High-Resolution Chromatin Profiling Using CUT&RUN

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Determining the genomic location of DNA-binding proteins is essential to understanding their function. Cleavage Under Targets and Release Using Nuclease (CUT&RUN) is a powerful method for mapping protein-DNA interactions at high resolution. In CUT&RUN, a recombinant protein A–micrococcal nuclease (pA-MN) fusion is recruited by an antibody targeting the chromatin protein of interest; this can be done with either uncrosslinked or formaldehyde-crosslinked cells. DNA fragments near sites of antibody binding are released from the insoluble bulk chromatin through endonucleolytic cleavage and used to build barcoded DNA-sequencing libraries that can be sequenced in pools of at least 30. Therefore, CUT&RUN provides an alternative to ChIP-seq approaches for mapping chromatin proteins, which typically have relatively high signal-to-noise ratios, while using fewer cells and at a lower cost. Here, we describe the methods for performing CUT&RUN, generating DNA-sequencing libraries, and analyzing the resulting datasets. © 2019 by John Wiley & Sons, Inc.

Keywords: chromatin • CUT&RUN • protein A–micrococcal nuclease • transcription factor

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INTRODUCTION

Genome-wide profiling of DNA-binding proteins is widely used to determine their targets and functions. Chromatin immunoprecipitation (ChIP) techniques (Raha, Hong, & Snyder, 2010) have been used to map the locations of chromatin-bound proteins for over three decades (Gilmour & Lis, 1984; Gilmour & Lis, 1985; Johnson, Mortazavi, Myers, & Wold, 2007). Traditional ChIP protocols involve formaldehyde crosslinking, to fix protein-DNA interactions, followed by nonspecific shearing of the crosslinked chromatin by sonication. The protein of interest is then immunoprecipitated with a specific antibody coupled to a magnetic or agarose bead via protein A, protein G, or a secondary antibody. Cleavage Under Targets and Release Using Nuclease (CUT&RUN) is a genome-wide derivative of chromatin immunocleavage (ChIC; Schmid, Durussel, & Laemmli, 2004) developed by Steven Henikoff and colleagues that maps native protein-DNA interactions at high resolution (Skene & Henikoff, 2017). The CUT&RUN method is diagrammed in Figure 1. Much as in ChIP, chromatin-bound proteins are identified using a primary antibody directed against the protein of interest. However, CUT&RUN does not require crosslinking or sonication (although crosslinking can be used; Skene, Henikoff, &

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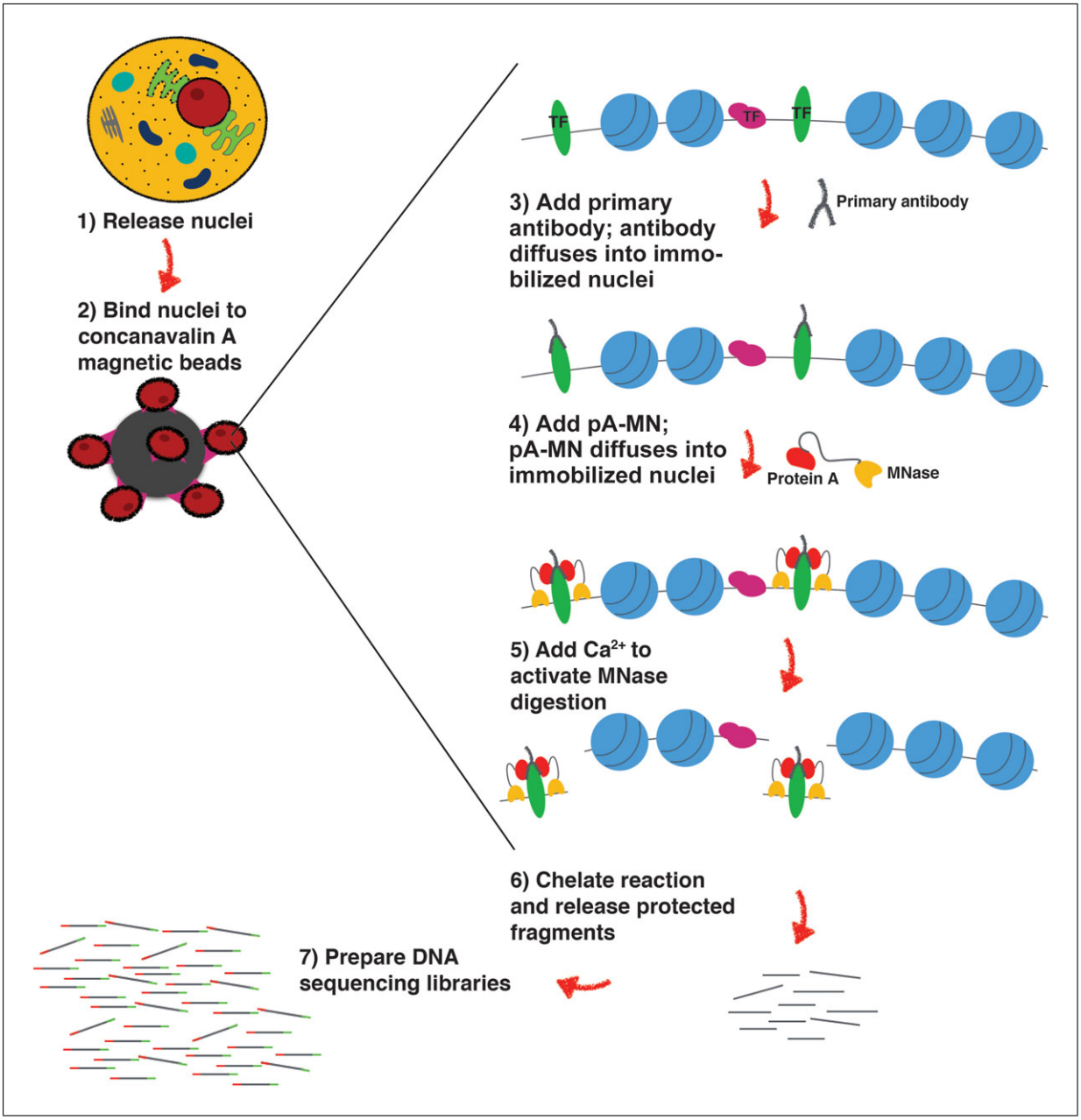

Figure 1 **Cartoon of workflow.** Shown is a diagram of the CUT&RUN approach.

Henikoff, 2018). Rather, CUT&RUN utilizes a recombinant protein A–micrococcal nuclease (pA-MN) fusion to specifically digest DNA fragments surrounding the protein of interest. DNA fragments underlying the specific chromatin-bound protein of interest are released from the bulk chromatin with high specificity due to the recruitment of pA-MN to the genomic locations of chromatin proteins through protein A–antibody interactions. Because the nonspecific association of pA-MN with chromatin is relatively low after appropriate washing, the background signal is often lower than those observed using ChIP-seq. Low background is advantageous as it leads to increased enrichment and a decreased requirement for high read coverage to map chromatin proteins genome-wide. CUT&RUN has been used to profile both histone proteins and transcription factors (TFs) from fewer cells than are required for ChIP-seq (Skene & Henikoff, 2017; Skene et al., 2018).

Here we include all the information necessary for performing CUT&RUN with mammalian cells. We describe our implementation of the original CUT&RUN protocol (Skene & Henikoff, 2017), which has been used extensively in our laboratories (Hainer, Boskovic, Rando, & Fazzio, 2018). However, alternative procedures in which cells are permeabilized with digitonin rather than lysed have also been reported to yield excellent results (Janssens et al., 2018; Skene et al., 2018). Basic Protocol 1 details the steps for performing CUT&RUN from mouse embryonic stem (mES) cells. Alternate Protocol 1 describes a variant including an additional step with a secondary antibody for use in detecting proteins that are not bound well by protein A. Basic Protocol 2 focuses on building libraries for paired-end Illumina sequencing. Basic Protocol 3 provides steps for analyzing the resulting datasets.

