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10X Multiome Profiling with CMO Hashing

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Abstract

The goal is to investigate a comprehensive view of gene
regulation during endothelial cell differentiation with a single cell multiome ATAC + Gene Expression . For multiplexing samples, we used CMO hashtags before 10x Capture.

10X Single Cell Multi-ome ATAC + Gene Expression

Cell Preparation

1. Prepare a 15 mL conical tubes with 5 mL of RT media.
2. Remove the cryovials from liquid nitrogen storage and place on dry ice.
3. Quickly thaw cryovials in a water bath at 37°C until only a small ice pellet is visible. Bring to the BSC.
4. Thaw 4 tubes per person → Spin 8 tubes at a time
5. Using a 5 mL serological pipette, slowly add 1 mL of RT media to the vial with the cells. Do not mix. Using the same pipette, slowly aspirate the now ~1.5 mL volume of diluted cells from the vial. Work carefully to remove all the liquid without creating extensive bubbles. Transfer the diluted cells from the vial into the prepared 15 mL conical tube from step 1 for a total of 5.5 mL. Do not mix; avoid disrupting the cells.
6. Centrifuge at 200×g for 3 min at RT.
7. Remove the supernatant without disrupting the cell pellet and gently resuspend in 1 ml PBS + 0.04% BSA using wide-bore tip. Transfer to a 1.5-ml microcentrifuge tube (lo-bind). Rinse the 15-ml tube with 0.5 ml PBS + 0.04% BSA and transfer the rinse to the 1.5-ml tube containing cells.
8. Place samples on ice at this point until all 25 tubes are thawed
9. Centrifuge cells at 200×g for 3 min (1st wash).
10. Remove the supernatant without disrupting the cell pellet.
11. Using a wide-bore tip, add 1 ml PBS with 0.04% BSA to the tube. Gently pipette mix 5 times and invert tubes to resuspend the cell pellet.
12. Centrifuge cells again at 200×g for 3 min (2nd wash).
13. Remove the supernatant without disrupting the cell pellet and resuspend in 1 ml PBS + 0.04% BSA with regular-bore tip.
14. Pass cell suspension through a 70 µm Flowmi Cell Strainer into a 1.5-ml tube (3rd wash).
15. Use the grey Flowmi Cell Strainer
16. Place the cells on ice.
17. Determine the cell concentration and viability using a Countess.
18. Proceed with at most 500k cells to Nuclei Isolation.

Nuclei Isolation

Follow 10x Genomics Protocol - Nuclei Isolation (Source)

1. Centrifuge all tubes at 300×g for 5 min at 4°C (preferably swinging bucket).
2. Remove ALL the supernatant without disrupting the cell pellet.
3. Add 100 µl chilled 0.5X Lysis Buffer. Pipette mix 10x.
4. Incubate for 2 min on ice.

5. Add 1 ml chilled Wash Buffer (with BSA) to the lysed cells. Pipette mix 5x.
6. Take 10 µl from the same 5 samples to take pictures on hemocytometer
7. Immediately centrifuge at 500×g for 5 min at 4°C.
8. Remove the supernatant without disrupting the nuclei pellet.
9. Resuspend nuclei in 180 µL Wash Buffer (with BSA). Proceed to CMO Hashing. (~500k or fewer cells per labeling condition)

CMO Hashing

In this section, we stain the samples with CMO oligos. Protocol from **McGinnis, et al. (2019)**.

CMO Hashing Reagents

Wash Buffer (without BSA)

Prepare fresh and maintain at 4°C.

	A	B	C	D	E
	Reagent	Stock Conc	Final Conc	Dilution Factor	40
	Nuclease-free water				37.96
	Tris-HCl (pH 7.4)	1M	10 mM	100	0.40
	NaCl	5M	10 mM	500	0.08
	MgCl2	1M	3 mM	333.3	0.12
	Tween-20	10%	0.1 %	100	0.40
	DTT	1M	1 mM	1000	0.04
	RNase Inhibitor	40 U/µL	1 U/µL	40	1.00

10x solution of CMO anchor:barcode ()
(20 µl per sample + extra) = this volume is applied to each CMO

	Reagent	Stock Conc	Final Conc	Dilution Factor	25 µL
	CMO Anchor Solution				24.5 µL

	Barcode	100 µM	2 µM	50	0.5 µL

CMO Anchor Solution

	A	B	C	D	E
	Reagent	Stock Conc	Final Conc	Dilution Factor	20
	Wash Buffer (without BSA)				19.6
	CMO Anchor	100 µM	2 µM	50	0.4

10x solution of CMO co-anchor
Multiplier = 1.12 × 45 Barcodes

	A	B	C	D	E
	Reagent	Stock Conc	Final Conc	Dilution Factor	20
	Wash Buffer (without BSA)				19.2
	CMO co-anchor	100 µM	4 µM	25	0.8

Protocol

1. Into cells in 180 µl Wash Buffer (with BSA) add 20 µl 10X Anchor:Barcode solution and pipette gently to mix.
2. Incubate on ice for 5 min.
3. Add 20 µl 10X Co-Anchor solution and pipette gently to mix.
4. Incubate on ice for 5 min.
5. Add 1 ml chilled Wash Buffer (with BSA).
6. Centrifuge at 500×g for 5 min at 4°C. Remove supernatant.
7. Resuspend nuclei pellet in 0.3 ml Wash Buffer (with BSA).
8. Filter nuclei with a 40 µm Flowmi into a new tube.
9. Centrifuge at 500×g for 5 min at 4°C.
10. Resuspend nuclei pellet in 0.15 ml Wash Buffer (with BSA).
11. Count nuclei to get the total number of nuclei.

12. Pool samples.
13. Proceed to 10X multiome (**Protocol**).