Jul 07, 2021

In vivo tissue-specific chromatin profiling in Drosophila

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 dx.doi.org/10.17504/protocols.io.buiqnudw

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ABSTRACT

Chromatin regulation plays an essential role in many nuclear processes, and genome-wide chromatin profiling approaches contribute to understanding how chromatin regulates cell homeostasis. Chromatin dysregulation lies in the heart of many human diseases, which most of them have a tissue-specific nature. Because of the physiological similarity of Drosophila and humans, tissue-specific studies can be performed using fruit flies. Here, we present an improved nuclear tagging approach that allows for efficient purification of cell-type specific nuclei from Drosophila increasing yield and stringency. Using this protocol, we purified photoreceptor neuron nuclei, and demonstrate the feasibility and high quality of chromatin accessibility profiling as well as profiling of histones and histone modifications, using Omni-ATAC and ChIP-seq, respectively. Last, we describe a modification to the nuclei purification protocol that allows for application of recently developed CUT&Tag and demonstrate that CUT&Tag outperforms traditional ChIP-seq, although protocol might require further optimization.

DOI

dx.doi.org/10.17504/protocols.io.buiqnudw

EXTERNAL LINK

https://www.biorxiv.org/content/10.1101/2021.03.23.436625v1

PROTOCOL CITATION

Vikki M. Weake, Juan P P Jauregui-Lozano 2021. In vivo tissue-specific chromatin profiling in Drosophila. **protocols.io** https://dx.doi.org/10.17504/protocols.io.buiqnudw

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

https://doi.org/10.1093/genetics/iyab079

CREATED

Apr 26, 2021

LAST MODIFIED

Jul 07, 2021

PROTOCOL INTEGER ID

49456

GUIDELINES

References:
Corces, M., Trevino, A., Hamilton, E.*et al.*An improved ATAC-seq protocol reduces background and enables interrogation of frozen tissues.*Nat Methods***14,**959–962 (2017). https://doi.org/10.1038/nmeth.4396

https://www.epicypher.com/products/epigenetics-reagents-and-assays/cutana-cut-and-tag-assays

MATERIALS TEXT

1X PBS (Phosphate-buffered saline) **Contributed by users**

Dynabeads™ Protein G for Immunoprecipitation **Thermo Fisher Catalog #10003D** In 4 steps

anti GFP antibody **Roche Catalog #11814460001** Step 5

Anti-Histone H3 antibody **Abcam Catalog #ab1791**

Anti-Histone H3 (tri methyl K4) antibody - ChIP grade **Abcam Catalog #Ab8580**

Anti-Histone H3 (tri methyl K36) antibody - ChIP grade **Abcam Catalog #Ab9050**

Illumina Tagment DNA TDE1 Enzyme and Buffer Kits **illumina Catalog #20034197**

IDT® for Illumina® DNA/RNA UD Indexes Set A Tagmentation (96 Indexes 96 Samples) **illumina Catalog #20027213**

CUTANA™ pAG-Tn5 for CUT&Tag **EpiCypher Catalog #15-1017** In 2 steps

Recipes

1

Homogenization/wash [WB] buffer
40 mM HEPES, pH 7.5
120 mM KCl
0.4% NP40 (IGEPAL)

Dilution buffer [cold]
40 mM HEPES, pH 7.5
120 mM KCl

Bead washing buffer [cold]
1X Phosphate Buffer Saline (PBS) buffer, pH 7.4
2.5 mM $MgCl_2$

Omni-ATAC
Omni-ATAC tagmentation mix
25 µL 2X buffer
2.5 µL Tn5
16.5 µL PBS
0.5 µL 1% digitonin
0.5 µL 10% Tween-20

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5 µL H_2O

ChIP-seq

A1 buffer

15 mM HEPES
15 mM NaCl
60 mM KCl
4 mM $MgCl_2$
0.5% Triton X-100

Nuclei Lysis Buffer

50 mM Tris
10 mM EDTA
1% SDS

X-ChIP dilution buffer

16.7 mM Tris-HCl, pH 8.0
167 mM NaCl
1% Triton X-100
1.2 mM EDTA

X-ChIP elution buffer

100 mM $NaHCO_3$
1% SDS

Low Salt Buffer

20 mM Tris-HCl, pH 8.0
150 mM NaCl
0.1% SDS
1% Triton X-100
2 mM EDTA

High Salt Buffer

20 mM Tris-HCl, pH 8.0
500 mM NaCl
0.1% SDS
1% Triton X-100
2 mM EDTA

LiCl wash buffer

10 mM Tris-HCl, pH 8.0
250 mM LiCl
0.1% Na-Deoxycholate
0.1% NP-40 or IGEPAL
1 mM EDTA

