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In vivo tissue-specific chromatin profiling in Drosophila V.2

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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 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.

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

- 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

- Ilumina Tagment DNA TDE1 Enzyme and Buffer Kits **Illumina, Inc.** **Catalog #**20034197
- CUTANA™ pAG-Tn5 for CUT&Tag **EpiCypher** **Catalog #**15-1017
- 1X PBS (Phosphate-buffered saline)
- Anti-Histone H3 antibody **Abcam** **Catalog #**ab1791
- Anti-Histone H3 (tri methyl K4) antibody - ChIP grade **Abcam** **Catalog #**Ab8580
- IDT® for Illumina® DNA/RNA UD Indexes Set A Tagmentation (96 Indexes 96 Samples) **Illumina, Inc.** **Catalog #**20027213
- Dynabeads™ Protein G for Immunoprecipitation **Thermo Fisher** **Catalog #**10003D
- anti GFP antibody **Roche** **Catalog #**11814460001
- Anti-Histone H3 (tri methyl K36) antibody - ChIP grade **Abcam** **Catalog #**Ab9050
- TRI Reagent **Zymo Research** **Catalog #**R2050-1-50
- Qubit RNA HS Assay Kit **Thermo Fisher Scientific** **Catalog #**Q32852
- Quick-DNA Microprep Plus Kit **Zymo Research** **Catalog #**D4074
- DNA Clean & Concentrator™-5 **Zymo Research** **Catalog #**D4003
- NEBNext High-Fidelity 2X PCR Master Mix - 50 rxns **New England Biolabs** **Catalog #**M0541S
- IDT for Illumina Nextera DNA Unique Dual Indexes **Illumina, Inc.** **Catalog #**20027213
- Agencourt AmPure XP beads **Catalog #**A63880
- Capillary electrophoresis instrument (e.g. Agilent Tapestation 4200)
- Ovation® SoLo RNA-Seq Library Preparation Kit **Tecan** **Catalog #**0502-32
- Pierce™ 16% Formaldehyde (w/v) Methanol-free **Thermo Fisher Scientific** **Catalog #**28906
- RNase A (10 mg/mL) **Thermo Fisher Scientific** **Catalog #**EN0531
- Proteinase K Solution (20 mg/mL) **Thermo Fisher Scientific** **Catalog #**AM2548
- 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
- CUTANA™ pAG-Tn5 for CUT&Tag **EpiCypher** **Catalog #**15-1017
- CUTANA™ High Fidelity 2X PCR Master Mix **EpiCypher** **Catalog #**15-1018
- Agencourt AmPure XP beads **Catalog #**A63880

- 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
- Ovation® Ultralow V2 DNA-Seq Library Preparation Kit **Tecan** **Catalog #** 0344NB-A01
- anti GFP antibody **Roche** **Catalog #**11814460001
- PureProteome™ Magnetic Stand **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**LSKMAGS08
- Dynabeads™ Pan Mouse IgG **Invitrogen - Thermo Fisher** **Catalog #**11041
- cOmplete™ EDTA-free Protease Inhibitor Cocktail **Roche** **Catalog #**4693132001

Troubleshooting

Recipes

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

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
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^{TM}, Mini, EDTA-free protease inhibitor (1 tablet/10mL Wash150 buffer)
Store at 4C for up to 1 week

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 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 1 mL **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%).

- *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 ***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.

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.

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.

RNA-seq

29 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

30 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

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

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

33 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.*

34 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.*

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

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

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

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

39 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

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

41 Place reaction on ice

42 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

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

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

ChIP-seq

45 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.

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

47 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.*

48 Rotate for 2 min at RT

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

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

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

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

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

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

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

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

57 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

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

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

57.3 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 55C (not higher temp).*

57.4 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

58 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.

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

60 *Day 2*

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

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

63 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.

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

65 Incubate for 5 minutes at 4C

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

67 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).

68 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

69 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.

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

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

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

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

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

75 Add 0.5 µg **Secondary antibody**

76 Incubate for 30 min at RT with constant rotation

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

78 Repeat step 70 two times

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

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

81 Incubate samples for 1 hour at RT with constant rotation

82 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

83 Repeat previous step for total of two washes

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

85 Incubate for 1 hour at 37C in thermocycler

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

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

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

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

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

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

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

93 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

94 CUT&Tag libraries are ready for sequencing