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ATAC-seq on nuclei from frozen, sliced, *Drosophila melanogaster* embryo halves V.2

PLOS One

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ABSTRACT

Protocol for performing ATAC-seq on nuclei isolated from *Drosophila melanogaster* stage 5 embryos that were flash frozen and then cut in half along the anterior-posterior midline. Data from this protocol are presented in the following paper: <https://www.biorxiv.org/content/early/2017/09/27/195073>.

This protocol is based adapted from the following sources: <https://www.nature.com/nmeth/journal/v10/n12/full/nmeth.2688.html> and <http://journals.plos.org/plosgenetics/article?>

MATERIALS

STEP MATERIALS

- ✂ Shandon™ Straight Point Teasing Needles, Straight Point, Wooden Handle, 5.5 in. (14.0cm) **Thermo Scientific Catalog #19010**
- ✂ Fisherbrand™ High Precision #10 Style Scalpel Blade **Fisher Scientific Catalog #12-000-162**
- ✂ Pellet pestles **Merck MilliporeSigma (Sigma-Aldrich) Catalog #Z359947**
- ✂ Nextera DNA library preparation kit 24 samples **Contributed by users Catalog #FC-121-1030**
- ✂ MinElute Reaction Cleanup Kit **Qiagen Catalog #28204**
- ✂ Corning® CoolRack CF15, Holds 15 Cryovial or FACS Tubes (Product #432049) **Corning Catalog #432049**
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SAFETY WARNINGS

- ⚠ Always wear proper PPE
- ⚠ Be careful to not get frostbite when working with dry ice. Double glove when handling the chilled scalpel.

Solutions

1 Lysis Buffer NO detergent

- 10mM Tris-Hcl, pH 7.4
- 10mM NaCl
- 3mM MgCl₂

Store at 4dC- make fresh weekly

Lysis Buffer Freezing Media

- 10ml Lysis Buffer No Detergent
- 1ml of 50% glycerol
- 1ul of bromoblue dye

Lysis Buffer + Spermine

- 10ml Lysis Buffer No Detergent
- 0.15mM spermine

Embryo Collection

- 2 Put several hundred adult OregonR *Drosophila melanogaster* (<http://flybase.org/reports/FBsn0000276.html>) into a fly cage at 25°C for three days. Feed flies with standard molasses plates a spread of yeast paste. Collect embryos for 2 hours and then let them age at 25°C for an hour and thirty minutes. This should yield embryos between 2:00 and 3:30 hours old.
 - 02:00:00 Embryo collection
 - 01:30:00 Aging
- 3 Bleach embryos for 3 minutes in 30%-50% bleach to remove the chorion.
 - 00:03:00 Bleaching
- 4 Rinse well with water. Pat dry on towel.
- 5 Rinse with 1xPBS + 0.5% Triton.
- 6 Brush embryos into an 1.5ml tube filled with 1xPBS + 0.5% Triton. Let embryos settle. Then use a pipette tip to transfer a drop of embryos to a slide.
- 7 Stage the embryos to the desired age. We usually go for Stage 5.

Freezing and Slicing embryos

- 8 Chill a cold block in the -80 for at least 30 minutes. Once it's cold, place in a box surrounded by dry ice to keep it cool while you slice

Corning® CoolRack CF15, Holds 15 Cryovial or FACS Tubes (Product #432049) **Corning Catalog #432049**

00:30:00 Freezing

Freezing and Slicing Embryos

- 9 Place staged embryos into ependorf tubes containing Lysis buffer freezing media.

- 10 Keep the slides, poker tool, and scalpel on dry ice to keep from melting.
✂ Shandon™ Straight Point Teasing Needles, Straight Point, Wooden Handle, 5.5 in. (14.0cm) **Thermo Scientific Catalog #19010**
✂ Fisherbrand™ High Precision #10 Style Scalpel Blade **Fisher Scientific Catalog #12-000-162**
- 11 Underneath a dissection scope, space out staged embryos on the glass slide such that they are evenly separated and can be cut easily with the blade. I affix a grid pattern to the bottom side of the glass slide to offer contrast to the white embryos.
- 12 Once the embryos are lined up on a slide, gently place the slide on dry ice for 2-5 minutes while you prepare the next slide. The embryos will turn from clear to completely white when frozen. I usually line up 10 embryos / slide and slice 5-6 slides at a time.
🕒 00:05:00 Placement on dry ice
- 13 Once embryos are frozen, immediately transfer the slide to a cold block that has been chilled. I surround the block with dry ice to keep it cool. Additionally, it is necessary to cool down the scalpel on dry ice before cutting. I leave the scalpel and 2 poker tools in dry ice while cutting to keep them cold. Be sure to wear gloves and keep the scalpel handle away from the dry ice to keep hands warm.
- 14 Cut embryos along the midline with the dry ice chilled blade or scalpel. Move each half to a 1.5ml tube filled with 50ul of ATAC Lysis buffer + Spermine with the chilled poker tool.
■ The embryos will thaw and dissolve as soon as they are transferred. To reduce contamination, we use 2 poker tools, one to handle anterior halves and one to handle posterior halves. Additionally, we wash pokers in 70% EtOH between slices
🧴 50 µL ATAC Lysis buffer + Spermine
- 15 Once your tube contains the desired number of embryo halves, move on to isolate the nuclei.

