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In vivo tissue-specific chromatin profiling in *Drosophila* V.3

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We use this protocol and it's working

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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 at the heart of many human diseases, most of which 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 (using Omni-ATAC) as well as profiling of histones and histone modifications using ChIP-seq, CUT&RUN, and CUT&Tag.

Materials

- 1X PBS (Phosphate-buffered saline)
- Dynabeads™ Protein G for Immunoprecipitation **Thermo Fisher Catalog #**10003D
- anti GFP antibody **Roche Catalog #**11814460001
- 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
- Ilumina Tagment DNA TDE1 Enzyme and Buffer Kits **Illumina, Inc. Catalog #**20034197
- IDT® for Illumina® DNA/RNA UD Indexes Set A Tagmentation (96 Indexes 96 Samples) **Illumina, Inc. Catalog #**20027213
- CUTANA™ pAG-Tn5 for CUT&Tag **EpiCypher Catalog #**15-1017

Protocol materials

- ChIP DNA Clean & Concentrator **Zymo Research Catalog #**D5205
- Qubit 1X dsDNA HS Assay Kit **Thermo Fisher Scientific Catalog #**Q33230
- Dynabeads™ Pan Mouse IgG **Invitrogen - Thermo Fisher Catalog #**11041
- Dynabeads™ Protein G for Immunoprecipitation **Thermo Fisher Catalog #**10003D
- Ovation® Ultralow V2 DNA-Seq Library Preparation Kit **Tecan Catalog #** 0344NB-A01
- PureProteome™ Magnetic Stand **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**LSKMAGS08
- TRI Reagent **Zymo Research Catalog #**R2050-1-50
- Qubit RNA HS Assay Kit **Thermo Fisher Scientific Catalog #**Q32852
- DNA Clean & Concentrator™-5 **Zymo Research Catalog #**D4003
- IDT for Illumina Nextera DNA Unique Dual Indexes **Illumina, Inc. Catalog #**20027213
- NEBNext High-Fidelity 2X PCR Master Mix - 50 rxns **New England Biolabs Catalog #**M0541S
- Capillary electrophoresis instrument (e.g. Agilent Tapestation 4200)
- Pierce™ 16% Formaldehyde (w/v) Methanol-free **Thermo Fisher Scientific Catalog #**28906
- pAG/MNase plasmid **addgene Catalog #**123461
- Quick-DNA Microprep Plus Kit **Zymo Research Catalog #**D4074
- Agencourt AmPure XP beads **Catalog #**A63880
- RNase A (10 mg/mL) **Thermo Fisher Scientific Catalog #**EN0531
- Proteinase K Solution (20 mg/mL) **Thermo Fisher Scientific Catalog #**AM2548
- CUTANA™ pAG-Tn5 for CUT&Tag **EpiCypher Catalog #**15-1017
- CUTANA™ High Fidelity 2X PCR Master Mix **EpiCypher Catalog #**15-1018
- 100ml TE Buffer [1X], pH 8.0, Low EDTA (Tris-EDTA; 10mM Tris base, 0.1mM EDTA) **G-Biosciences Catalog #**786-150
- Dynabeads™ Pan Mouse IgG **Invitrogen - Thermo Fisher Catalog #**11041
- anti GFP antibody **Roche Catalog #**11814460001
- cOmplete™ EDTA-free Protease Inhibitor Cocktail **Roche Catalog #**4693132001
- NEBNext® RNA Depletion Core Reagent Set with RNA Sample Purification Beads **New England Biolabs Catalog #**E7870L
- NEBNext® Ultra™ II Directional RNA Library Prep Kit for Illumina® **New England Biolabs Catalog #**E7760

Buffer preparation

1 **Homogenization/Wash 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 $MgCl_2$
- 0.02% Tween-20

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 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 or overexpression.

Bead-antibody coupling

30m

3 ***Note:*** *The following amounts of Dynabeads and antibody are for* ***400 fly heads****. These amounts can be scaled depending on input.*

Incubate 20 µL/reaction of

Dynabeads™ Pan Mouse IgG **Invitrogen - Thermo Fisher Catalog #**11041 in 1 mL

Bead Washing Buffer for 10 min at RT with constant rotation.

