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Adult_Nvectensis_scATAC-seq

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Nematostella_snOMICS

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We use this protocol and it's working

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Abstract

This protocol describes the steps for isolating single nuclei from whole adult *Nematostella vectensis* polyps for scATAC-seq using 10x Chromium platform. *N. vectensis* is a brackish-water sea anemone (Cnidaria) that is maintained in the laboratory in 1/3 artificial sea water (~350mOsm/L), as previously reported[1]. This nuclei isolation protocol combines a slightly hypertonic buffer (TST lysis buffer, ~380mOsm/L)[2] with mechanical dissociation using a dounce homogenizer. The resulting cell suspension is mildly fixed with 0.1% PFA[3] to mitigate damage during sorting and then purified from debris and aggregates by FACS. Finally, sorted nuclei are permeabilized using a modified hypotonic OmniATAC lysis buffer[4] containing 1/10 the standard detergent concentration and supplemented with 70µM Pitstop2 to increase nucleus permeability to Tn5[5] . After washing, nuclei are visually inspected under the microscope, counted, and tagmented in bulk prior to single-nuclei encapsulation using 10x Chromium platform. scATAC-seq libraries are subsequently prepared following manufacturer`s instructions.

Guidelines

Clean and cool down dounce homogenizer

Decontaminate dounce homogenizer right after each use by rinsing thoroughly with tap water to remove gross debris. Then, soak the tube and pestle in 10% bleach (freshly prepared) for 15min. Rinse 7-8 times with nuclease-free water to remove all traces of bleach, and one more time with 70% ethanol. Let the dounce homogenizer dry on a clean kimwipe inside a clean container (eg nuclease-free pipette tip box). Cool down the douncer at least 1h at 4 C before use.

Materials

- Nuclease-free water (ThermoFisher, #W4502)
- Tween-20 10% (Sigma # 11332465001).
- NP40 10% (Sigma # 11332473001)
- 1M Tris-HCl, pH 7.5 (Invitrogen #15567-027)
- 1M Tris-HCl, pH 8 (Invitrogen #15568-025)
- 5M NaCl (Ambion #AM9760G)
- 1M MgCl2 (Sigma # M1028)
- 1M CaCl2 (Sigma # 21115)
- BSA nuclease- and protease-free (Sigma #126609)
- cOmplete, Mini tablet EDTA-free Protease inhibitor (Roche #11836170001)
- DAPI (ThermoFisher #D1306)
- 10x PBS Ca/Mg- and nuclease-free (ThermoFisher #AM9624)
- Digitonin 20mg/mL (Promega #G9441)
- Pitstop2 (Abcam #120687).
- 16% methanol-free formaldehyde (PFA) (ThermoFisher #28906)
- Glycine (Sigma-Aldrich #G7126)
- 20x Nuclei Buffer (10x genomics)
- OTHER: LoBind protein tubes, razor blades, 2mL douncer homogenizer, 70µm cell strainer, hemocytometer, 40µm Flowmi strainer

BUFFERS/STOCK SOLUTIONS

- **Salts Tris Resuspension Buffer (ST buffer).** Can be stored at RT long term. Prepare 50ml by mixing:
  500uL 1M Tris-HCl, pH 7.5 (final 10mM)
  1460uL 5M NaCl (final 146mM)
  50uL 1M CaCl2 (final 1mM CaCl2)
  1050uL 1M MgCl2 (final 21mM)
  46.94mL Nuclease-free water
  50mL Total

- **ATAC Resuspension Buffer (ATAC-RSB).** Can be stored at RT long term, but better at 4C. Prepare 50ml by mixing:
  500uL 1M Tris-HCl, pH 7.5 (final 10mM)
  100uL 5M NaCl (final 10mM)
  150uL 1M MgCl2 (final 3mM)
  49.25mL Nuclease-free H2O
  50mL Total

- **Salts Tris Resuspension Buffer + 10% BSA (ST-10%BSA).** Make 1mL aliquots and store at -20C
- **ATAC-RSB-10%BSA.** Make 1mL aliquots and store at -20C
- **PBS-10%BSA.** Make 1mL aliquots and store at -20C
- **Pitstop2 30mM**. Dissolve Pitstop2 in DMSO, make 10uL aliquots and store at -20C

- **Digitonin 1%**. Dilute stock solution (20mg/mL) 1:1 with water to make 1% working stock, aliquot, store at -20C for up to 6 months, do not freeze/thaw
- **2.5M Glycine:** Dissolve 0.94gr in 5mL nuclease-free water and filter sterilize through a 0.22. Store at 4C
- **DAPI 1mg/mL**. Dissolve DAPI in UP water, make aliquots and store at 4C (short-term use) / -20C (long-term)

FRESH BUFFERS
Prepare fresh buffers the day of experiment and keep them on ice/4 C

-**ST-protInhibitor**. Dissolve 1 Mini tablet EDTA-free Protease inhibitor in 10mL ST buffer.

