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# Gastrula_Nvectensis_scATAC-seq

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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 gastrula-stage embryos of the cnidarian *Nematostella vectensis* for scATAC-seq using 10x Chromium platform. Nuclei are directly isolated and permeabilized by mechanically dissociating gastrula embryos in hypotonic OmniATAC lysis buffer[1] supplemented with 70µM Pitstop2 to increase nucleus permeability to Tn5[2]. After washing, nuclei are visually inspected under the microscope, counted, and tagmented in bulk prior to single-nucleus encapsulation using 10x Chromium platform. scATAC-seq libraries are then prepared according to 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)
- 5M NaCl (Ambion #AM9760G)
- 1M MgCl2 (Sigma # M1028)
- BSA nuclease- and protease-free (Sigma #126609)
- DAPI (ThermoFisher #D1306)
- 10x PBS Ca/Mg- and nuclease-free (ThermoFisher #AM9624)
- Digitonin 20mg/mL (Promega #G9441)
- Pitstop2 (Abcam #120687).
- 20x Nuclei Buffer (10x genomics)
- OTHER: LoBind protein tubes, 2mL douncer homogenizer, 70µm cell strainer, hemocytometer, 40µm Flowmi strainer

**BUFFERS/STOCK SOLUTIONS**

- **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 |

- **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
- **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

-**PBS-1%BSA**. Prepare 10mL by mixing 1mL of 10%BSA-PBS in 9mL 1xPBS

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

-**1x OmniATAC lysis buffer supplemented with PitStop2**, 400uL:

| | |
|---|---|
| 320uL | ATAC-RSB |

4uL 10% NP-40 (final 0.1% v/v)
4uL 10% Tween20 (final 0.1% v/v)
4uL 1% Digitonin (final 0.01% v/v)
28uL PitStop2 1mM in PBS (70µM final)
40uL ATAC-RSB-10%BSA (final 1%)

- **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%)

- **1x Diluted Nuclei Buffer.** Prepared from 20x stock (10x genomics)

## Before start

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.

1 **Collect** 300 gastrula-stage embryos of *N.vectensis* into protein LoBind tube.

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

3 **Add** 300 µL **of ice-cold OmniATAC Lysis buffer** and transfer embryos into 2 mL **cold dounce homogenizer**

3.1 **Gently** homogenize the tissue using the dounce homogenizer with **pestle A** (~3-4strokes)

3.2 **Gently** homogenize the tissue using the dounce homogenizer with **pestle B** (~3-4strokes).

3.3 Further dissociate the homogenate by **pipetting with a p1000 tip** until a homogenous suspension is obtained.

Dissociate in **OmniATAC lysis buffer for 3min on ice**

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.

4 Add 1.7mL **ice-cold ATAC Wash Buffer**.

5 **Filter** the homogenate **through 40µm cell strainer** into 2mL protein LoBind tube.

6 **Centrifuge** nuclei suspension at 500xg for 5 min at 4 C using a **swinging-bucket rotor** to minimize cell loss (set break 1). **Remove SN** without disturbing nuclei pellet.

7 Add 1mL of ice-cold **PBS-1%BSA** and flick the tube to **resuspend the pellet**

8 **Centrifuge** nuclei at 500xg for 5 min at 4 C. **Remove SN,** carefully leaving ~10uL behind

9 **Gently ressuspend nuclei** in 300µL 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 (based on the number and developmental stage of embryos dissociated) 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 ~1.5 x $10^6$ nuclei out of 300 gastrula-stage embryos.

10 (OPTIONAL) **Filter nuclei suspension** through **40µm Flowmi strainer** if aggregates are present

11 Take an aliquot and **determine the Nuclei Stock Concentration** using DAPI counterstaining. Count nuclei with a hemocytometer under a fluorescence microscope.

12 **Prepare** at least 5ul (one reaction) 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.

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

## Protocol references

1- Corces, M. R. et al. An improved ATAC-seq protocol reduces background and enables interrogation of frozen tissues. Nat Methods 14, 959 (2017).
2- 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.