4 Transfer tube to magnet stand and remove supernatant.

- *For this step, and all steps involving the magnet - briefly spin tubes in a microcentrifuge to remove any liquid from the cap before placing tube on the magnet stand.*

- *We use a 1 mL pipettor to remove the supernatant at all bead/magnet steps.*
- *Wait at least 1 min to make sure all beads are cleared from solution before pipetting liquid out.*

PureProteome™ Magnetic Stand **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**LSKMAGS08

5 Resuspend beads in 500 µL **Bead Washing Buffer** and add 2 µg anti GFP antibody **Roche Catalog #**11814460001 .

6 Incubate at room temperature for 30 min with constant rotation.

7 Transfer tube to magnet and remove supernatant.

8 Resuspend beads in 100 µL **0.1% Homogenization/Wash Buffer** (mix 3 parts dilution buffer and 1 part homogenization/wash buffer for a final NP-40 concentration of 0.1%).

Note

The resuspension volume can be altered depending on how many samples are being processed. Aim for 50 µL/sample. e.g., if you are processing 4 samples for NIE, resuspend in 200 µL 0.1% homogenization/wash buffer.

Homogenization

10m

9

Note

Homogenization notes:
- *Always keep homogenizer on ice*
- *Use 3 mL of homogenization buffer for any number up to 400 fly heads - from flies snap frozen in liquid nitrogen and stored at -80°C*
- *Fresh samples can also be used e.g., 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*

Add 3 mL of **Homogenization/Wash Buffer** and 100 µL 25X cOmplete™ EDTA-free Protease Inhibitor Cocktail **Roche Catalog #**4693132001 to

a Dounce homogenizer. Keep on ice.

10 Transfer frozen flies into 15 mL conical tubes. Pre-chill the sieves, flies, and paint brush using liquid nitrogen.

11 Vortex conical tubes containing flies for 3 seconds, then place tubes back into liquid nitrogen. Repeat 4 more times to separate fly heads from bodies.

Keep sieves cold by pouring a small amount of liquid nitrogen over them between vortexing tubes.

12 Pour flies into pre-cooled top sieve and tap top sieve so fly heads fall into middle sieve. Remove top sieve and tap middle sieve to get rid of any smaller debris.

13 Transfer separated fly heads from middle sieve into 7 mL glass Dounce homogenizer using a paint brush chilled using liquid nitrogen.

14 Grind samples in Dounce homogenizer with 5 "loose" pestle strokes.
- *Homogenize slowly, lifting pestle all the way out to the wide bulb each time*
- *Twisting the pestle in the bottom may improve homogenization*

15 Incubate samples on ice for 1 min.

16 Grind samples with 5 "tight" pestle strokes.

17 Filter homogenate using 40 µm cell strainer or Miracloth and glass funnel into two microcentrifuge tubes.

Note

If using Miracloth:
- Cut ends off 1000 µL pipette tips (one per sample) using scissors sterilized with Ethanol - tips are cut so fly heads can be pipetted easily
- Put small glass funnel into microcentrifuge tube
- Cut a square of Miracloth (large enough to fit into funnel and extend slightly over the sides) and place into funnel
- Pipette half of homogenate through the Miracloth and funnel into one microcentrifuge tube using cut tip (head debris will stay in Miracloth, while nuclei will pass through)
- Transfer funnel and Miracloth into second microcentrifuge tube, and pipette rest of homogenate into tube

18 Centrifuge homogenate at 800 x g for 5 min at 4°C.

19 Carefully decant supernatant containing cell debris and resuspend the two pellets from each sample in 0.5 mL **0.1% homogenization/wash buffer** total, combining the two pellets into one microcentrifuge tube.

20 Pipette combined pellets up and down ~10 times, or until pellet is completely dispersed.

21 Add 20 µL 25X protease inhibitor to each tube.

Nuclei - bead incubation

1h 30m

22 Add 50 µL antibody-bound beads into each tube that has homogenate.

Or, split antibody-bound beads evenly between all tubes depending on the resuspension volume.

