by pipetting (can use plate or tubes as described in step 1.11).
- Pipette 10 µL of the mixture into a Countess cell counting chamber slide. Follow User guide instructions and recommendations.

2.4 Calculate volume to be used per sample to have equalized representation across all samples when pooled 1m

- Volume to use is calculated as:
((Lowest Concentration)/(Live Concentration of Sample n))∗(Total Volume per sample)

$$\frac{C_{\text{lowest}}}{C_{\text{live}_n}} \times V_{\text{total}}$$

where C_{lowest} is the lowest concentration measured, C_{live_n} is the live concentration of sample n and V_{total} is the total volume per sample.

Note

Calculation is done according to the total volume of the sample with lowest concentration.

Example:

	Live Concentration (per mL)	Volume to pool (ml)
	2.05∗10^5	0.45
	2.64∗10^5	0.35

	Live Concentration (per mL)	Volume to pool (ml)
	8.56*10^5	0.10
	9.38*10^4	1

Above: example calculation of volume to use (if samples were resuspended in 1 ml)
In the table, sample #4 has the lowest live cell concentration, therefore all of the volume is used (1 ml), the volume for every other sample is calculated such that there is an equal number of live cells compared to the lowest sample when combined.

2.5 Combine the appropriate volume of each sample into a single tube. 1m

2.6 Pass the entire volume of the combined (multiplexed) samples through a cell strainer and into a FACS tube with a pipette. **Keep on ice.** 1m

3 ***FACS Protocol*** 1h

Follow user guide instructions for FACS start-up protocol according to operating manual(s).
For our purposes we used a BD FACSAria Fusion Cell Sorter with accompanying BD FACSDiva Software.

- **Pre-load** 200 µL cold FACS Buffer to two protein-LoBind collection tubes (one tube per sorted cell population) before sorting starts. **Keep on ice**.

Software

BD FACSDiva™ Software — NAME

BD Biosciences — DEVELOPER

Equipment

NAME	TYPE	BRAND
BD FACSAria™ Fusion Cell Sorter	Cell sorter	BD Biosciences

Note

Cell sorting machine preparation and start-up can begin during the hashtags and antibody incubation step (during the 20 minute incubation, see step 1.18).
Be sure to turn on the cooling system (set to 4°C) early, at least 1h before "real" cell sorting starts (**primary conical tube**).

3.1 Gating strategy may vary with cell populations, cell types of interest, sorting machine, etc. Please refer to expert/core facility guidance. Below is a description of the strategy we applied. 10m

For our purposes we used:
1. SSC-A versus FSC-A to visualize the entire pool and define all cells and exclude debris
2. FSC-H versus FSC-A to select singlets only
3. FITC-A versus SSC-A to exclude dead/dying (live versus dead) cells
4. PE-A versus APC-A to define the sorting populations

CALIBRATION CONTROLS:

- Use the "no fluorescent staining" control (from step 1.9) to have a baseline/background fluorescent signal and also to set the gate to exclude debris and doublets. Ready-up the live versus dead plot and gate. Record events.
- Unload the "no fluorescent staining" control and keep on ice.
- Switch to using the "fluorescent stained" control (from step 2.3) to set the gate for rare cell population of interest (antibody-positive cells) versus all other cells. Record events.
- Unload "fluorescent stained" control and keep on ice
- Make a fresh dilution from stock of live/dead dye in a UV-blocked tube with cold PBS. Keep on ice.

We used 1:1000

SYTOX™ Green Nucleic Acid Stain **Thermo Fisher Scientific Catalog #**S7020

- Add an initial volume (20-60 µL) of diluted live/dead dye to "fluorescent stained" control to set the gate for live versus dead cells.
- Resume acquisition.
- Incrementally add the 1:1000 diluted live/dead dye by 20-60 µL until an optimal/observable separation between live/dead cells is achieved. Record events.

Note

The initial and subsequent incremental volumes of diluted live/dead dye to be added depends on total number of cells expected in the control. That is, if the control is not very concentrated stay on the lower end of the volume range. Record the total amount of live/dead dye used in the control.

After this point the gates should be in a relatively "finalized" form but will take some minor adjustments when the real sample is run.

Note

Rationale behind this approach is to use these controls for calibration and gate-setting so as not to use/waste sample volume or cells from the "real" pool.

3.2 **"Real" pooled sample:** 1m

Add an initial volume of 1:1000 diluted sytox Green (in UV-blocked tube) to sample pool, dependent on the estimated total number of cells and also using the total volume needed in the calibration control in previous step as a starting point.

Note

It is likely that the "fluorescent stained" calibration control has less cells than the real sample, so the volume needed there to separate live/dead is a safe starting point for the volume to add to the "real" sample.

3.3 Pay attention to flow rate and events per second (in accordance to FACS operating manuals) and adjust gating positions as needed. 1m

Incrementally add live/dead dye as needed to achieve good separation between live and dead cells.
Record a similar total number of events as with the controls for comparison later.