-**TST Lysis buffer** (0.03% Tween20- ST-protInhibitor).
Prepare 8mL by mixing 24uL of 10%Tween-20 in 7976uL of ST buffer-protInhibitor

-**ST-2%BSA**. Prepare 2mL by mixing 400uL of ST-10%BSA in 1.6mL ST-protInhibitor

-**PBS-2%BSA**. Prepare 10mL by mixing 2mL of 10%BSA-PBS in 8mL PBS

-**1mM PitStop2**. Mix 2uL of 30mM PitStop2 stock with 58uL PBS.

-**ATAC-RSB-1%BSA**. Mix 450ul ATAC-RSB with 50ul ATAC-RSB-10%BSA

- **1x OmniATAC lysis buffer**, 200uL:
174uL ATAC-RSB
2uL 10% NP-40 (final 0.1% v/v)
2uL 10% Tween20 (final 0.1% v/v)
2uL 1% Digitonin (final 0.01% v/v)
20uL ATAC-RSB-10%BSA (final 1%)

- **0.1x OmniATAC Lysis Buffer supplemented with Pitstop2**, 300uL:
30uL 1x OmniATAC lysis buffer
21uL PitStop2 1mM in PBS (70µM final)
249uL ATAC-RSB-1%BSA

- **ATAC Wash Buffer**, 1mL:
790 uL ATAC-RSB
10 uL 10% Tween20 (final 0.1% v/v)
200 uL 10% BSA-ATAC-RSB (final 2%)

- **1% PFA**. Mix 100uL 16% PFA with 1500uL PBS

- **Nuclei staining buffer for FACS** (10ug/mL DAPI in PBS-2%BSA)**.** Mix 10uL of 1mg/mL DAPI stock in 1mL of PBS containing 2%BSA.

- **1x Diluted Nuclei Buffer.** Prepared from 20x stock

Before start

Use animals that have been starved at least 3 days, as well as recently spawned individuals, to avoid any possible contamination with gametes and artemia.

Prepare all buffers and reagents as described in the "Materials" section and refrigerate a clean dounce homogenizer in advance. Work quickly, keeping buffers and samples on ice unless otherwise specified. Use protein LoBind tubes and a refrigerated centrifuge with a swinging-rotor to minimize cell loss throughout the protocol.

Tissue homogenization

1 **Transfer** 2-4 adult *N.vectensis* polyps into protein LoBind tube.

> Note
>
> Use animals starved at least 3 days and spawned the day before to avoid any possible contamination with gametes and artemia.

2 **Wash** with ice-cold 1xPBS**.** Remove as much PBS as possible before adding ice-cold Lysis Buffer.

3 **Add** 500 µL **ice-cold TST Lysis buffer** to fresh tissue.

4 Transfer the polyps onto a clean slide placed on ice, and **mince the tissue** into small pieces **using a pre-chilled knife/razor blade.**

5 Using a p1000 tip, **transfer** minced tissue into a 2 mL **cold dounce homogenizer.**

6 **Add** up to 1 mL i**ce-cold TST Lysis buffer.**

7 **Gently homogenize** the tissue using the **dounce homogenizer with pestle A** (~15-18 strokes). Wait 2 min.

8 **Gently dounce homogenize with pestle B** (~5 strokes)**,** and then further dissociate the homogenate by **pipetting with a p1000 tip** until a homogenous suspension is obtained. Do not exceed **max 12 min total incubation in TST lysis buffer** (since it was added in Step 3).

> Note
>
> Keep the dounce homogenizer on ice at all times and avoid bubble formation. The required number of strokes will vary depending on the amount of minced tissue and the applied force; the aim is to obtain a homogenous suspension.

9 Add 1 volume of **cold ST-2%BSA.**

10 **Filter** cell suspension through a **70µm cell strainer** (pre-wet) into a cold 2 mL LoBind tube.

11 **Centrifuge** the sample at 800 x g for 5 min at 4C using a **swinging-bucket rotor** to minimize cell loss (set break 1).

12 **Remove carefully supernatant** (SN), leaving ~20uL.

13 Add **900µL PBS-2%BSA.** Do not mix; **incubate on ice 5min** to allow buffer exchange.

14 **Gently pipette mix** to resuspend pellet properly before fixing.

Note

If tissue chunks remain, filter the suspension again through 70µm strainer and adjust the volume to 900µL

15 **Mildly fix the suspension with 0.1% PFA** by adding:

	Volume Stock	Stock	Final Concentration
	100ul	1% PFA (fresh)	0.1% PFA

Incubate 5min at room temperature (RT) . Invert the tube 3-4 times during the incubation.