CUT&RUN OF TRANSCRIPTION FACTORS IN MAMMALIAN CELLS

BASIC PROTOCOL 1

CUT&RUN is a useful and versatile technique to map the occupancy of DNA binding proteins on chromatin. The following section describes the protocol for CUT&RUN from mES cells. This protocol is written for 500,000 E14 mES cells (RRID:CVCL_C320) but is also amenable to the use of other cell types or lower cell numbers (see step 9).

Materials

Mammalian cells in culture
Phosphate-buffered saline (PBS; e.g., Corning, cat. no. 21031CV), ice cold
Nuclear extraction (NE) buffer (see recipe)
Concanavalin A beads (Polysciences, cat. no. 86057)
Binding buffer (see recipe)
Blocking buffer (see recipe)
Wash buffer (see recipe)
Primary antibody against protein of interest
Recombinant pA-MN, expressed from Addgene plasmid no. 86973 and purified as described by Schmid et al. (2004)
100 mM $CaCl_2$ (Moore, 1996)
2× STOP buffer (see recipe; be sure to add RNase A, glycogen, and spike-in heterologous DNA fresh before use in step 56)
10% (w/v) SDS (see recipe)
20 mg/ml proteinase K (Bioline, cat. no. 37085)
Tris-buffered 25:24:1 (v/v/v) phenol/chloroform/isoamyl alcohol (PCI; e.g., Fisher, cat. no. BP1752I400)
Chloroform (e.g., Fisher, cat. no. C298500)
20 mg/ml glycogen (e.g., VWR, cat. no. 9005792)
100% ethanol
0.1× TE buffer (Moore, 1996)

Cell counter (Bio-Rad TC20, cat. no. 1450102)
Refrigerated centrifuge for 15-ml conical tubes (e.g., Eppendorf, cat. no. 2231000382)
Vortex mixer (e.g., Fisher, cat. no. 02215414)
DynaMag-2 magnetic stand (Life Technologies, cat. no. 12321D)
Refrigerated (4°C) rotator (e.g., VWR, cat. no. 10136084)
Refrigerated microcentrifuge (e.g., Eppendorf, cat. no. 5404000537)
Thermomixer (Eppendorf, cat. no. 2231000574)
Phase-lock tubes (Quanta Bio, cat. no. 2302830)

IMPORTANT NOTE: Chill all buffers on ice. Work efficiently and keep tubes on ice unless otherwise indicated.

Table 1 Examples of Concanavalin A Bead Slurry/Cell Number Ratios

Number of cells	Amount of Concanavalin A bead slurry
500,000	150 µl
50,000	100 µl
5,000	75 µl

Cell harvesting and lysis

1. Harvest cells and count their density using a cell counter. Add an appropriate number of cells to a 15-ml conical tube. The following protocol is written for 500,000 E14 mES cells, but this number can be reduced as necessary.

   Our lab routinely uses a Bio-Rad TC20 cell counter according to the manufacturers' instructions. Other methods of cell counting (e.g., hemocytometer; Phelan & May, 2017) are also fine.

2. Centrifuge the cells 5 min at 600 × *g*, 4°C.
3. Discard supernatant and resuspend cells in 1 ml ice-cold PBS by gently pipetting the cells. Transfer to a 1.5-ml microcentrifuge tube.
4. Centrifuge the cells 5 min at 600 × *g*, 4°C.
5. Discard supernatant and resuspend cells in 1 ml ice-cold NE buffer by gently pipetting the cells without introducing bubbles.
6. Centrifuge the sample 5 min at 600 × *g*, 4°C.
7. Discard supernatant and resuspend cells in 600 µl ice-cold NE buffer by gently pipetting the sample without introducing bubbles.

Preparation of concanavalin A beads

Beads can be prepared during the waiting periods in the previous section of the protocol.

8. Resuspend concanavalin A beads with gentle vortexing and/or pipetting.
9. Transfer 150 µl concanavalin A bead slurry to a 1.5-ml microcentrifuge tube containing 850 µl ice-cold binding buffer.

   The amount of bead slurry needed depends upon the number of cells. See Table 1 for example ratios of bead slurry volumes to cell numbers.

10. Place the microcentrifuge tube containing concanavalin A beads on a magnetic stand for at least 2 min, until the solution is clear.

    For this and all bead separation steps, confirm the solution is completely clear to avoid loss of beads. After beads accumulate at the magnet-proximal side of the tube, one can invert the tube to capture any beads that may be on the lid and incubate further to allow collection of the remaining beads.

    See Video 1 for an example of separation using a magnetic stand.

11. After the solution has completely cleared, remove and discard the supernatant without disturbing the beads.
12. Remove microcentrifuge tube from magnetic stand and resuspend the concanavalin A beads in 1 ml ice-cold binding buffer by gently pipetting the sample.
13. Place microcentrifuge tube on magnetic stand for ~2 min.

14. After the solution has completely cleared, remove and discard supernatant without disturbing beads.

15. Remove microcentrifuge tube from magnetic stand and resuspend beads in 1 ml ice-cold binding buffer by gently pipetting the sample.

16. After the solution has completely cleared, remove and discard supernatant without disturbing beads.

17. Remove microcentrifuge tube from magnetic stand and resuspend beads in 300 µl ice-cold binding buffer.

Binding of nuclei to magnetic beads

18. Take the nuclei that were previously resuspended in 600 µl NE buffer (in step 7), and while gently vortexing them, slowly add the 300 µl of washed and resuspended concanavalin A beads (from step 17). During vortexing and bead addition, pipet up and down to assist in complete mixing.

    For this and subsequent steps (such as addition of primary antibody and pA-MN), gentle vortexing is crucial. Vortex only vigorously enough to gently mix the beads, and be sure the beads mix completely. (Mixing is crucial to evenly distribute beads on nuclei, but harsh vortexing can damage nuclei, potentially leading to higher background or otherwise poor performance.) Set the vortex mixer to setting ~3. See Video 2 for an example of gentle vortexing during sample addition.

19. Incubate the combined nuclei and beads on a rotating platform for 10 min at 4°C.

Blocking of beads

20. Place microcentrifuge tube containing bead-bound nuclei on a magnetic stand for ~5 min.

    The amount of time required for bead separation increases once the nuclei are bound. This helps prevent loss of nuclei.

21. After the solution has completely cleared, remove and discard supernatant without disturbing the beads.

22. Remove microcentrifuge tube from magnetic stand and resuspend beads in 1 ml blocking buffer by gently pipetting the sample.

    Gentle pipetting is preferable to vortexing at this step. Take care to minimize the introduction of bubbles during this action.

23. Incubate the resuspended beads 5 min at room temperature.

Primary antibody incubation

24. Place microcentrifuge tube containing the bead-bound nuclei on a magnetic stand for ~5 min.

25. After the solution has completely cleared, remove and discard supernatant without disturbing beads.

26. Remove microcentrifuge tube from magnetic stand and resuspend beads in 1 ml wash buffer with gentle pipetting.

27. Place microcentrifuge tube containing bead-bound nuclei on magnetic stand for ~5 min.

28. After the solution has completely cleared, remove and discard supernatant without disturbing beads.

29. Remove microcentrifuge tube from magnetic stand and resuspend beads in 250 µl wash buffer with gentle pipetting.

30. Dilute the primary antibody recognizing the protein of interest in 250 µl wash buffer.

    The amount of primary antibody used depends on the efficiency of the antibody. A titration of antibody may be necessary (see Critical Parameters). Typically, a dilution of 1:100 final (5 µl total antibody per 500 µl final binding volume) works well. A master mix of primary antibody should be made when working with more than one sample.

31. While gently vortexing the bead-bound nuclei, slowly add the mixture of 250 µl wash buffer + primary antibody to each sample.

    A critical control that should be included in all CUT&RUN experiments is the inclusion of a no-primary-antibody or nonspecific IgG control. This control sample is treated identically to the experimental sample, except that 250 µl wash buffer alone (no antibody) or containing nonspecific IgG should be added to this sample in lieu of a primary antibody.

32. Incubate the microcentrifuge tube containing the bead-bound nuclei and primary antibody on a rotating platform for 2 hr at 4°C.

33. Place the microcentrifuge tube on a magnetic stand for ~5 min.

    After rotation, it is essential to capture all bead-bound nuclei. After initial separation, invert tube to capture any beads on the lid, and allow the microcentrifuge tube to sit on the magnetic stand for additional time.