3.4 Begin sort once collection tubes have been correctly loaded 20m

Note

- Monitor collection tube for overflowing
- To reduce the risk of sample degradation and/or clogging during sort: occasionally pause the sorting to vortex the FACS tube and do not exceed 25 mins total sort time.

4 ***Post-FACS***

Note

Cell sorting results in these collection tubes:
- sorted pDC **(i)**
- all other PBMCs **(ii)**

4.1 Add **300 µL** 0.1% BSA in PBS to **collection tube (i)** which contain the sorted pDCs. 1m
Pipette half of this volume to a new protein-LoBind tube.
Leave on ice.

Note

Resulting in two tubes **"A"** and **"B"** each containing an aliquot of sorted pDCs where one of these tubes is the "original" collection tube from sorting - this is done to minimize loss due to pipetting. Each tube should now contain:
~250 µL (FACS Buffer + BSA) + "x/2" µL sorted pDCs volume
since the collection tube (i) has these components at this step:
200 µL FACS Buffer + 300 µL BSA + "x" µL sorted pDCs volume

4.2 Centrifuge **collection tube (ii)** which contains the sorted all other PBMCs 5m
800 rcf, 4°C, 00:05:00

Equipment

Eppendorf 5417R Refrigerated Centrifuge	NAME
Micro-centrifuge (1.5-2.0 ml tubes)	TYPE
Eppendorf	BRAND

4.3 Remove supernatant from **collection tube (ii)**.
Resuspend the pellet in **collection tube (ii)** immediately in **100 µL** 0.1% BSA in PBS

1m

4.4 Check cell concentration and viability in **collection tube (ii)** and record the values**.**

2m

Note

Counting Cells with Countess using default setting or a specific (custom) profile:
- For counting the cells, add 10 µL of each sample to the corresponding well/tube containing Trypan Blue and mix by pipetting (can use plate or tubes as described in step 1.11).
- Pipette 10 µL of the mixture into a Countess cell counting chamber slide. Follow User guide instructions and recommendations.

4.5 Add a volume from **collection tube (ii)** equivalent to ~33,000 cells (live cell concentration) into each of the tubes **"A"** and **"B"** containing the aliquots of sorted pDCs = **two replicates of the pooled cell suspension**.

1m

Note

This results in two protein LoBind tubes containing aliquots of the sorted pDCs from **collection tube (i)** plus a volume of the sorted all other PBMCs from **collection tube (ii)** resulting in a total volume of least:
~250 µL + "x/2" µL sorted pDCs volume + "y" µL sorted all other PBMCS
in each tube: "**A**" and "**B**".

4.6 800 rcf, 4°C, 00:05:00 5m

Centrifuge **"A"** and **"B"**

4.7 Carefully pipette-off the supernatant from each tube until the remaining volume is **≤ 38.7 µL**. 2m

Note

Specifically **38.7 µL** is used because this is the total allowable volume of Cell Suspension Stock per reaction + Volume of Nuclease-free Water per reaction, according to the **Cell Suspension Volume Calculator Table**

Carefully remove as much of the supernatant as possible without disturbing the pellet.

Keeping track of the approximate volume in each tube prior to this stage as described in step 4.1 and 4.5 can help with predetermining how much volume can be safely removed by pipetting to leave approximately 38.7 µL behind.

Since the pellet may not be visible, avoid disturbing the area where it is expected to form, according to the centrifugation position.

4.8 Thoroughly resuspend pellet based on expected position in the tube with the remaining volume and **measure the exact remaining volume (v) by pipetting**. 1m

If resuspension volume of cells < 38.7 µL **calculate the volume of ddH2O needed** such that **cells + ddH2O = 38.7 µL.**

- Tube "A":

$$Volume of dH2O to add = 38.7 \mu L - \nu_{a}$$

- Tube "B":

$$Volume of dH2O to add = 38.7 \mu L - \nu_{b}$$

Note

The ddH20 is calculated per tube [(ii) + $(i)_a$] and [(ii) + $(i)_b$] and added independently to the corresponding tubes containing the **NEXT GEM kit master mix** as instructed in the **Chromium Next GEM Single Cell 5' v2 (Dual Index) with Feature Barcode technology for Cell Surface Protein & Immune Receptor Mapping User Guide** see here

4.9 Proceed with chip loading step in accordance with 10x protocols. Where **"A"** and **"B"** represent two replicates of the pooled cell suspension to be loaded into two separate channels (channel splitting). 2m

Note

Channel splitting allows us to increase the target number of recovered cells while keeping the residual doublet rate low.

5 Materials below are provided by the **Chromium Next GEM Single Cell 5' v2 (Dual Index) with Feature Barcode technology for Cell Surface Protein & Immune Receptor Mapping** kit**.**

Note

The following sections of the protocol apply to the **Chromium Next GEM Single Cell 5' v2 Chromium Next GEM Single Cell 5' v2 (Dual Index) with Feature Barcode technology for Cell Surface Protein & Immune Receptor Mapping** protocol.
For a full list of reagents and materials please review the relevant section in the user guide **here**.

