ChIPmentation CeMM v1.14 (September 2016)

This protocol works well for efficient histone modification and transcription factor antibodies. For less efficient antibodies sonication different sonication-, IP- and wash-buffers might be necessary!

Before starting

1. Precool a centrifuge
2. Dissolve glycine crystals that may have formed in the solution
3. Set up Covaris for sonication

Fixing cells for ChIPmentaiton

(small cell numbers up to 5 x 10^5, large cell numbers up to 10 x 10^6 per IP)

1. Collect cells spinning 5 min at 500 x g and wash cells once with 1 ml PBS. Resuspend cells in 1 ml room tempered PBS. For small cell numbers: collect cells and resuspend in 150µl PBS/10% FCS (omit washing with PBS). Do not use DNA-low bind tubes.
2. Add fresh formaldehyde to a concentration of 1 % final to cell suspension, mix carefully and rock the cell suspension for 10 minutes at room temperature.
3. Quench the formaldehyde by adding glycine to a 0.125 M final concentration, mix and rock the cell suspension for 5 minutes at room temperature.
4. Collect cells by centrifugation at 300 x g for 5 minutes at 4°C. Wash twice with 1000 µl ice-cold PBS + inhibitors (see appendix). For small cell numbers: Wash once with 150 µl ice-cold PBS/PMSF.
5. *The cell pellets can be snap-frozen in liquid N_2 and stored at -80°C.*

Sonicate cells

6. Resuspend cell pellets in 1 ml (up to 20 mio cells) 0.25 % SDS sonication buffer + 1 x protease inhibitors + PMSF, small cell numbers (10,000 to 3 x 10^6 cells) are resuspended in only 130 µl of this buffer.
7. Sonicate in a Covaris MilliTUBE (1ml) or MicroTUBE (130µl) until most fragments are in the range of 200-700 bp.
8. Dilute lysate 1:1.5 with equilibration buffer (10mM Tris, 233 mM NaCl, 1.66 % TritonX-100, 0.166 % DOX, 1 mM EDTA, inhibitors), e.g. use 1 ml Lysate and dilute with 1.5 ml equilibration buffer.
9. Spin samples at 14,000 x g, 4 °C for 10 minutes to pellet insoluble material and transfer supernatant to a new tube.
10. Optional: quantify DNA content of chromatin on a nanodrop.
11. Top up chromatin with RIPA-LS + inhibitors for desired IP volumes. Volumes should be around 500 – 1000 µl for large cell numbers and 100 - 200 µl for small cell numbers per IP.
12. Preserve an input and gel sample.
13. Dispense lysate in 0.5 or 1.5 ml tubes, add antibody and incubate on a rotator o/n at 4°C. For small cell number experiments use 0.2 ml PCR stripes.

Bead preparation

14. Per IP, wash 10 µl (small cell numbers) or 25 µl protein A Dynabeads beads (large cell numbers) (or protein G Dynabeads, e.g. when using goat pAB or mouse IgG1/IgG3 or IgM) 2 x with 0.1 % BSA/RIPA and incubate o/n 4°C to block beads.

Next day: ChIP with magnetic beads

15. Transfer beads to tubes with chromatin and incubate at 4°C for 2 h rotating.
16. Precool magnet on ice. Prepare wash buffers by adding protease inhibitors and other appropriate inhibitors. Wash buffers should be ice-cold.
17. Wash the beads with 150 µl (small cell numbers) - 1000 µl (high cell numbers) by adding ice-cold buffer and moving the samples on the magnet 5 times so that the beads move through the solution:

buffer	Times washing
RIPA-LS	2
RIPA-HS	2
RIPA-LiCl	2
10 mM Tris pH8	1

18. Very gently resuspend beads in 150 µl Tris and transfer to a new tube/plate (now it's a good time to prepare the tagmentation reaction).
19. Magnetize the beads and discard the supernatant.