34. After the solution has completely cleared, remove and discard supernatant without disturbing beads.

35. Remove microcentrifuge tube from magnetic stand and resuspend beads in 1 ml wash buffer by gentle pipetting.

36. Place microcentrifuge tube on magnetic stand for ~5 min.

37. After the solution has completely cleared, remove and discard supernatant without disturbing beads.

38. Remove microcentrifuge tube from magnetic stand and resuspend beads in 1 ml wash buffer by gentle pipetting.

Protein A–micrococcal nuclease (pA-MN) coupling

pA-MN should be added to both the antibody-containing experimental samples and the no-antibody or IgG controls, in order to control for untargeted pA-MN digestion.

39. Place microcentrifuge tube on magnetic stand for ~5 min.

40. After the solution has completely cleared, remove and discard supernatant without disturbing beads.

41. Remove microcentrifuge tube from magnetic stand and resuspend beads in 250 µl wash buffer using gentle pipetting.

42. Dilute pA-MN in 250 µl wash buffer.

    The amount of pA-MN used depends on the concentration and activity of the purified recombinant protein. A titration of fusion protein must be performed upon each purification to optimize DNA recovery and digestion kinetics, as described (Skene & Henikoff, 2017). See Critical Parameters for a description of pA-MN titration. A master mix of pA-MN should be made when working with more than one sample.

43. While gently vortexing the bead-bound nuclei, slowly add the 250 µl wash buffer + pA-MN. Pipet up and down during addition to ensure mixing.

44. Incubate microcentrifuge tube on a rotating platform for 1 hr at 4°C.

45. Place microcentrifuge tube on magnetic stand for ~5 min.

    After rotation, it is essential to capture all bead-bound nuclei. After initial separation, invert tube to capture any beads on the lid, and allow the microcentrifuge tube to sit on the magnetic stand for additional time.

46. After the solution has completely cleared, remove and discard supernatant without disturbing magnetic beads.

47. Remove microcentrifuge tube from magnetic stand and resuspend beads in 1 ml wash buffer by gentle pipetting.

48. Place microcentrifuge tube on magnetic stand for ~5 min.

49. After the solution has completely cleared, remove and discard supernatant without disturbing magnetic beads.

Targeted micrococcal nuclease cleavage

50. Place microcentrifuge tube on magnetic stand for ~5 min.

51. After the solution has completely cleared, remove and discard supernatant without disturbing magnetic beads.

52. Remove microcentrifuge tube from magnetic stand and resuspend beads in 150 µl wash buffer by gentle pipetting.

53. Equilibrate to 0°C by incubating tubes for 5 min in an ice-water bath.

54. Working with one tube at a time, briefly remove tubes from ice water and add 3 µl 100 mM $CaCl_2$ while gently vortexing. Flick briefly three times to quickly mix and return to 0°C ice water.

    See Video 3 for demonstration. Working rapidly to minimize the time tubes are held outside of the ice water slurry is essential. In addition, complete mixing is essential for proper pA-MN cleavage.

55. Incubate samples in ice-water bath for 30 min.

    Digestion can be completed in <2 min. However, additional digestion time allows higher recovery of DNA fragments with no effect on background (Skene & Henikoff, 2017).

56. Stop each reaction by addition of 150 µl 2× STOP buffer.

    Be sure to add the RNase A, glycogen, and spike-in DNA fresh to the STOP buffer prior to use. See recipe for instructions.

Fragment release and cleanup

57. Incubate samples for 20 min at 37°C on thermomixer (with no shaking) to digest RNA and release cleaved DNA fragments from insoluble nuclear chromatin.

    The incubation can also be done at 37°C on a heated block or any instrument that fits 1.5-ml microcentrifuge tubes well.

58. Centrifuge 5 min in a microcentrifuge at 16,000 × *g*, at 4°C.

59. Place samples on a magnetic stand, and after separation, immediately transfer supernatants to fresh 1.5-ml microcentrifuge tubes.

    Supernatant contains the released fragments. Discard beads/pellet. Pellet can be difficult to visualize.

60. Add 3 µl 10% SDS and 2.5 µl 20 mg/ml proteinase K to the 1.5-ml tube containing each sample.

61. Invert each tube well and incubate 10 min at 70°C on a thermomixer (without shaking).

    The 70°C incubation can be done on any instrument that fits 1.5-ml microcentrifuge tubes well.

62. Add 300 µl Tris-buffered 25:24:1 (v/v/v) phenol/chloroform/isoamyl alcohol (PCI) to the sample and vortex well.

    The solution should become somewhat opaque.

63. Transfer to a phase-lock microcentrifuge tube and microcentrifuge 5 min at 16,000 × *g*, 4°C.

64. Transfer aqueous phase to fresh (non-phase-lock) microcentrifuge tube and discard the organic phase in an appropriate receptacle.

65. Add 300 µl chloroform to the sample and vortex well.

66. Centrifuge 5 min at 16,000 × *g*, 4°C.

67. Carefully transfer the aqueous phase to a fresh 1.5-ml microcentrifuge tube and discard the organic phase in an appropriate receptacle.

    Minimize contamination with phenol and chloroform, as both can inhibit downstream reactions.

68. Add 2 µl 2 mg/ml glycogen to each sample and mix by vortexing well.

69. Add 750 µl 100% ethanol to each sample and mix by vortexing well.

70. Incubate samples on ice for ~20 min.

    Sample can also be stored in −20°C for at least several days.

71. Centrifuge 10 min at 16,000 × *g*, 4°C.

72. Carefully discard the supernatant without disturbing the glycogen-DNA pellet.

73. Wash the pellet with 1 ml 100% ethanol.

    The pellet is hardly visible, so be careful when removing the supernatant not to disturb the pellet.

74. Centrifuge 5 min at 16,000 × *g*, 4°C.

75. Discard supernatant.

76. Briefly centrifuge for 1 min at 16,000 × *g*, 4°C, to spin down any residual ethanol.

77. Discard any residual supernatant.

78. Air dry ~5 min at room temperature.

    Do not overdry samples.

79. Dissolve the pellet in 36.5 µl 0.1× TE.

    At this point, the experimenter can store the sample at −20°C or proceed immediately to building a DNA sequencing library.

CUT&RUN WITH ADDITION OF SECONDARY ANTIBODY FOR pA-MN RECOGNITION

ALTERNATE PROTOCOL 1

Some IgG classes bind poorly to protein A. Rabbit IgG is strongly recognized by protein A, as are mouse IgG2a, human IgG1, IgG2, and IgG4. However, antibodies raised in mouse with IgG1, IgG2b, or IgG3 subtypes, goat IgG, or sheep IgG are not recognized well by protein A. Therefore, if the primary antibody utilized is not rabbit IgG or mouse IgG2a subtype, after primary antibody addition and washes and before pA-MN addition, an additional step of secondary antibody addition can be included. This allows the use of primary antibodies that otherwise would not be strongly bound by protein A.

Additional Materials *(see Basic Protocol 1)*

Secondary antibody: e.g., rabbit anti-mouse (Millipore, cat. no. 06-371)

1. Perform steps 1 to 38 of Basic Protocol 1 (through primary antibody addition and washes).
2. Place microcentrifuge tube on a magnetic stand for ~5 min.
3. After the solution has completely cleared, remove and discard supernatant without disturbing beads.
4. Remove tube from magnetic stand and resuspend beads in 250 µl wash buffer by gently pipetting the sample.
5. Dilute secondary antibody in 250 µl wash buffer.

   Typically, a dilution of 1:100 final (5 µl total antibody per reaction) works well. A master mix of secondary antibody should be made when working with more than one sample. No-antibody controls should also include secondary antibody when using this protocol.
6. While gently vortexing the bead bound nuclei, slowly add the 250 µl wash buffer + secondary antibody.
7. Incubate on a rotating platform for 1 hr at 4°C.
8. Place on magnetic stand for ~5 min.