Alternatively, you may proceed with other protocols, such as the 10x Single Cell 3' protocol.

Thaw reagents at RT

- Reducing Agent B 2000087 - stored in -20°C **10x Genomics**
- poly-dT RT Primer 2000007 - stored in -20°C **10x Genomics**
- Single Cell VDJ 5 Gel Bead 1000264 - stored in -80°C **10x Genomics**
- RT reagent B 2000165 - stored in -20°C **10x Genomics**

5.1 Place reagents on ice

- RT Enzyme C 2000085/2000102 - stored in -20°C **10x Genomics**

5.2 Have at hand: other materials & reagents

- 10x Vortex Adapter **10x Genomics Catalog #**120251/ 330002
- Chromium Next GEM Secondary Holder **10x Genomics Catalog #**1000142/ 3000332

10X Gasket 3000072 **10x Genomics**

Glycerin (glycerol), 50% (v/v) Aqueous Solution **Ricca Chemical Company Catalog #**3290-32

Chromium Next GEM Chip K 2000182 **10x Genomics**

Partitioning Oil 2000190 **10x Genomics**

6 ***Assembly and Loading of Chromium Next GEM Chip K*** 3h

Note

"Step 1": Follow the **Chromium Next GEM Single Cell 5' v2 (Dual Index) with Feature Barcode technology for Cell Surface Protein & Immune Receptor Mapping User Guide** These steps are thoroughly explained in the **Chromium Next GEM Single Cell 5' v2 (Dual Index) User Guide**, which can be accessed here.

Equipment

Chromium Controller	NAME
microfuidic based single-cell capture	TYPE
10x Genomics	BRAND
1000171	SKU
https://www.10xgenomics.com/instruments/chromium-controller	LINK

Alternatively,

Equipment

NAME	TYPE	BRAND	SKU
Chromium iX/X series	microfluidic based single-cell capture	10x Genomics	1000332/1000329

7 ***Post GEM-RT Cleanup & cDNA Amplification*** 6h

Note

"Step 2": Follow the **Chromium Next GEM Single Cell 5' v2 (Dual Index) with Feature Barcode technology for Cell Surface Protein & Immune Receptor Mapping User Guide** which can be accessed **here**.

Equipment

NAME	TYPE	BRAND
Eppendorf Mastercycler Pro S	Thermocycler	Eppendorf

Equipment

Qubit™ 4 Fluorometer, with WiFi	NAME
Fluorometer	TYPE
Invitrogen	BRAND
Q33238	SKU
https://www.thermofisher.com/order/catalog/product/Q33238#/Q33238	LINK

Pause: freeze cDNA (-20°C) after verifying and recording concentration. Can resume library generation protocol on the next day.

Day 2

12h

8 ***V(D)J Amplification from cDNA***

3h

Time dependent on stop options used and may vary with cycling conditions

Note

"Step 3": Follow the **Chromium Next GEM Single Cell 5' v2 (Dual Index) with Feature Barcode technology for Cell Surface Protein & Immune Receptor Mapping User Guide** which can be accessed **here**.

9 ***V(D)J Library Construction***

3h 20m

Time dependent on stop options used and may vary with cycling conditions

Note

"Step 4": Follow the **Chromium Next GEM Single Cell 5' v2 (Dual Index) with Feature Barcode technology for Cell Surface Protein & Immune Receptor Mapping User Guide** which can be accessed **here**.

10 ***5' Gene Expression (GEX) Library Construction***

4h

Time dependent on stop options used and may vary with cycling conditions

Note

"Step 5": Follow the **Chromium Next GEM Single Cell 5' v2 (Dual Index) with Feature Barcode technology for Cell Surface Protein & Immune Receptor Mapping User Guide** which can be accessed **here**.

11 ***Cell Surface Protein/Immune Receptor Mapping Library Construction*** 1h 40m

Time dependent on stop options used and may vary with cycling conditions

Note

"Step 6": Follow the **Chromium Next GEM Single Cell 5' v2 (Dual Index) with Feature Barcode technology for Cell Surface Protein & Immune Receptor Mapping User Guide** which can be accessed **here**.

After this step the libraries are ready for quality checking (bioanalyzer) and sequencing.

Equipment

Bioanalyzer	NAME
Bioanalyzer	TYPE
Agilent	BRAND
G2991AA	SKU
https://www.agilent.com/en/product/bioanalyzer-automated-electrophoresis/bioanalyzer-instrument/2100-bioanalyzer-instrument-228250	LINK
Any bioanalyzer will suffice.	SPECIFICATIONS

Protocol references

10x Genomics. (2024, July 29). *Chromium Single Cell 5' Reagent Kits User Guide (v2 Chemistry Dual Index) with Feature Barcoding technology for Cell Surface Protein and Immune Receptor Mapping* (Document number: CG000330 Rev G) [User guide].

BD FACSDiva Flow Cytometry Software, BD Life Sciences, 2019.

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