ChIPmentation reaction

20. Prepare tagmentation reaction:

Component Tagmentation	volume
5x Tagmentation buffer	5 µl
nuclease free water	19 µl
Tagment DNA Enzyme	1 µl

21. Gently resuspend beads in tagmentation reaction.
22. Incubate 1-10 minutes at 37°C (see appendix for more information), then cool on ice**.**
    For short tagmentation times: make sure the sample itself is at 37°C for the full time, so when putting into a thermocycler add 10 seconds to ensure thorough heating of the sample. For long tagmentation times: Mix beads once after 5 minutes by gentle pipetting.
23. Add 150 µl (small cell numbers) - 1000 µl (high cell numbers) of RIPA-LS to the tagmentation reaction and incubate for 5 minutes on ice to inactivate Tn5.
24. Wash the beads with 150 µl (low cell numbers) - 1000 µl (high cell numbers) by adding ice-cold buffer and moving the samples on the magnet 5 times so that the beads move through the solution:

buffer	Times washing
RIPA-LS	2
TE	2

25. After washing, resuspend beads in 48 µl ChIP elution buffer + 2 µl Proteinase K.
26. Incubate at 55°C 1 h and 65°C for 6-10 hours (de-crosslinking).
27. Don't forget the input! Also bring input sample to 0.4 % SDS 300 mM NaCl final and add 2 µl Proteinase K and incubate 1 h 55°C followed by 65°C 6-10 hours.
28. Magnetize beads and transfer supernatant to new 1.5 ml DNA lo-bind tube.
29. Add another 19 µl of ChIP elution buffer + 1 µl Proteinase K and incubate 1h for 55°C.
30. Magnetize beads and combine supernatant with first eluate.
31. Purify with Qiagen MinElute kit and elute in 22 µl EB or, alternatively purify by a 1.8:1 SPRI cleanup eluting in 22 µl Qiagen EB (10 mM Tris pH 8.5).

Instructions for SPRI bead cleanup:

1. Add 1.8 x volume of room temperature AMPureXP beads to each reaction (here: 126 µl) by mixing 15 x with a pipette.
2. Incubate for 10 min.
3. Magnetize for 10 min, discard supernatant.
4. On magnet: wash beads 2 x with 100 µl 80% EtOH (5 s each wash).
5. Air-dry beads for 5 min on magnet.
6. Remove tubes from magnet, add 22 µl H2O to beads and mix to elute sample.
7. Incubate for 10 minutes.
8. Magnetize beads, wait for 5 minutes and transfer supernatant to a new tube.

Library amplification

1. Pipet qPCR (**critical:** preheat 2 x KAPA HiFi HotStart Ready Mix 98°C 30s before preparing the mastermix - KAPA is a hotstart polymerase and not suitable for nick translation in the first PCR step):

Component qPCR	volume
Nextera custom primer Ad1_noMX 25 µM	0.3 µl
Nextera custom primer Ad2.1 25 µM	0.3 µl
2 x Kapa HiFi HotStart Ready Mix	5 µl
100 x SYBR	0.1 µl
nuclease free water	2.3 µl
ChIPmentation DNA	2 µl

2. Perform PCR with the following program:
    a. 72°C 5 minutes
    b. 98°C 30 seconds
    c. 25 cycles of:
        i. 98°C 10 seconds
        ii. 63°C 30 seconds
        iii. 72°C 30 seconds
    d. 72°C 1 minute
    e. hold at 10 °C

3. The Ct-value of the qPCR indicates the number of enrichment cycles to amplify the rest of the ChIPmentation DNA. Usually Cq (rounded up + 1) is a sufficient number of cycles.

4. Pipet in the following order in a PCR tube for each reaction (**critical:** preheat 2 x KAPA HiFi HotStart Ready Mix 98°C 30s before preparing the PCR - KAPA is a hotstart polymerase and not suitable for nick translation in the first PCR step).