TE buffer

10 mM Tris-HCl, pH 8.0
1 mM EDTA

CUT&Tag

Wash 150 buffer

20 mM HEPES, pH 7.5
150 mM NaCl
0.5 mM Spermidine
1X Roche cOmplete™, Mini, EDTA-free protease inhibitor (1 tablet/10mL Wash150 buffer)
Store at 4C for up to 1 week

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Digitonin150 buffer
Wash buffer + 0.01% Digitonin
Prepare fresh each day and store at 4C

Antibody150 buffer
Digitonin buffer + 2 mM EDTA
Prepare fresh each day and store at 4C

Wash300 buffer
20 mM HEPES, pH 7.5
300 mM NaCl
0.5 mM Spermidine
1X Roche cOmplete^{TM}, Mini, EDTA-free Protease Inhibitor (1 tablet/10 mL Wash300 buffer)
Store at 4C for up to 1 week

Digitonin300 buffer
Wash 300 Buffer + 0.01% Digitonin
Prepare fresh each day and store at 4C

Tagmentation buffer
Wash buffer + 10 mM $MgCl_2$
Store at 4C for up to 1 week

TAPS buffer
10 mM TAPS, pH 8.5
0.2 mM EDTA
Store at RT for up to 6 months

SDS Release Buffer
10 mM TAPS, pH 8.5
0.1% SDS
Store at RT for up to 6 months

SDS Quench Buffer
0.67% Triton-X 100 in Molecular grade H_2O
Store at RT for up 6 months

Primers:
Nextera P1: AATGATACGGCGACCACCGAGA
Nextera P2: CAAGCAGAAGACGGCATACGA
Universal i5: AATGATACGGCGACCACCGAGATCTACACTCGTCGGCAGCGTCAGATGTG
Indexed i7-1: CAAGCAGAAGACGGCATACGAGATTCGCCTTAGTCTCGTGGGCTCGGAGATGT
Indexed i7-2: CAAGCAGAAGACGGCATACGAGATCTAGTACGGTCTCGTGGGCTCGGAGATGT
Indexed i7-3: CAAGCAGAAGACGGCATACGAGATTTCTGCCTGTCTCGTGGGCTCGGAGATGT
Indexed i7-4: CAAGCAGAAGACGGCATACGAGATGCTCAGGAGTCTCGTGGGCTCGGAGATGT

Drosophila stocks

2 Generate flies expressing GFP^{KASH} protein in the cell type of interest by crossing UAS-GFP^{KASH} or QUAS-GFP^{KASH} flies with the appropriate Gal4 or QF driver. Confirm expression patterns by microscopy. We typically generate recombinant flies expressing the driver and GFP^{KASH} where both transgenes are homozygous (two copies) because this is

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convenient for expanding the flies, and we have found that the IP efficiency improves with higher expression. However, we can also obtain nuclei from flies expressing only a single copy of the driver and GFP^{KASH}, even when combining these with other UAS-transgenes such as RNAi, overexpression.

Bead-antibody coupling 10m

3 ☒Dynabeads™ Protein G for Immunoprecipitation **Thermo**

Incubate 40 µL of **Fisher Catalog #10003D** in 1 mL

Bead washing buffer for 10 min at RT with constant rotation

4 Transfer tube to magnet and remove supernatant. *For this step, and all steps involving the magnet - invert the tube several times on the magnet to ensure all beads are bound and remove any remaining supernatant from inside the lid of the tube using a pipettor. We use a 1 mL pipettor to remove the supernatant at all bead/magnet steps.*