23 Incubate nuclei and antibody-bound beads for 60 min at 4°C with constant rotation.

24 Remove supernatant using a magnet.

25 Gently resuspend bead-bound nuclei with 1 mL **0.1% homogenization/wash buffer**.

26 Incubate for 5 min at 4°C with constant rotation.

27 Repeat wash steps 23-24 two more times.

If proceeding to CUT&RUN, use CUT&RUN Dig-wash Buffer on final wash (See Janssens & Henikoff protocol linked below in CUT&RUN section).

28 After final wash, samples contain bead-bound GFP-expressing nuclei and can be used subsequently for RNA-seq, Omni-ATAC, ChIP-seq, CUT&Tag, or CUT&RUN.

CUT&RUN

29 Our CUT&RUN protocol is based on the following protocol, proceeding from nuclei-bead incubation directly to Step 9: "Permeabilize cells and bind primary antibodies":

Citation

Derek Janssens, Steven Henikoff (2026)
. CUT&RUN: Targeted in situ genome-wide profiling with high efficiency for low cell numbers.
protocols.io.

https://protocols.io/view/cut-amp-run-targeted-in-situ-genome-wide-profiling-zcpf2vn
LINK

We make the following modifications to the linked protocol:

- We purify our own pAG/MNase enzyme (pAG/MNase plasmid **addgene Catalog #**123461) using the protocol outlined in the following paper:

Citation

Meers MP, Bryson TD, Henikoff JG, Henikoff S (2019)
. Improved CUT&RUN chromatin profiling tools.

https://doi.org/10.7554/eLife.46314 LINK

- **Step 11:** Use 150 µL antibody buffer.
- **Step 13:** Place microcentrifuge tubes containing samples into 50 mL conical tubes. Place conical tubes on a roller mixer overnight. This prevents beads from settling and ensures liquid stays in motion at this small volume.
- Skip "**Bind secondary antibody as required**" section.
- Follow "**Chromatin Digestion and Release Option 1: Standard CUT&RUN**"
- **Step 32:** Place samples on a metal cold block cooled to 0°C in an ice bath (a heater block from a dry bath works for this purpose).
- **Step 34:** Incubation time should be assayed for each target using a pilot experiment We have used the following incubation times:

	A	B	C
	Target	**Antibody**	**Incubation time (min)**
	GFP	Abcam, ab6556	5

	A	B	C
	H3K4me2/3	Abcam, ab8580	20
	H3K36me3	Abcam, ab9050	20
	H3K9me3	Abcam, ab176916	20
	H3K27me3	EpiCypher, 13-0055	20
	H3K4me1	Abcam, ab8895	30
	H3K4me2	Thermo Fisher, 710796	30
	H3K4me3	EpiCypher, 13-0041	30
	RNA Pol II CTD	Millipore Sigma, 050623	2

Antibody incubation time table.

- **Step 35:** Make master mix of stop buffer ahead of time that contains spike-in DNA for normalization.
- **Step 36:** Halfway through 30 min incubation, remove tubes from heat block and tap to resuspend beads, then place back on heat block.
- **Step 58:** Increasing original sample volume with water and scaling all steps to the increased volume can reduce sample loss when pipetting aqueous layer (if not using phase-lock tube).

30 For DNA library preparation, use the following protocol:

Citation

Nan Liu (2026)
. Library Prep for CUT&RUN with NEBNext® Ultra™ II DNA Library Prep Kit for Illumina® (E7645).
protocols.io.

https://protocols.io/view/library-prep-for-cut-amp-run-with-nebnext-ultra-ii-bagaibse
LINK

We make the following modification to the linked protocol:

- **Step 16**: Before proceeding with PCR amplification, take 1/5 of the reaction volume for qPCR to determine appropriate cycle number for each sample. Choose a cycle

number corresponding to 1/2 - 2/3 up the amplification curve within the exponential phase of the qPCR.

	A	B
	Component	**Volume (µL)**
	Adaptor Ligated Fragments	2.6
	NEBNext Ultra II Q5 Master Mix	3
	Index Primer/i7 Primer	0.2
	Universal PCR Primer/i5 Primer	0.2
	20X EvaGreen	0.3

1x qPCR reaction mix to determine PCR cycle number.

