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Intestinal Organoid Dissociation and Nuclei Isolation for Single Cell ATAC-Seq V.1

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Protocol status: In development
We are still developing and optimizing this protocol

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Abstract

This protocol provides a procedure for human intestinal organoid dissociation into a single cell suspension and nuclei isolation prior to Single Cell ATAC-Sequencing.

Guidelines

Nuclei isolation for Chromium Next GEM Single Cell ATAC Sequencing was performed following the protocol provided by 10X Genomics. For further guidelines and tips reference the original protocol below. (https://assets.ctfassets.net/an68im79xiti/5g035d2ngCW1aB9DFqPphO/71445a59fb282ea273a866c26cb5d319/CG000169_DemonstratedProtocol_NucleiIsolation_ATAC_Sequencing_RevD.pdf)

Chromium Next GEM Single Cell ATAC Sequencing was performed following the protocol provided in the user guide from 10X Genomics. (https://assets.ctfassets.net/an68im79xiti/7L2MU4QSWfrEgd2h13Efac/d5326fcdc6363aa04e4fdf11b2a1f2f8/CG000209_Chromium_NextGEM_SingleCell_ATAC_ReagentKits_v1.1_UserGuide_RevD.pdf)

The primary human tissue that generates the organoids, are obtained from endoscopic biopsies after patient's consent and approval from Institutional Review Board at the University of Chicago (IRB Number: 15573A).

Materials

MATERIALS

- Magnesium chloride solution for molecular biology (1.00 M) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M1028
- TrypLE™ Express Enzyme **Thermo Fisher Scientific Catalog #**12604013
- Wheat Germ Agglutinin, Alexa Fluor™ 594 Conjugate **Thermo Fisher Catalog #**W11262
- Trizma Hydrochloride Solution pH 7.4 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**T2194
- Sodium Chloride Solution 5 M **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**59222C
- Magnesium Chloride Solution 1 M **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M1028
- Nonidet P40 Substitute **Merck MilliporeSigma (Sigma-Aldrich) Catalog #** 74385
- MACS BSA Stock Solution **Miltenyi Biotec Catalog #** 130-091-376
- Flowmi Cell Strainer 40 µm **Bel-Art Catalog #**H13680-0040
- Nuclei Buffer 20X **10x Genomics Catalog #**2000153/2000207
- DAPI **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**D9542

Note: 10x Genomics Nuclei Buffer 20X (2000153/2000207) is included in the 10x Genomics Single Cell ATAC Library Kits

Diluted Nuclei Buffer 1mL

Nuclei Buffer (20X)	50 ul (final concentration 1X)
Nuclease free water	950 ul

Wash Buffer Prepare fresh, maintain at 4°C	Stock	Final	2 ml
Tris-HCl (pH 7.4)	1 M	10 mM	20 µl
NaCl	5 M	10 mM	4 µl
$MgCl_2$	1 M	3 mM	6 µl
BSA	10%	1%	200 µl
Tween-20	10%	0.1%	20 µl
Nuclease-free Water	-	-	1.75 ml
Lysis Buffer **Prepare fresh, maintain at 4°C**	**Stock**	**Final**	**2 ml**
Tris-HCl (pH 7.4)	1 M	10 mM	20 µl
NaCl	5 M	10 mM	4 µl
$MgCl_2$	1 M	3 mM	6 µl
Tween-20	10%	0.1%	20 µl
Nonidet P40 Substitute (if using Sigma (74385) 100% solution, prepare a 10% stock)	10%	0.1%	20 µl
Digitonin (incubate at 65°C to dissolve precipitate before use)	5%	0.01%	4 µl
BSA	10%	1%	200 µl
Nuclease-free Water	-	-	1.726 ml

Troubleshooting

Before start

Prepare diluted nuclei buffer, wash buffer, lysis buffer, and PBS with 0.04% BSA

Organoid Dissociation

1 Incubate organoid in TrypLE for up to 20 minutes at 37°C

1.1 Every 5 minutes, pipette the cell suspension up and down 5-10x and check the digestion progress with a hemocytometer until enough single cells are present

Nuclei Isolation

2 For freshly dissociated cells, perform 1-2 washes with PBS + 0.04% BSA (20ul BSA/1mL 1X PBS).

> Note
>
> Consult 10X Genomics protocol for using frozen cells

3 Determine the cell count after washing using a hemocytometer.

4 Add cell suspension of 100,000-1,000,000 cells to a 2-ml tube. For our experiment we started with **200,000** cells per sample.

5 Centrifuge at 300 rcf for 5 min at 4°C

300 x g, 4°C, 00:05:00

6 Remove ALL the supernatant without disrupting the cell pellet

7 Add 100 µl chilled Lysis Buffer. Pipette to mix 10x

100 µL Lysis Buffer

8 Incubate for 4 min on ice

On ice 4 min

Note

Time may vary depending on cell type; 4 minutes is specific for organoid samples

9 Add 1 ml chilled Wash Buffer to the lysed cells. Pipette to mix 5x

1 mL Wash Buffer

10 Centrifuge at 500 rcf for 5 min at 4°C

500 x g, 4°C, 00:05:00

11 Remove the supernatant without disrupting the nuclei pellet

12 Based on your targeted nuclei recovery, cell concentration in step 4 and assuming ~50% nuclei loss during cell lysis, resuspend in chilled Diluted Nuclei Buffer (1x). Maintain on ice.
(See Nuclei Stock Concentration Table and Example Calculation below)

For our experiment, we targeted **5,000 nuclei**.

Nuclei Stock Concentration Table

Based on the Targeted Nuclei Recovery, prepare the nuclei suspension in Diluted Nuclei Buffer to achieve the corresponding Nuclei Stock concentrations.

Targeted Nuclei Recovery	Nuclei Stock Concentration (nuclei/µl)
500	155-390
1,000	310-780
2,000	610-1,540
3,000	925-2,300
4,000	1,230-3,075
5,000	1,540-3,850
6,000	1,850-4,600
7,000	2,150-5,400
8,000	2,460-6,150
9,000	2,770-6,900
10,000	3,080-7,700

Example Calculation

Cell count at step 2a: **200,000**
Estimated nuclei count at step 2h (~50% loss):**100,000**
If targeting 5,000 Nuclei Recovery, nuclei pellet at step 2h may be resuspended in **30 µl** Diluted Nuclei Buffer for Nuclei Stock Concentration of 1,540-3,850 nuclei/µl (see Table above)

13 Check nuclei integrity by staining with WGA and DAPI. Also determine the nuclei concentration using a hemocytometer

14 OPTIONAL: If cell debris and large clumps are observed, pass through a cell strainer. For low volume, use a 40 µm Flowmi Cell Strainer to minimize volume loss

15 Proceed immediately to Chromium Next GEM Single Cell ATAC Sequencing protocol (found in the Chromium Single Cell ATAC Solution User Guide)