☒PureProteome™ Magnetic Stand **Millipore**

Sigma Catalog #LSKMAGS08

5 Resuspend beads in 1 mL **Bead washing buffer** and add 4 µg

☒anti GFP

antibody **Roche Catalog #11814460001**

6 Transfer tube to magnet and remove supernatant

7 Resuspend beads in 200 µL 0.1% **homogenization/wash buffer** (mix 3 parts **dilution buffer** and 1 part **homogenization buffer/wash** - final NP-40 concentration of 0.1%)

Homogenization

8 (Always keep homogenizer on ice)
(1 mL of homogenization buffer is good for 400 fly heads - from flies snap frozen in liquid nitrogen and stored at -80C)
(fresh samples can also be used i.e. partially dissecting tissues from larvae, whole embryos - but nuclei can be isolated successfully from frozen flies and this is our standard approach for neuronal cell types in the adult head)

Transfer fly samples to 1 mL dounce homogenizer that contains 1 volume of cold **homogenization/wash buffer**

9 Grind samples with 5 "loose" pestle strokes

10 Incubate samples on ice for 5 min

11 Repeat 5 "loose" pestle strokes and follow with 10 "tight" pestle strokes

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12 Filter homogenate using a Corning® 40µm Cell Strainer **Corning Catalog #431750** and add 3 volumes of **dilution buffer**

Nuclei - bead incubation 10m

13 Split 4 mL lysate into four microcentrifuge (1.5 mL) tubes

14 Add a 1/4 of resuspended bead-antibody complex into each tube

15 Incubate nuclei-bead-antibody solution for 30 min at 4C with constant rotation

16 Using a magnet, remove supernatant

17 Gently resuspend bead-bound nuclei with 1 mL **homogenization/wash buffer**

18 Incubate for 5 min at 4C with constant rotation

19 Using a magnet, remove supernatant

20 Gently resuspend bead-bound nuclei with 1 mL **homogenization/wash buffer** and transfer to new centrifuge tube

21 Incubate for 5 min at 4C with constant rotation

22 Repeat wash 1 more time (no need to switch tubes again). At this step, samples contain bead-bound nuclei and can be used for RNA-seq (see step 22), Omni-ATAC (see step 25), ChIP-seq (see step 39), or CUT&Tag (see step 63: note that different magnetic beads should be used for CUT&Tag in step 2).

RNA-seq

23 Resuspend beads in TRI Reagent **Zymo Research Catalog #R2050-1-50** and purify according to the manufacturers' instructions. We typically use Direct-Zol microprep kit, eluting in 15 µL, and quantify 2 µL of eluted RNA

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Qubit RNA HS Assay Kit **Thermo Fisher**

using **Scientific Catalog #Q32852**

24 Libraries for RNA-seq can be generated using Ovation® SoLo RNA-Seq Library Preparation Kit **Tecan Catalog #0502-32** . This kit allows RNA inputs : [10 pg- 10 ng]. This library kit has ribodepletion step incorporated into the protocol using Drosophila anyDeplete, and libraries are therefore total nuclear RNA depleted for rRNA (not mRNA). We also recommend to use a library kit that has an in-solution DNAse step as part of the initial protocol because in our hands, the gDNA removal in the Direct-Zol kit is not 100% efficient.

Omni-ATAC

25 Begin the Omni-ATAC protocol at this step using the bead-bound nuclei obtained in Step 22.

26 After third wash, resuspend nuclei in 500 µL **homogenization/wash buffer**

27 Quantify gDNA from 10% of nuclei suspension or count nuclei using hemocytometer. *We typically determine gDNA using* Quick-DNA Microprep Plus Kit **Zymo Research Catalog #D4074** *kit, and use this to determine the amount of nuclei suspension to use for Omni-ATAC. For comparisons between samples under different experimental conditions, the same amount of nuclei (DNA) should be used.*

28 Based on quantification, aliquot nuclei according to desired input amount. *We have successfully used 50 ng or 100 ng DNA equivalent for Omni-ATAC, but it is likely that much lower input DNA levels will also work well using this protocol.*

29 Using magnet, remove supernatant and resuspend nuclei in 50 µL of Omni-ATAC tagmentation mix

30 Perform Omni-ATAC as described in this publication: (Corces, 2017)

31 Incubate reaction for 30 minutes at 37C in a thermal shaker using 1000 RPM shaking speed.

32 Purify DNA using DNA Clean & Concentrator™-5 **Zymo Research Catalog #D4003** and elute in 15 µL elution buffer (from the Zymo kit).