16 **Quench PFA fixation** by adding:

	Volume Stock	Stock	Final Concentration
	60ul	2.5M glycine	125mM
	60ul	1M Tris-HCl pH 8.0	50mM

	Volume Stock	Stock	Final Concentration
	80ul	PBS-10%BSA	1.7%

Mix gently, and **incubate 5min at 4 C**

17 **Centrifuge** the sample at 500xg for 5 min at 4 C. Remove SN

18 Add 1mL **PBS-2%BSA.** Do not mix; **incubate on ice 2min**

19 Gently flick the tube to **resuspend pellet**

20 **Centrifuge** the sample at 500xg for 5 min at 4 C. Remove SN, leaving ~50uL

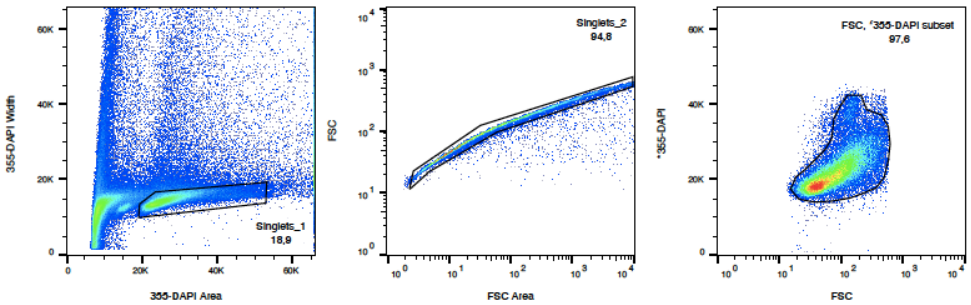
21 **Resuspend** the pellet in 0.5-1mL of **nuclei staining buffer for FACS** (10ug/mL DAPI in PBS-2%BSA).

22 Using a hemocytometer and a fluorescence microscope, **count and adjust the nuclei suspension** to ~2-3 × 10^6 nuclei/mL**. Proceed immediately** to purify single cells/nuclei from debris and aggregates by FACS

Nuclei purification by FACS

23 **FACS sort immediately single nuclei into PBS-2%BSA.**
Prior to sort, filter again tissue homogenate through 40µm cell strainer. Sort 1×10^6 single events into a pre-coated tube containing 0.5-1mL of PBS-2%BSA.

To purify single nuclei, we used a Influx Fluorescence-Activated Cell Sorter (FACS) equipped with a 100um nozzle to sort single nuclei (2n and 4n DNA content) at 12psi under cold conditions. Using the sorting strategy shown below and a flow rate of ~4000 events/sec, approximately 700×10^3 to 1×10^6 single events can be sorted in ~20min (purity mode).



Example of the gating strategy used to isolate single nuclei from debris and aggregates following a double gating of singlet discrimination (DAPI-A vs DAPI-W and FSC-A vs FSC-H). The last gate narrows the population of interest to single events containing 2n and 4n DNA content.

Nuclei permeabilization

24 **Centrifuge** the sample at 500xg for 10 min at 4 C. **Remove SN, leaving ~20uL**

25 **Gently resuspend the pellet** in 200uL of **0.1x OmniATAC Lysis buffer** by pipetting up and down 5 times. **Incubate** on ice **2min.**

26 Add 1mL **ice-cold ATAC Wash Buffer** and invert.

27 **Centrifuge** the sample at 500xg for 5 min at 4 C. **Remove SN** without disturbing nuclei pellet.

28 **Add** 100µL **1x Diluted Nuclei Buffer** and flick tube to mix.

29 **Centrifuge** nuclei at 500xg for 5 min at 4 C. **Remove SN, carefully leaving ~5uL behind**

30 **Gently resuspend** in ~15-20uL of **1x Diluted Nuclei Buffer** by pipetting up and down 5 times.

Note

The volume of 1× diluted nuclei buffer to add depends on the expected yield of nuclei and should be adjusted to obtain a working concentration suitable for the desired target number of nuclei for encapsulation on the 10x Chromium platform. Expect a recovering of ~15% of sorted events after filtering, but it has to be empirically determined by each user. The aim is to get 2500-6000 nuclei/µL

31 **Filter nuclei suspension** through 40µm Flowmi strainer.

32 **Count Nuclei Stock Concentration.**

33 **Prepare** at least 5ul of **nuclei suspension at a concentration needed to encapsulate the target nuclei of interest.** Accordingly to manufacturer's instructions (Next GEM, Chromium scATAC v1.1):

	Volume Nuclei Stock Concentration	1x Diluted Nuclei Buffer
	Y (ul) = Target Nuclei Recovery X 1.53 / Nuclei Stock Concentration	Z (ul)= 5ul - Y (ul)

Gently mix Y (ul) of Nuclei Stock Concentration and Z (ul) of 1x Diluted Nuclei Buffer.

34 Proceed with your scATAC-seq experiment **following manufacturer's instructions**.

Protocol references

1- Hand, C. \u0026 Uhlinger, K. R. The Culture, Sexual and Asexual Reproduction, and Growth of the Sea Anemone Nematostella vectensis. Biol Bull 182, 169 6176 (1992).
2- Drokhlyansky, E. et al. The Human and Mouse Enteric Nervous System at Single-Cell Resolution. Cell 182, 1606-1622.e23 (2020).
3- Ma, S. et al. Chromatin potential identified by shared single-cell profiling of RNA and chromatin. Cell 183, 1103 61116.e20 (2020).
4- Corces, M. R. et al. An improved ATAC-seq protocol reduces background and enables interrogation of frozen tissues. Nat Methods 14, 959 (2017).
5- De Rop, F. V et al. Hydrop enables droplet-based single-cell ATAC-seq and single-cell RNA-seq using dissolvable hydrogel beads. 11 (2022) doi:10.7554/eLife.