RNA-seq

31 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 using Qubit RNA HS Assay Kit **Thermo Fisher Scientific Catalog #**Q32852 .

32 Libraries can be generated using:

- rRNA depletion kit
  NEBNext® RNA Depletion Core Reagent Set with RNA Sample Purification Beads **New England Biolabs Catalog #**E7870L
- RNA library prep kit
  NEBNext® Ultra™ II Directional RNA Library Prep Kit for Illumina® **New England Biolabs Catalog #**E7760
- Custom ssDNA probes for rRNA depletion from IDT

This kit allows RNA input: [10ng-1ug]. Our custom ribodepletion has reduced Drosophila melanogaster rRNA reads to less than 10% in the raw data. Probe sequences are available upon request. Additionally, we incorporated a qPCR step using 1/5 of the adaptor-ligated cDNA to optimize the cycle number required for the final amplification, see step 30 for details.

Omni-ATAC

33 Reagents

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
- 5 µL H_2O

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

35 After third wash, resuspend nuclei in 500 µL **Homogenization/Wash Buffer.**

36 Quantify gDNA from 10% of nuclei suspension or count nuclei using hemocytometer.

Note

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.*

37 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.*

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

39 Perform Omni-ATAC as described in this publication:

Citation

Corces MR, Trevino AE, Hamilton EG, Greenside PG, Sinnott-Armstrong NA, Vesuna S, Satpathy AT, Rubin AJ, Montine KS, Wu B, Kathiria A, Cho SW, Mumbach MR, Carter AC, Kasowski M, Orloff LA, Risca VI, Kundaje A, Khavari PA, Montine TJ, Greenleaf WJ, Chang HY
(2017)
. An improved ATAC-seq protocol reduces background and enables interrogation of frozen tissues.

https://doi.org/10.1038/nmeth.4396 LINK

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

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

42 PCR amplify Omni-ATAC libraries:

25 µL
NEBNext High-Fidelity 2X PCR Master Mix - 50 rxns **New England Biolabs Catalog #**M0541S
15 µL purified DNA
10 µL
IDT for Illumina Nextera DNA Unique Dual Indexes **Illumina, Inc. Catalog #**20027213

43 Amplify for 5 cycles.

	A	B	C
	Temperature	**Time**	
	72°C	5 min	
	98°C	30 sec	
	98°C	10 sec	5 cycles

	A	B	C
	63°C	30 sec	
	72°C	1 min	

Thermocycler conditions.

44 Place reaction on ice.

45 Determine additional PCR cycles using qPCR:

	A	B
	Component	**Volume (µL)**
	25 uM Nextera P1	0.25
	25 uM Nextera P2	0.25
	100X Syber Green I	0.09
	NEBnext 2X	5
	diH2O	4.4

1x qPCR reaction mix.

46 Purify DNA using Agencourt AmPure XP beads **Catalog #**A63880 using double size selection (0.5 - 1X ratio).

47 Assess tagmentation patterns using Capillary electrophoresis instrument (e.g. Agilent Tapestation 4200)
Libraries can be directly sequenced after this step.

ChIP-seq

48 Reagents

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

49 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.

50 Resuspend bead-bound nuclei in 1 mL **A1 Buffer**.

51 Add
Pierce™ 16% Formaldehyde (w/v) Methanol-free **Thermo Fisher 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.*

52 Rotate for 2 min at RT.

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

54 Resuspend in 140 µL of **Nuclei Lysis Buffer**.

55 Transfer to sonication tube (MicroTube (6×16mm), AFA fiber with Snap-Cap 520045).

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

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

58 Add **X-ChIP Dilution Buffer** to make up to 1mL final volume.

59 Centrifuge supernatant 10min at 20,000 x g at 4ºC.

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

61 Take a 5% fraction (for input prep go to step 61.1) and flash-freeze remaining chromatin in liquid nitrogen or continue to step 62.

61.1 Fill up to 200 µL with **X-ChIP Elution Buffer**

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

61.3 Add 2 µL of 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 55ºC (not higher temp).*

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

62 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.

63 Add 1 µg antibody of interest and incubate at 4ºC with constant rotation overnight.

64 *Day 2*

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

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

67 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.