   After rotation, it is essential to capture all DNA. After initial separation, invert the tube to capture any beads on the lid, and allow additional time on the magnetic stand.
9. After the solution has completely cleared, remove and discard supernatant without disturbing beads.
10. Remove microcentrifuge tube from magnetic stand and resuspend beads in 1 ml wash buffer by gently pipetting the sample.
11. Place tube on magnetic stand for ~5 min.
12. After the solution has completely cleared, remove and discard supernatant without disturbing beads.
13. Remove microcentrifuge tube from magnetic stand and resuspend beads in 1 ml wash buffer by gentle pipetting.
14. Proceed to pA-MN addition, starting at step 39 of Basic Protocol 1.

CUT&RUN LIBRARY PREPARATION FOR PAIRED-END ILLUMINA SEQUENCING

BASIC PROTOCOL 2

The following section describes the protocol for preparation of CUT&RUN DNA libraries for Illumina paired-end sequencing.

Table 2 Adapters and Primers Used in Basic Protocol 3

Adapter or primer	Sequence
TruSeq adapters and primers	
TruSeq universal adapter	5′-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTAC ACGACGCTCTTCCGATC*T
TruSeq indexed adapter	5′-[Phos]GATCGGAAGAGCACACGTCTGAACTCCAGTCAC NNNNNNATCTCGTATGCCGTCTTCTGCTT*G
TruSeq PCR primers for library enrichment	
P5 PCR primer	5′-AATGATACGGCGACCACCGA*G
P7 PCR primer	5′-CAAGCAGAAGACGGCATACGA*G
PE inline adapters	
Adapter oligo 1	5′-[Phos]NNNNGATCGGAAGAGCGGTTCAGCAGGAATG CCGAG
Adapter oligo 2	5′-ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNN
PE PCR primers for library enrichment	
PE PCR 1.0	5′-CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCC TGCTGAACCGCTCTTCCGATCT
PE PCR 2.0	5′-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTAC ACGACGCTCTTCCGATCT
PE sequencing primers	
Forward	5′-ACACTCTTTCCCTACACGACGCTCTTCCGATCT
Reverse	5′- CGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT

[Phos] indicates a 5′ phosphate group on the indexed adapter; * indicates a phosphorothioate bond between the last two bases at the 3′ end; N indicate barcodes (of the indicated length) used for multiplexing samples.

Materials

36.5 µl of CUT&RUN-enriched DNA (from Basic Protocol 1)
10× T4 DNA ligase buffer (NEB cat. no. B0202S)
5 U/µl T4 DNA polymerase (NEB, cat. no. M0203S)
10 mM dNTPs (NEB, cat. no. N0447S; or use the KAPA dNTPs listed below—the two types of dNTPs are fungible)
10 mM ATP (NEB, cat. no. P0756S)
40% (w/v) PEG 4000 (see recipe)
10 U/µl T4 PNK (NEB, cat. no. M0201S)
Taq DNA polymerase (NEB, cat. no. M0273S)
Commercially available paired-end adapters and PCR primers for Illumina TruSeq *or* inline PE adapters and primers (Table 2)
TruSeq adapters and primer sequences (Table 2)
Quick ligation kit (NEB, cat. no. M2200S)
AMPure XP beads (Beckman Coulter, cat. no. A63881), equilibrated to room temperature before use
80% (v/v) ethanol
10 mM Tris·Cl, pH 8.0 (Moore, 1996)
5× KAPA HiFi Fidelity Buffer (Kapa, cat. no. KK2502)
10 mM KAPA dNTPs (Kapa, cat. no. KK2502)
KAPA HiFi polymerase (Kapa, cat. no. KK2502)
Primers: P5 and P7 *or* PE PCR 1.0 and 2.0 primers (Table 2)
100-bp size ladder (NEB, cat. no. N3231S) *or* NEB low-molecular-weight ladder (NEB, cat. no. N3233S)

Qiagen gel-extraction kit

0.5-ml PCR tube
DynaMag-2 magnetic stand (Life Technologies, cat. no. 12321D)
Thermocycler without heated lid (Eppendorf, cat. no. 6311000010), precooled to 12°C
UV lamp
dsDNA-specific assay or analysis instrument (e.g., Thermo Fisher Qubit analyzer)
Fragment Analyzer capillary electrophoresis genetic analyzer (Agilent) or equivalent
Thermomixer (e.g., Eppendorf, cat. no. 2231000574)

Additional reagents and equipment for agarose gel electrophoresis (Voytas, 2001)

End repair, phosphorylation, and A-tailing

1. Transfer 36.5 µl CUT&RUN-enriched DNA into a 0.5-ml PCR tube.
2. Dilute T4 DNA polymerase 1:20 as follows:

   17.0 µl nuclease-free water
   2.0 µl 10× T4 DNA ligase buffer
   1.0 µl 5 U/µl T4 DNA polymerase.

   Pipet up and down to mix.
3. Prepare the end repair/adenylation master mix by combining the following for each sample:

   5 µl 10× T4 DNA ligase buffer
   2.5 µl 10 mM dNTPs (e.g., dNTP mix from NEB)
   1.25 µl 10 mM ATP
   3.13 µl 40% PEG 4000
   0.63 µl 10 U/µl T4 PNK
   0.5 µl diluted T4 DNA polymerase
   0.5 µl Taq DNA polymerase.

   Carefully and gently pipet up and down to mix.
4. Add 13.5 µl of the master mix to 36.5 µl CUT&RUN-enriched DNA in 0.5-ml PCR tube (step 1). Mix by carefully pipetting up and down.

   Keep samples on ice while adding master mix.
5. Incubate the mixture as follows in a thermal cycler without a heated lid that was precooled to 12°C (using maximum ramp rate of the instrument for ramping):

   15 min 12°C
   15 min 37°C
   20 min 72°C
   Ramp to 4°C.
6. Immediately remove samples and proceed immediately to the next step for adapter ligation.

Adapter ligation

7. Place A-tailed library samples on ice.
8. Add 5 µl 1.5 µM annealed adapters (either TruSeq or inline PE Illumina adapters).

The choice of adapters depends on the choice of Illumina platform for sequencing. Inline PE adapters require no extra indexing run but necessitate demultiplexing (see step 1 of Basic Protocol 3), whereas TruSeq adapters allow automated demultiplexing of barcoded libraries after sequencing.

9. Make a ligation master mix by combining the following (from quick ligation kit; amounts per sample are shown):

   55 µl 2× quick ligase buffer
   5 µl quick ligase.

   Gently pipet up and down to mix.

10. Add 60 µl ligation master mix to the DNA + adapter mixture in the 0.5-ml PCR tube (from step 8). Mix by carefully pipetting up and down.

11. Incubate 15 min at 20°C in precooled thermocycler with no heated lid.

12. Immediately remove samples and proceed to AMPure bead purification.

    Over-ligating can lead to poor library preparation, so it is important to proceed immediately to the purification step.

AMPure DNA purification

13. Transfer the adapter-ligated samples to 1.5-ml microcentrifuge tubes.

14. Add 38 µl AMPure XP bead solution to each tube containing adapter-ligated DNA.

    Before use, bring AMPure beads to room temperature and resuspend well by vortexing.

15. Thoroughly resuspend the beads by pipetting them up and down gently.

16. Incubate the sample 15 min at room temperature to allow DNA to precipitate onto the beads.

17. Place sample on a magnetic stand and let stand ~5 min.

18. After the solution has completely cleared, remove and discard the supernatant without disturbing the magnetic beads.

19. While leaving the microcentrifuge tube on the magnetic stand, add 200 µl 80% ethanol without disturbing the beads.

20. Incubate 30 sec on the magnetic stand.

21. Remove and discard the ethanol without disturbing magnetic beads.

22. While leaving the microcentrifuge tube on the magnetic stand, add 200 µl 80% ethanol without disturbing the beads.

23. Incubate 30 sec on the magnetic stand.

24. Remove and discard the ethanol without disturbing magnetic beads.

25. Briefly spin the tube (5 sec at 1,000 × *g*).

26. Place sample on the magnetic stand ~2 min.

27. Remove any residual ethanol without disturbing the magnetic beads.

28. Air dry the beads ~5 min at room temperature.

    Do not overdry beads. They should be a chocolate-brown, without a gleam from solution.