33 PCR amplify Omni-ATAC libraries:

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☒NEBNext High-Fidelity 2X PCR Master Mix - 50 rxns **New England**

25 µL **Biolabs Catalog #M0541S**

15 µL purified DNA

☒IDT for Illumina Nextera DNA Unique Dual Indexes **Illumina,**

10 µL **Inc. Catalog #20027213**

34 Amplify for 5 cycles
72C 5min
98C 30 sec
Then, 5 cycles of:
98C 10 sec
63C 30 sec
72C 1 min

35 Place reaction on ice

36 Determine additional PCR cycles using qPCR:

qPCR mix	**1 rxn**
25 uM Nextera P1	0.25 µL
25 uM Nextera P2	0.25 µL
100X Syber Green I	0.09 µL
NEBnext 2X	5 µL
diH2O	4.4 µL

37 ☒Agencourt AmPure XP beads **Contributed by**

Purify DNA using **users Catalog #A63880** using double size selection (0.5-1X ratio)

38 Assess tagmentation patterns using
☒Capillary electrophoresis instrument (e.g. Agilent Tapestation 4200) **Contributed by users** Libraries can be directly sequenced after this step.

ChIP-seq

39 Begin the ChIP-seq protocol at this step using the bead-bound nuclei obtained in Step 22.
After third wash, use a magnet to remove supernatant.

40 Resuspend bead-bound nuclei in 1 mL **A1 buffer**

41 ☒Pierce™ 16% Formaldehyde (w/v) Methanol-free **Thermo Fisher**

Add **Scientific Catalog #28906** to a final concentration of 1%. *We use these small ampules for ChIP experiments and discard ~2 weeks after opening, storing at 4C.*

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42 Rotate for 2 min at RT

43 Add Glycine to a final concentration of 125 mM for quenching and rotate for 5 min at RT

44 Resuspend in 140 uL of **Nuclei Lysis Buffer**

45 Transfer to sonication tube (MicroTube (6x16mm), AFA fiber with Snap-Cap 520045)

46 Sonicate chromatin with E220 Covaris
Conditions: 10 min with 2% duty cycle 105W, 200 CPB

47 Transfer the sonicated lysate to an eppendorf tube using a magnet to discard beads

48 Add **X-ChIP dilution buffer** to make up to 1mL final volume.

49 Centrifuge supernatant 10min at 20,000 x g at 4C.

50 Transfer supernatant [soluble chromatin] to new centrifuge tube on ice

51 Take a 5% fraction (for input prep go to step 51.1) and flash-freeze remaining chromatin in liquid nitrogen or continue to step 52

51.1 Fill up to 200 µL with **X-ChIP elution buffer**

51.2 Add 2 µL of RNase A (10 mg/mL) **Thermo Fisher Scientific Catalog #EN0531** and incubate at 37C for 1 hour

Add 2 µL of

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51.3 Proteinase K Solution (20 mg/mL) **Thermo Fisher Scientific Catalog #AM2548** and incubate at 55C overnight. *It is important to do this incubation step at 55C (not higher temp).*

51.4 ChIP DNA Clean & Concentrator **Zymo Research Catalog #D5205** and Purify DNA using quantify 2 µL using Qubit 1X dsDNA HS Assay Kit **Thermo Fisher Scientific Catalog #Q33230**

52 Divide soluble chromatin based on number of antibodies to be used and fill up each tube to 1 mL with **X-ChIP dilution buffer.** We recommend using ~100ng equivalent of DNA (chromatin) per antibody for histone mark antibodies (*eg H3K4me3*), but lower amounts may be sufficient for bulk histone (*eg histone H3*). Higher amounts may be required depending on the epitope of interest.

53 Add 1 µg antibody of interest and incubate at 4C with constant rotation overnight

54 *Day 2*

55 Wash 25 µL of beads with 1 mL of X-ChIP dilution buffer to get rid of the slurry

56 Immuno-precipitate the antibody-chromatin complex with 25 µL G agarose beads (Santa Cruz) for 2 hours at 4C

57 Wash the beads with the following buffers for 5 min at RT with constant rotation: Low Salt Buffer, High Salt Buffer, LiCl Wash Buffer. Use 1 mL of each wash buffer, and remove supernatant using magnet as in other steps.