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

69 Incubate for 5 minutes at 4ºC.

70 Using a magnet, remove supernatant and resuspend beads in 200 µL of **X-ChIP Elution Buffer**.

71 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, Proteinase K, purification).

72 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

73 Reagents

Wash 150 Buffer
- 20 mM HEPES, pH 7.5
- 150 mM NaCl
- 0.5 mM Spermidine
- 1X Roche cOmplete^{TM}, Mini, EDTA-free protease inhibitor (1 tablet/10mL Wash150 buffer)

Store at 4°C for up to 1 week

Digitonin150 Buffer
- Wash buffer + 0.01% Digitonin

Prepare fresh each day and store at 4°C

Antibody150 Buffer
- Digitonin buffer + 2 mM EDTA

Prepare fresh each day and store at 4°C

Wash300 Buffer
- 20 mM HEPES, pH 7.5

- 300 mM NaCl
- 0.5 mM Spermidine
- 1X Roche cOmplete™, Mini, EDTA-free Protease Inhibitor (1 tablet/10 mL Wash300 buffer)

Store at 4°C for up to 1 week

Digitonin300 Buffer

- Wash 300 Buffer + 0.01% Digitonin

Prepare fresh each day and store at 4°C

Tagmentation Buffer

- Wash buffer + 10 mM $MgCl_2$

Store at 4°C 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

74 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.

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

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

77 Add 0.5 µg **Primary Antibody** and gently pipette up and down to mix.

78 Incubate for 1 hour at RT at 4C with constant rotation.

79 Using magnet, remove supernatant, resuspend bead-bound nuclei in 50 µL cold **Digitonin 150 Buffer**.

80 Add 0.5 µg **secondary antibody**.

81 Incubate for 30 min at RT with constant rotation.

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

83 Repeat step 70 two times

84 Remove from magnet, add 50 µL cold **Digitonin 300 Buffer**.

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

86 Incubate samples for 1 hour at RT with constant rotation.

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

88 Repeat previous step for total of two washes.

89 Remove from magnet, add 50 µL cold **Tagmentation Buffer**.

90 Incubate for 1 hour at 37°C in thermocycler.

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

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

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

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

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

96 Amplify in a thermocycler using the following conditions:

	A	B	C
	Temperature	**Time**	
	58°C	5 min	
	72°C	5 min	
	98°C	45 sec	
	98°C	*15 sec*	*Repeat 14 - 21 cycles*
	60°C	*10 sec*	
	72°C	1 min	
	4°C	hold	

Thermocycler conditions.

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

97 Clean CUT&Tab libraries using 1.3X
Agencourt AmPure XP beads **Catalog #**A63880 .

98 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

and quantify using

Qubit 1X dsDNA HS Assay Kit **Thermo Fisher Scientific Catalog #**Q33230 .

99 CUT&Tag libraries are ready for sequencing.

Citations

Step 29

Derek Janssens, Steven Henikoff. CUT&RUN: Targeted in situ genome-wide profiling with high efficiency for low cell numbers
https://protocols.io/view/cut-amp-run-targeted-in-situ-genome-wide-profiling-zcpf2vn

Step 29

Meers MP, Bryson TD, Henikoff JG, Henikoff S. Improved CUT&RUN chromatin profiling tools.
https://doi.org/10.7554/eLife.46314

Step 30

Nan Liu. Library Prep for CUT&RUN with NEBNext® Ultra™ II DNA Library Prep Kit for Illumina® (E7645)
https://protocols.io/view/library-prep-for-cut-amp-run-with-nebnext-ultra-ii-bagaibse

Step 39

Corces MR, Trevino AE, Hamilton EG, Greenside PG, Sinnott-Armstrong NA, Vesuna S, Satpathy AT, Rubin AJ, Montine KS, Wu B, Kathiria A, Cho SW, Mumbach MR, Carter AC, Kasowski M, Orloff LA, Risca VI, Kundaje A, Khavari PA, Montine TJ, Greenleaf WJ, Chang HY. An improved ATAC-seq protocol reduces background and enables interrogation of frozen tissues.
https://doi.org/10.1038/nmeth.4396