29. Remove microcentrifuge tube from magnetic stand and resuspend beads completely with 29 µl 10 mM Tris·Cl, pH 8.0.

30. Incubate for ~5 min at room temperature to allow DNA to elute from the beads.

31. Place the tube on magnetic stand ~2 min.

32. Transfer 27.5 µl of eluate to a fresh 0.5-ml PCR tube.

    Be sure to avoid any disruption of the beads by leaving a small volume behind. Eluates can be stored at –20°C after purification and prior to library enrichment.

Library enrichment

33. Place samples on ice.

34. Prepare a PCR master mix by combining the following (amounts per sample are shown):

    10.0 µl 5× KAPA HiFi Fidelity Buffer
    1.5 µl 10 mM KAPA dNTPs
    5 µl 20 µM P5 or PE PCR 1.0 primer (depending on adapters used)
    5 µl 20 µM P7 or PE PCR 2.0 primer (depending on adapters used)
    1 µl 1 U KAPA HiFi HotStart DNA Polymerase.

    Carefully and gently pipet up and down to mix

35. Add 22.5 µl PCR mix to 27.5 µl purified DNA.

36. Pipet up and down to mix well.

37. Amplify the CUT&RUN libraries using the following PCR conditions:

    14 cycles: 45 sec 98°C
    15 sec 98°C
    10 sec 60°C
    Final step: 1 min 72°C
    Ramp to 4°C and hold.

    Use the maximum ramp rate of the instrument for ramping. The 14 PCR cycles listed may or may not be sufficient, depending on the protein of interest and the antibody used. We recommend performing qPCR following 5 cycles of initial amplification using a procedure analogous to previously described protocols for ATAC-seq (Buenrostro, Wu, Chang, & Greenleaf, 2015) to determine the number of cycles required. Furthermore, using lower cell numbers may necessitate additional PCR cycles. Samples can be stored at −20°C after library enrichment.

Size selection and purification of library enriched samples

38. Pour a 1.5% agarose gel, preferably 15 to 20 cm in length.

39. Add loading dye and run the entire sample on the agarose gel, using a 100-bp ladder or NEB-low-molecular weight ladder for comparison. Run the dye front close to the end of the agarose gel to get maximum separation of the library from any adapter dimers.

    If necessary, the sample can be precipitated through standard ethanol precipitation and resuspended in a smaller volume prior to running on the agarose gel.

40. Working under long-wave UV light, cut out a gel slice that includes the size range from ~150 bp to ~650 bp.

Since adapters on each side of the insert correspond to ∼120 bp total, the size range described will include inserts ∼30 to 530 bp in size.

41. Use a Qiagen gel-extraction kit as recommended by the manufacturers. Elute each library in 15 µl nuclease-free water.

    Samples may be stored at −20°C prior to sequencing.

Quality control of library samples

42. Quantify the concentration of the CUT&RUN DNA libraries using a dsDNA-specific assay (for example, using a Thermo Fisher Qubit analyzer).

43. Determine the size distribution of libraries using a Fragment Analyzer instrument or equivalent.

    Assessing read size for the factor is obtained is the most informative quality control step.

44. Optional: Clone and sequence ∼10 fragments per library by Sanger sequencing to ensure libraries contain adapters and appropriate inserts.

BASIC PROTOCOL 3

EXAMPLE ANALYSIS PIPELINE OF CUT&RUN DATASETS

CUT&RUN libraries should be sequenced using paired-end Illumina sequencing (minimum 25 bp each side; longer reads provide minimal additional information). The following protocol provides an example step-by step method for analysis of resulting datasets. Alternative programs may be used for analysis.

Materials

Unix-compatible computer
Appropriate software; for example:
- Bowtie2 (*http://bowtie-bio.sourceforge.net/bowtie2/index.shtml*), to map reads to the genome
- Novocode (*http://www.novocraft.com/documentation/*), to split reads by PE barcode and trim PE barcode
- Samtools (*http://samtools.sourceforge.net*), for making size classes and converting files
- Picard (*https://broadinstitute.github.io/picard/*), to remove duplicates
- HOMER (*http://homer.ucsd.edu/homer/*), for generating genome browser tracks, calling peaks, and averaging data over genomic locations

Sufficient processing power for whole-genome sequence analysis

1. If inline adapters (such as Illumina PE adapters) were used, Split samples by barcode and remove the barcode sequences using Novocode.

   If Illumina TruSeq adapters are used, this is unnecessary.

2. Use the awk command in Unix to trim paired-end reads to 21 bases.

3. Align to annotated genome with Bowtie2 using the -X 1000 parameter.

4. Remove duplicates using Picard.

5. Use Samtools to remove low-quality reads (MAPQ < 10).

6. Use the Unix awk command, combined with Samtools, to separate reads into two size classes: <120 bp for TFs and 150 to 500 bp for nucleosomes.

7. Generate Tag directories and UCSC genome browser tracks using HOMER (Heinz et al., 2010) using the "makeTagDirectories" and "makeUCSCfile" commands (see Fig. 4 for an example of a genome browser generated by HOMER).

8. Align over specific regions of the genome using the "annotatePeaks" command in HOMER.
9. Call peaks using the "findPeaks" command in HOMER.

REAGENTS AND SOLUTIONS

Binding buffer

For 20 ml binding buffer, mix 400 µl 1 M HEPES-KOH, pH 7.9 (see recipe), 200 µl 1 M KCl (Moore, 1996), 20 µl 1 M $CaCl_2$ (Moore, 1996), 20 µl 1 M $MnCl_2$ (see recipe), and 19.36 ml pure water. This stock solution can be stored at 4°C for up to 1 year.

Blocking buffer

To 5 ml of wash buffer (see recipe) containing protease inhibitors, add 20 µl 0.5 M EDTA (Moore, 1996). Invert well to mix.

BSA, 30%

Add 3 g BSA to 8 ml filtered deionized water. Stir at room temperature until dissolved. Bring to final volume of 10 ml and filter sterilize. Solution can be stored at room temperature for up to 1 year.

EGTA, 0.2 M

Add 76.07 g EGTA to ~800 ml filtered deionized water. Stir at room temperature. Add NaOH pellets to reach pH 8.0. Filter sterilize. Solution can be stored at room temperature for up to 1 year.

HEPES-KOH, pH 7.5 and 7.9, 1 M

Add 238.3 g HEPES to 800 ml filtered deionized water. Stir at room temperature. Add KOH to reach pH 7.5 or 7.9 (prepare both separately). Stir at room temperature until dissolved. Bring to final volume of 1 liter and filter sterilize. Solution can be stored at room temperature for up to 1 year.

$MnCl_2$, 1 M

Dissolve 1.98 g $MnCl_2$ in 10 ml filtered deionized water. Stir at room temperature until dissolved and filter sterilize. Solution can be stored at room temperature for up to 1 year in a 15-ml conical tube covered with foil.

Nuclear extraction (NE) buffer

For 50 ml NE buffer, mix 1 ml 1 M HEPES-KOH, pH 7.9 (see recipe), 500 µl 1 M KCl (Moore, 1996), 12.5 µl 2 M spermidine (see recipe), 500 µl 10% Triton X-100 (see recipe), 12.5 ml 80% glycerol (see recipe), and 35.48 ml pure water. This stock solution can be stored at 4°C for up to 1 year.

On the day of the experiment, add 1× protease inhibitors (e.g., Life Technologies, cat. no. 78439) to appropriate amount of NE buffer.

PEG 4000, 40%

Add 4 g PEG 4000 to 8 ml filtered deionized water. Stir at room temperature until dissolved. Bring to final volume of 10 ml and filter sterilize. Solution can be stored at room temperature for up to 1 year.

SDS, 10%

Add 100 g sodium dodecyl sulfate to 800 ml filtered deionized water. Stir at room temperature until dissolved. Bring to final volume of 1 liter and filter sterilize. Solution can be stored at room temperature for up to 1 year.

CAUTION: *Wear a mask when working with SDS powder!*

Spermidine, 2 M

Add 2.9 g spermidine to 10 ml filtered deionized water. Stir at room temperature until dissolved and filter sterilize. Solution can be stored at room temperature for up to 1 year in a 15-ml conical tube covered with foil.