58 After LiCl wash, resuspend beads in 1 mL of TE buffer and transfer to new centrifuge tube (1.5 mL tube).

59 Incubate for 5 minutes at 4C

60 Using a magnet, remove supernatant and resuspend beads in 200 µL of X-ChIP Elution buffer

61 Extract the DNA from each ChIP sample obtained at step 60 using the same method as described for the input fraction (5%): steps 51.2 to 51.4 (RNAse, proteinaseK, purification).

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62 Use purified DNA for library construction. We use ☒Ovation® Ultralow V2 DNA-Seq Library Preparation Kit **Tecan Catalog # 0344NB-A01** and have found that 100 pg and 2 ng of DNA yield comparable libraries.

CUT&Tag

63 If nuclei will be used for CUT&Tag, perform the nuclear immuno-enrichment (starting at step 3) using ☒Dynabeads™ Pan Mouse IgG **Invitrogen - Thermo Fisher Catalog #11041** instead of ☒Dynabeads™ Protein G for Immunoprecipitation **Thermo Fisher Catalog #10003D** since Protein G coupled dynabeads might interfere with downstream steps in CUT&Tag.

64 After third wash, remove supernatant using a magnet and wash nuclei with 1 mL of cold **Antibody 150 buffer** three times

65 Using magnet, remove supernatant, resuspend bead-bound nuclei in 50 µL **Antibody 150 buffer** and transfer to PCR tube

66 Add 0.5 µg **Primary antibody** and gently pipette up and down to mix

67 Incubate for 1 hour at RT at 4C with constant rotation

68 Using magnet, remove supernatant, resuspend bead-bound nuclei in 50 µL cold **Digitonin 150 buffer**

69 Add 0.5 µg **Secondary antibody**

70 Incubate for 30 min at RT with constant rotation

71 Using a magnet, remove supernatant and add 200 µL cold **Digitonin 150 Buffer**

72 Repeat step 70 two times

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73 Remove from magnet, add 50 µL cold **Digitonin 300 buffer**

74 Add 2.5 µL CUTANA™ pAG-Tn5 for CUT&Tag **EpiCypher Catalog #15-1017** and pipette up and down to mix

75 Incubate samples for 1 hour at RT with constant rotation

76 Using a magnet, remove supernatant and add 200 µL cold **Digitonin 300 buffer.** Thoroughly resuspend by pipetting, return to magnet then pipet to remove supe

77 Repeat previous step for total of two washes

78 Remove from magnet, add 50 µL cold **Tagmentation Buffer**

79 Incubate for 1 hour at 37C in thermocycler

80 Using a magnet, remove supernatant and resuspend beads in 50 µL RT **TAPS Buffer**

81 Using a magnet, remove supernatant, add 5 uL RT **SDS Release Buffer** and vortex on max speed for 7 seconds. Quick spin to collect

82 Add 15 µL RT **SDS Quench Buffer** and vortex on max speed.

83 Add 2 µL each of Universal i5 and barcoded i7 primers (10 µM stocks)

84 Add 25 µL CUTANA™ High Fidelity 2X PCR Master Mix **EpiCypher Catalog #15-1018** and mix

85 Amplify in a thermocycler using the following conditons:
a. 58C - 5 min

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b. 72C - 5 min
c. 98C - 45 sec
d. 98C - 15 sec
e. 60C - 10 sec
f. Repeat d-e for a total of 14-21.
g. 72C - 1min
h. hold at 4C

We have found that 20 cycles yield optimal libraries when a H3K4me3 CUT&Tag reaction is started using nuclei corresponding to 100 ng gDNA

86 Clean CUT&Tab libraries using 1.3X am
☒Agencourt AmPure XP beads **Contributed by users Catalog #A63880**

87 Elute DNA in 15 µL
☒100ml TE Buffer [1X], pH 8.0, Low EDTA (Tris-EDTA; 10mM Tris base, 0.1mM EDTA) **G-Biosciences Catalog #786-150**
☒Qubit 1X dsDNA HS Assay Kit **Thermo Fisher Scientific Catalog #Q33230**
and quantify using

88 CUT&Tag libraries are ready for sequencing

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