Embryo Lysing

- 16 Crush using a plastic pestle upwards of 20 times vigorously.
✂ Pellet pestles **Merck MilliporeSigma (Sigma-Aldrich) Catalog #Z359947**
- 17 After homogenization, rinse the pestle with 50ul of lysis buffer to make sure not to lose any material. This will bring the total volume up to 100ul.
🧴 50 µL Lysis buffer
- 18 Vortex slightly at low speed and spin down on a table top centrifuge.
- 19 Add 1ul of 10% IGEPAL CA-630 drop-wise to a final concentration of 0.1%. Mix well by flicking tube with a finger. **Let sit for about 10 minutes**
🧴 1 µL 10% IGEPAL CA-630
🕒 00:10:00 Wait
- 20 Spin in microcentrifuge at 800xg for 10 minutes at 4°C. Remove as much supernatant as possible to avoid nuclei loss.

00:10:00 Centrifugation

- 21 Resuspend nuclei in enough DNase and RNase free water for the transposition reaction (below).

Tagmentation

- 22 Make sure the cell pellet is set on ice.

To make the transposition reaction mix, combine the following:

Samples	20 halves-10 embryos
2xTDBuffer	25
Tn5 Enzyme	7.5
H ₂ O / DNA	17.5
Total	50

Nextera DNA library preparation kit 24 samples **Contributed by users** Catalog #FC-121-1030

- 23 Gently pipette to resuspend nuclei in the transposition reaction mix.

- 24 Incubate the transposition reaction at **37°C for 30 min**. Immediately following transposition, purify using a **Qiagen MinElute Kit**.

00:30:00 Tagmentation

MinElute Reaction Cleanup Kit **Qiagen Catalog #28204**

- 25 Purified DNA can be stored at -20°C

I quantify DNA at this point with the qubit.

PCR amplification

26 To amplify transposed DNA fragments, combine the following in a PCR tube:

- 10 µL Transposed DNA
- 5 ul 25uM Primer 1
- 5 ul 25uM Primer 2
- 25ul NebNext 2x Master Mix
- 5ul of PPC

50 µL Total

Run PCR as follows:

- (1) 72°C, 5 min
- (2) 98°C, 30 sec
- (3) 98°C, 10 sec
- (4) 63°C, 30 sec
- (5) 72°C, 1 min
- (6) Repeat steps 3-5, **5x**
- (7) Hold at 4°C

Optional - QPCR

27 You can stop at this point and run a small qPCR reaction to calculate the number of additional cycles to use to avoid overamplification.

Take out 5 ul of PCR reaction and run the qPCR reaction with the same conditions.

5 µL Transposed DNA
0.25 ul 25uM Primer 1
0.25 ul 25uM Primer 2
5ul NebNext 2x Master Mix
3.9 ul of H2O
0.09 100x Sybr Green I Dye

15 µL Total

Put in the QPCR machine and run:

1 cycle: 30 sec 98° C
20 cycles: 10 sec 98° C
 30 sec 63° C
 1 min 72° C.

To calculate the additional number of cycles needed, plot linear Rn (fluorescence) versus cycle and determine the cycle number that corresponds to one-third of the maximum fluorescent intensity.

28 Once you decide how many additional cycles to run, run the rest of the PCR :

- (2) 98°C, 30 sec
- (3) 98°C, 10 sec
- (4) **63°C**, 30 sec
- (5) 72°C, 1 min **For X cycles**
- (7) Hold at 4dC

Ampure (or Spri) bead Cleanup

29 Bring Beads to RT.

30 Add 1.25x Beads, Mix well, let stand for 5 minutes.

00:05:00 Wait

31 Put on a magnet, let stand for 2 minutes.

00:02:00 Magnet

32 Wash with 70% EtOH. (1/2)

33 Wash with 70% EtOH. (2/2)

34 Let dry for 10 minutes.

00:10:00 Drying

35 Resuspend in 20ul H₂O.

20 µL H₂O

36 Let sit for one minute, put on magnet, take of super.

00:01:00 Wait

Library Validation

37 To validate your libraries, quantify them with qubit HS DNA and evaluate them with the Bioanalyzer High Sensitivity DNA assay.

Sometimes they look like this:

Sometimes they look like this: I usually see this when I am working with smaller amounts of embryos. Below is from one embryo half. I am not 100% sure what this means (whether it's actually undertagged or what) but when I sequenced this sample the data was comparable to my other samples. If you have any ideas let me know! I usually try not to sequence these samples but they could be perfectly fine.