STOP buffer, 2×

For 5 ml, mix 200 µl 5 M NaCl (Moore, 1996), 200 µl 0.5 M EDTA (Moore, 1996), 100 µl 0.2 M EGTA (see recipe), and 4.46 ml filtered, deionized water. Buffer can be stored at room temperature for at least 1 year.

Separately, prepare heterologous DNA from a different organism from the sample of interest: for example, when performing CUT&RUN on mammalian cells, DNA from *S. cerevisiae* can be used. Prepare the DNA by crosslinking cells, digesting it with micrococcal nuclease to mononucleosomes, purifying it DNA, and diluting it to 10 ng/ml.

On the day of the experiment, add 25 µl 10 mg/ml RNase A (e.g., Thermo Scientific, cat. no. EN0531), 10 µl 20 mg/ml glycogen (e.g., VWR, cat. no. 9005792), and 5 µl 10 ng/ml heterologous DNA.

Triton X-100, 10%

Add 10 g Triton X-100 to 80 ml filtered deionized water. Stir at room temperature until dissolved. Bring to final volume of 100 ml and filter sterilize. Solution can be stored at room temperature for up to 1 year.

Wash buffer

For 100 ml wash buffer, mix 2 ml 1 M HEPES-KOH, pH 7.5 (see recipe), 3 ml 5 M NaCl (Moore, 1996), 25 µl 2 M spermidine (see recipe), 333 µl 30% BSA (see recipe), and 94.642 ml pure water. Stock solution can be stored at 4°C for up to 1 year.

On the day of the experiment, add 1× protease inhibitors (e.g., Life Technologies, cat. no. 78439) to appropriately sized aliquot(s) of wash buffer.

COMMENTARY

Background Information

Transcription factors (TFs) are DNA-binding proteins that recognize short DNA sequence motifs and regulate gene expression through a variety of mechanisms. Histone proteins interact with ~147 bp of DNA to form the basic unit of chromatin, the nucleosome, which also modulates gene expression, typically by preventing TF binding through the tight interaction of histones with DNA. Profiling of chromatin protein and nucleosome occupancy is essential to understanding DNA templated processes and the activities of these proteins.

Chromatin immunoprecipitation (ChIP) is the most widely used technique for exploring protein-DNA interactions on chromatin. Traditional ChIP includes crosslinking of protein to DNA using formaldehyde, shearing of crosslinked chromatin with sonication, and immunoprecipitation of the protein of interest (Gilmour & Lis, 1984, Gilmour & Lis, 1985). Crosslinking and sonication of chromatin can lead to biases in traditional ChIP techniques including false positives from crosslinking and increased release of fragments in nucleosome-depleted regions of the genome from sonication (Baranello, Kouzine, Sanford, & Levens, 2016; Meyer & Liu, 2014).

In addition to potential artifacts from crosslinking and sonication, the resolution of ChIP techniques is limited by chromatin shearing. In crosslinking ChIP, the DNA fragments that are generated are usually 200 to 400 bp in size. Since many TFs bind sequences of only 6 to 20 bp, additional information regarding binding motifs is often necessary to infer the precise locations of TFs within broad peaks of

ChIP enrichment. The ChIP-exo method increases the resolution of ChIP while maintaining the basic features of ChIP-based methods for enrichment of chromatin (Rhee & Pugh, 2011). ChIP-exo involves immunoprecipitation of chromatin fragments from a large number of crosslinked cells, followed by the use of an exonuclease to degrade the regions of immunoprecipitated DNA not bound by the chromatin protein. Exonuclease digestion allows precise localization of TF binding sites to the single-nucleotide level. However, the large number of cells required for this technique are not always readily available.

Modifications to traditional ChIP protocols have been developed to increase the sensitivity and thereby reduce the number of cells required. These modifications include ChIPmentation (Schmidl, Rendeiro, Sheffield, & Bock, 2015), carrier-assisted ChIP-seq (Zwart et al., 2013), ULI-NChIP (Brind'Amour et al., 2015), μChIP (Dahl et al., 2016), and DROP-ChIP (Rotem et al., 2015). Several of these techniques enable mapping of abundant histone modifications in fewer than 1,000 cells. However, ChIP-based methods for mapping chromatin occupancy of TFs currently require a minimum of 10,000 cells, and often two to three orders of magnitude more.

An orthogonal approach termed DamID utilizes overexpression of the bacterial Dam methylase fused to chromatin binding proteins to profile their genomic locations (van Steensel, 2000). This approach has been effectively utilized in many systems but requires expression of a Dam-fusion protein for each factor of interest. Furthermore, overexpression of fusion proteins may not be feasible in some settings as it could potentially lead to occupancy at non-physiological locations. Similar to DamID, ChEC (Schmid et al., 2004) and ChEC-seq (Zentner, Kasinathan, Xin, Rohs, & Henikoff, 2015) require fusion of chromatin proteins to micrococcal nuclease.

CUT&RUN is a recently described method for genome-scale profiling that results in high-resolution maps of chromatin factors (Skene & Henikoff, 2017). CUT&RUN was derived from the ChIC technique, developed in 2004 by Ulrich Laemmli and colleagues, which utilized recombinant pA-MN to map chromatin proteins at individual loci (Schmid et al., 2004). In 2017, Steven Henikoff and colleagues systematically modified the ChIC approach to accommodate genome-wide profiling of factors (Skene & Henikoff, 2017). The authors showed that the background signal for Cut&RUN is extremely low, leading to increased enrichment of factors and a decreased requirement for high read coverage relative to ChIP-seq approaches. The technique itself is rapid and easily performed, with few steps that require optimization for each protein profiled. Consequently, CUT&RUN facilitates profiling of chromatin factors with increased fidelity and at lower cost than traditional approaches. In sum, CUT&RUN is an excellent alternative to traditional ChIP-seq techniques, especially when collecting large numbers of cells is unfeasible.

Critical Parameters and Troubleshooting

Harvesting cells and cell lysis

Membrane lysis is essential for proper binding of nuclei to lectin-coated beads in subsequent steps. Increasing the incubation time in NE buffer may result in in more efficient lysis for some cell types. When performing CUT&RUN on multiple samples, the same number of cells needs to be used in order for appropriate comparisons to be made later on.

Preparing concanavalin A beads and nuclei binding

Concanavalin A beads must be completely mixed prior to use. Gentle vortexing of nuclei is important to prevent crushing of nuclei.

Blocking beads

Addition of EDTA at this step prevents micrococcal nuclease from cleaving chromatin prior to activation with $CaCl_2$. Complete resuspension of the beads in blocking buffer allows all nuclei to be blocked equally.

Primary antibody use

Another important consideration is the amount of primary antibody used. Typically, one starts with a 1:100 final dilution of primary antibody (i.e., 5 μl in the 500 μl reaction volume). However, because different antibodies exhibit different affinities for their substrates, unequal background binding to chromatin, and other differences, one should typically titrate new antibodies (e.g., 1:50, 1:100, 1:200, and 1:400) to identify the optimal dilution for CUT&RUN.

Primary antibody and pA-MN coupling

The most critical steps of the CUT&RUN protocol are the proper mixing of nuclei-bound beads during addition of reagents. Incomplete mixing can result in improper and uneven incorporation or spatial differences in the

concentrations of primary antibody, pA-MN, or $CaCl_2$. See Video 2 for an example of primary antibody and pA-MN addition.

pA-MN titration

The pA-MN must be expressed and purified. Details of purification can be found in the original ChIC protocol (Schmid et al., 2004). Once recombinant pA-MN has been generated, the nuclease efficiency should be tested. pA-MN activity should be titrated using standard procedures (Skene & Henikoff, 2017; Zaret, 2005). Briefly, cells should be gently lysed and while cell numbers are held constant, a range of pA-MN should be added to lysates (from 0 to 30 U). After digestion for a fixed period of time, the reaction is terminated by the addition of EDTA. After purification of DNA, the size distribution should be determined by agarose gel electrophoresis or bioanalyzer analysis. Commercial micrococcal nuclease (such as Clontech 2910A) should be used as a side-by-side control for nuclease activity. After the endonuclease activity of pA-MN is determined, the amount of pA-MN to be used in a CUT&RUN experiment can be further titrated as previously described (Skene & Henikoff, 2017). Briefly, a CUT&RUN experiment (Basic Protocol 1) should be performed, but a titration of pA-MN should be used (e.g., 1:50, 1:100, 1:200, 1:400, and 1:800 final dilutions). Libraries should be built (Basic Protocol 2) and fragment enrichment assessed and compared between the different pA-MN dilutions (Basic Protocol 3) to identify the conditions optimal for mapping of chromatin proteins.

