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

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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 **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 NaC1
- 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.

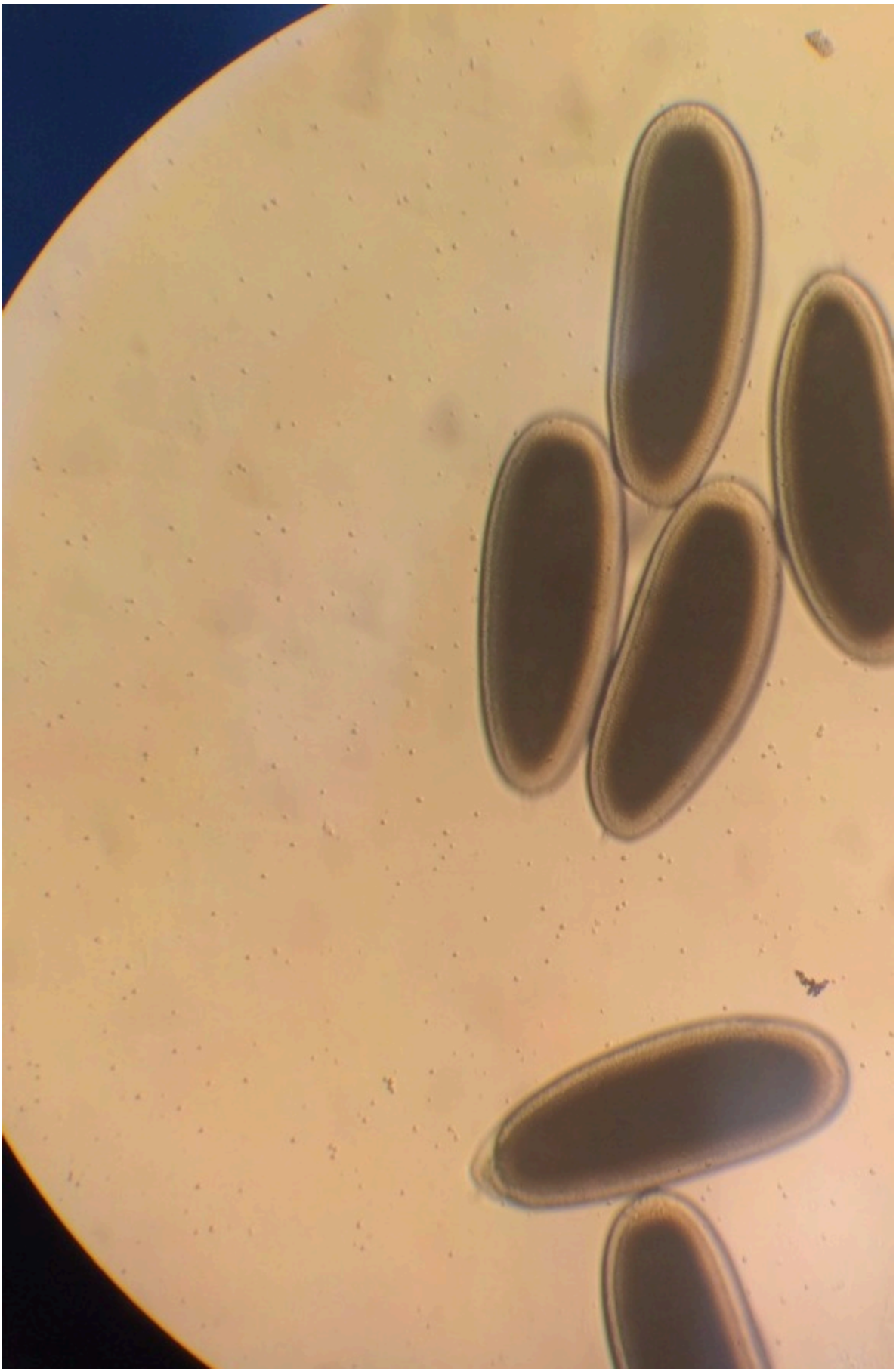
00:03:00 Bleaching

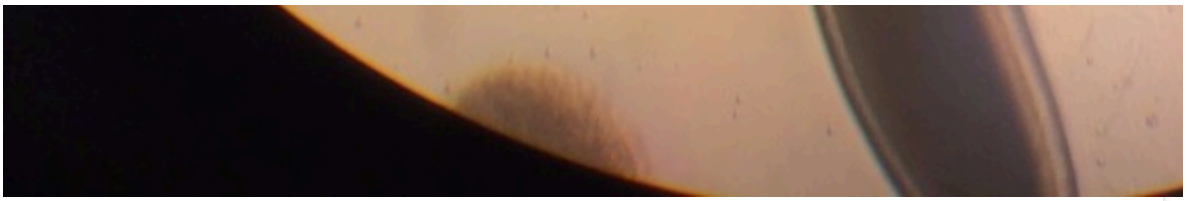
4 Rinse well with water. Pat dry on towel.

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 transfered. 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 4dC. Remove as much supernatant as possible to 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:

	Sample	20 halves- 10 embryos
	2x TD Buffer-	25

Tn5 Enzyme	7.5
H20/ DNA	17.5
Total	50

Nextera DNA library preparation kit 24 samples **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 H20
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 H_2O.

20 µL H2O

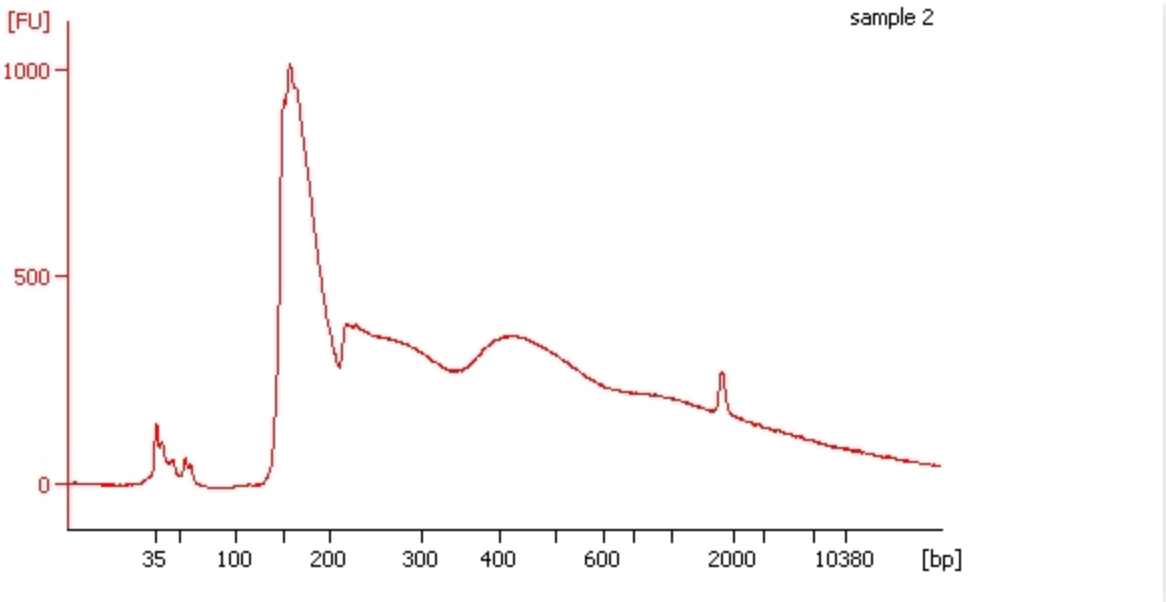
36 Let sit for one minute, put on magnet, take of super.

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 undertagmented or what) but when I sequenced this sample the data was comprable 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.