Targeted micrococcal nuclease cleavage

Because of the small volume of $CaCl_2$ added, proper mixing of $CaCl_2$ upon addition is critical. We recommend vortexing and flicking the sample during and immediately after addition, respectively. Furthermore, pA-MN cleavage is performed at 0°C, which is suboptimal for nuclease activity. This is intentional to prevent over-digestion and off-target digestion by pA-MN. Therefore, it is important to act quickly and carefully when performing this step, without heating the sample with one's hand. We recommend equilibrating the samples to 0°C by incubation in an ice water bath for at least 5 min, turning the vortex mixer on, and having 3 µl of $CaCl_2$ within your pipet tip before removing the sample from the ice water. Also, holding the upper half of the microcentrifuge tube with your fingers prevents heating of the sample from your hand. Finally, by starting a timer upon addition of $CaCl_2$ to the first sample, and stopping the reactions in the same order, appropriate timing is more easily maintained. See Video 3 for an example of $CaCl_2$ addition.

Fragment release and clean up

Preheating the thermomixer or other incubator to 37°C and 70°C prior to addition of RNase and proteinase K addition, respectively, assists in rapid clean up. Carefully taking all of, and only, the aqueous phase during the PCI and chloroform purification steps is important to prevent DNA loss and degradation.

Library preparation

Generating master mixes for each step is highly recommended as it helps to control for variation in sample preparation. Continuing with the library build immediately following end repair/adenylation and adapter ligation is important to promote high quality of DNA libraries as over-ligation can result in the generation of inappropriate ligation intermediates.

Understanding the results

The method described here permits the mapping of TFs and histone proteins rapidly to high resolution. Prior to gel extraction at the end of the library build, the libraries will be visualized on the agarose gel. Here, a strong "smear" should be observed (Fig. 2). Often, adapter dimers and/or PCR dimers are also observed, and these should be excluded from portion of the gel subjected to DNA extraction.

Quality control steps can be taken at the end of the library build. These include quantification of DNA concentration and assessment of the size distribution of the library (Fig. 3). For profiling of TFs, fragment sizes will be enriched under 120 bp, although large shoulders can occur at over 150 bp when adjacent nucleosomes are profiled (as with CTCF CUT&RUN). For profiling histone proteins, a large proportion of the reads will usually be over 150 bp. Furthermore, prior to deep sequencing, libraries can be cloned, and individual fragments can be sequenced by Sanger sequencing to confirm that adapters are appropriately ligated, the fragments map to multiple locations, and the fragments map to the correct genome of interest.

Once genome-wide sequencing reads have been obtained and reads have been mapped to the appropriate reference genome, the distribution of recovered fragments can be assessed to

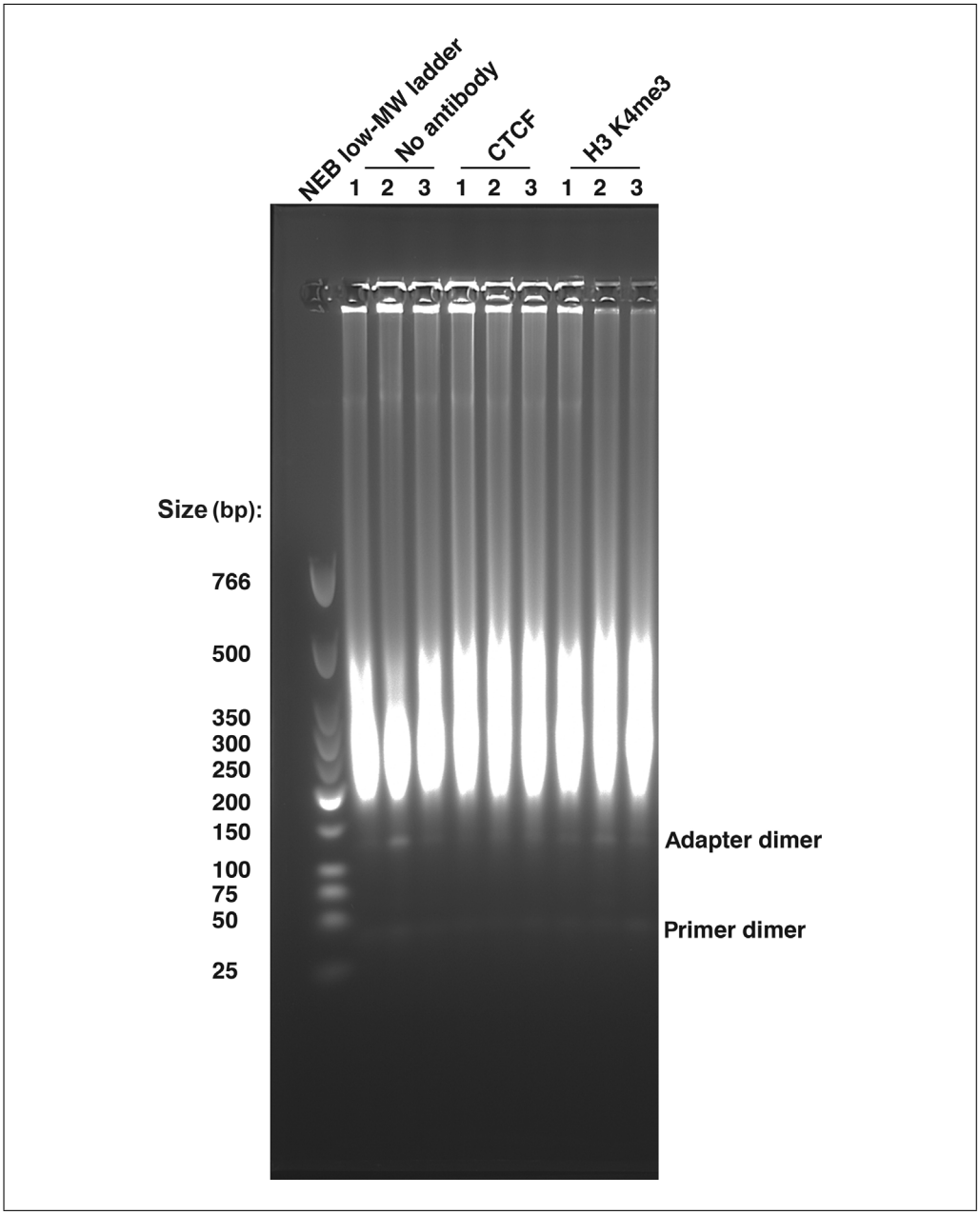

Figure 2 Visual example of CUT&RUN libraries on a gel. Nine CUT&RUN libraries are shown separated on an agarose gel after experimentation and library preparation, just before gel extraction for size selection and purification. The marker included in the leftmost lane is the NEB low-molecular-weight ladder. Both adapter dimers and PCR dimers are labeled for clarity.

confirm that appropriate fragment sizes were obtained. Mapping efficiency for 500,000 cells should be ~60% to 70%. Reads can also be mapped to a genome browser (such as UCSC or IGV) to confirm enrichment of the protein of interest at expected locations (Fig. 4). Furthermore, if ChIP-seq data are available for the factor profiled, CUT&RUN data can be compared with them either through visualization on a genome browser track or by aligning the CUT&RUN datasets over the available ChIP-seq peaks. Bound sites can be identified across the genome using peak calling algorithms.

Time Considerations

The time needed to complete a CUT&RUN experiment and library is significantly less than that for traditional crosslinking ChIP experiments. The steps prior to building the library can be completed in 1 day. The library preparation can be completed in one additional day. Detailed time estimates for each step are included below. Quality control and sequencing of the libraries can take as little as one additional day, depending upon available resources. Data analysis depends on familiarity with the software packages and datasets but is

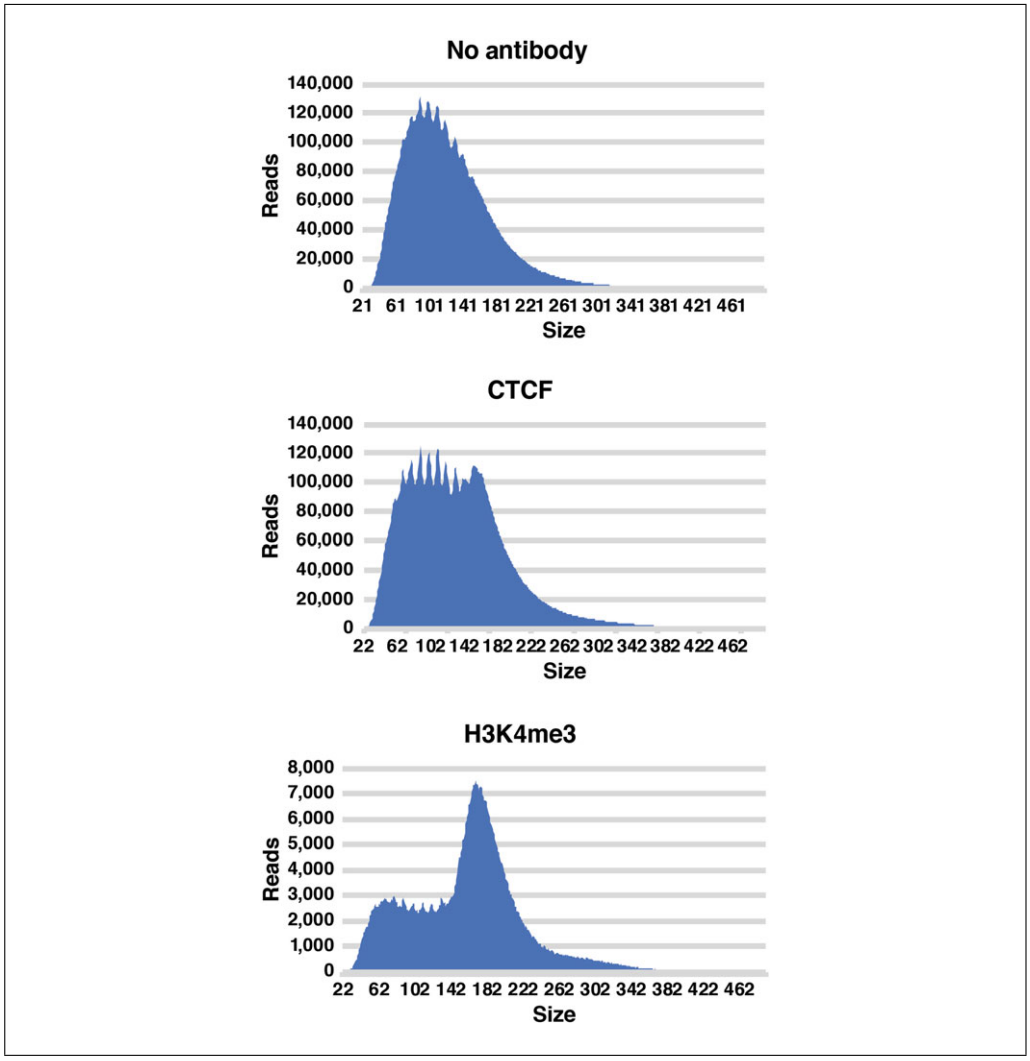

Figure 3 Example CUT&RUN library size distributions. After sequencing, the size distributions of fragments in the CUT&RUN library are assessed to confirm enrichment profiles. No-primary-antibody libraries typically show reduced size enrichment over 150 bp relative to factor-specific libraries. CTCF CUT&RUN libraries (representative of many TF libraries) show a slight right "shoulder" over 150 bp, due to pA-MN cleavage of DNA on either side of nucleosomes immediately flanking CTCF binding sites. H3K4me3 libraries show strong enrichment of library fragments of over 150 bp, but also include smaller inserts corresponding to open chromatin regions near sites of H3K4me3 enrichment.

generally not substantially more complex than analysis of ChIP-seq data.

Basic Protocol 1

Buffer preparation: 0.5 hr (0.5 hr bench time)

Lysis and binding to beads: 1 hr (1 hr bench time)

Primary antibody use: 2.5 hr (2 hr incubation, 0.5 hr bench time)

pA-MN coupling: 1.5 hr (1 hr incubation, 0.5 hr bench time)

pA-MN digestion: 1 hr (0.5 hr incubation, 0.5 hr bench time)

Fragmentation and purification: 2 hr (1 hr incubation, 1 hr bench time)

Alternate Protocol 1

Secondary antibody use: 1.5 hr (1 hr incubation, 0.5 hr bench time)

Basic Protocol 2

Buffer preparation: 0.5 hr (0.5 hr bench time)

End repair and adenylation: 1.5 hr (50 min incubation, 40 min bench time)

Adapter ligation: 0.5 hr (15 min incubation, 15 min bench time)

AMPure purification: 0.5 hr (20 min incubations, 10 min bench time)

Library enrichment: 40 min (20 min PCR, 20 min bench time)

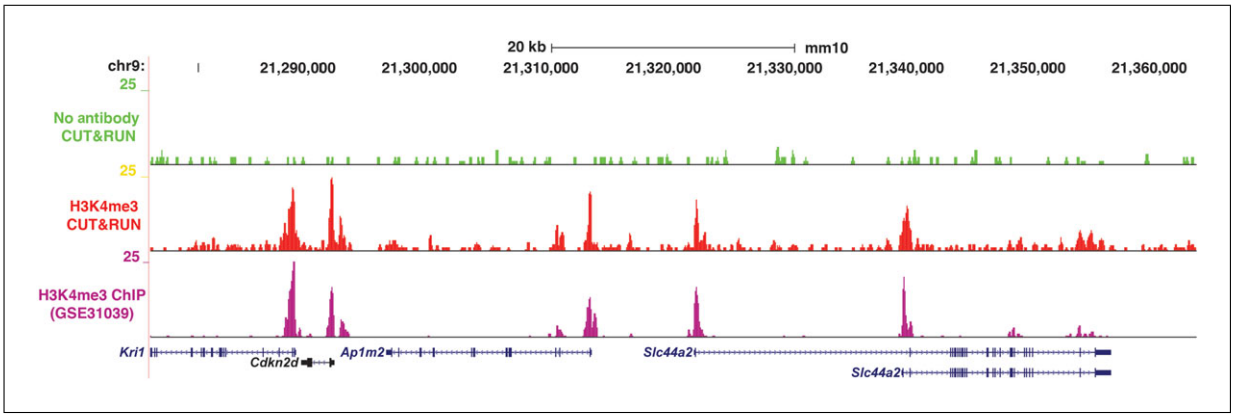

Figure 4 Genome browser track for H3K4me3 CUT&RUN compared to available ChIP-seq datasets. Indicated ChIP-seq datasets from mES cells were downloaded and aligned, and browser tracks were generated. CUT&RUN experiments were performed from 500,000 mES cells. Included are no-antibody controls for the matching size distribution.

Gel extraction: 3 hr (2 hr running time, 1 hr extraction)

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Schmid, M. et al. 2004. See above.

Describes the development of recombinant protein A–micrococcal nuclease and described its use in a ChIC technique to map the chromatin-binding protein Gbd in yeast.

Skene, P. et al. 2017. See above.

Describes the initial development of CUT&RUN as a novel genome-wide mapping technique that leads to high-resolution mapping of TFs and histone proteins in yeast and human K562 cells.

Internet Resources

Information on IgG affinities: Retrieved from *https://www.neb.com/tools-and-resources/selection-charts/affinity-of-protein-ag-for-igg-types-from-different-species*

Picard website: Retrieved from *http://broadinstitute.github.io/picard/*

HOMER website: Retrieved from *http://homer.ucsd.edu/homer/*

Samtools website: Retrieved from *http://samtools.sourceforge.net*

Bowtie2 website: Retrieved from *http://bowtie-bio.sourceforge.net/bowtie2/index.shtml*