Component qPCR	volume
Nextera custom primer Ad1_noMX 25 µM	1.5 µl
Nextera custom primer Ad2.x 25 µM	1.5 µl
2 x Kapa HiFi HotStart Ready Mix	25 µl
nuclease free water	2 µl
ChIPmentation DNA	20 µl

5. Perform PCR with the following program:
    f. 72°C 5 minutes
    g. 98°C 30 seconds
    h. x cycles (calculated from qPCR) of:
        i. 98°C 10 seconds
        ii. 63°C 30 seconds
        iii. 72°C 30 seconds
    i. 72°C 1 minute
    j. hold at 10 °C

Purify and size select libraries

Purify the libraries with a normal AmpureXP cleanup, then size select the libraries with a double-sided SPRI

SPRI bead cleanup:

1. Add 1.8 x volume of room temperature AMPureXP beads to each reaction (here: 90 µl) by mixing 15 x with a pipette.
2. Incubate for 10 min.
3. Put on magnet for 10 min, discard supernatant.
4. On magnet: wash beads 2 x with 100 µl 80% EtOH (5 s each wash).
5. Air-dry beads for 5 min on magnet.
6. Remove tubes from magnet, add 50 µl H2O to beads and mix to elute sample.
7. Incubate for 10 minutes.
8. Put tubes back on magnet, wait for 5 minutes and transfer supernatant to a new tube.

Size-selection:

1. Add 0.65 x volume of room temperature AMPureXP beads to each sample (here: 32.5 µl) by mixing 15 x with a pipette.
2. Incubate for 10 min.
3. Put on magnet for 10min, TRANSFER supernatant to new tube.
4. Add to the supernatant 12.5 µl of room temperature AMPureXP beads each and mix 15 x with a pipette.
5. Incubate for 10 min.
6. Place tubes on magnet, incubate for 10 minutes, discard supernatant.
7. On magnet: wash beads 2 x with 100 µl 80% EtOH (5sec each wash).
8. Air-dry beads for 5 min on magnet
9. Remove tubes from magnet, add 15 µl H2O to beads and mix to elute sample
10. Incubate for 10 minutes.
11. Put tubes back on magnet, wait for 5 minutes and transfer supernatant to a new tube.

Determine library concentration using the Qbit and quality control library with a Bioanalyzer/Experion.

Reagents

- Pierce™ 16% Formaldehyde (w/v), Methanol-free (Thermo Scientific #28906)
- SYBR green: SYBR® Green I Nucleic Acid Gel Stain - 10,000X concentrate in DMSO (Thermo Scientific/Invitrogen, #S7563)
- SPRI beads for purifications: Agentcourt AMPure XP - PCR purification (Beckman Coulter) or Qiagen MinElute kit (Qiagen, #28004)
- Nextera DNA Library Preparation Kit (Illumina, #FC-121-1030)
- KAPA HiFi Hotstart Ready mix 6.25 ml (Peqlab, #07-KK2601-02)

Buffers

Sonication buffer: 10 mM Tris pH 8.0, 0.25% SDS, 2 mM EDTA

5x Tagmentation buffer: 50 mM Tris pH 8.0, 25 mM MgCl2, 50 % v/v dimethylformamide

RIPA-LS (store at 4°C)

	1X	Stock	Add for 50 ml
Tris-HCl pH 8.0	10mM	1M	500 ul
NaCl	140mM	5M	1.4 ml
EDTA pH 8.0	1 mM	500 mM	100 ul
SDS	0.1%	10%	500 ul
Na-Deoxycholate	0.1%	5%	1 ml
Triton x-100	1%	10%	5 ml
H2O			41.5 ml

1 x TE (store at 4C):

	Stock	Final	For 100ml	For 250ml
Tris-HCl pH 8.0	1M	10mM	1ml of 100xTE	2.5ml of 100xTE
EDTA pH 8.0	100mM	1mM		
H2O			99ml	247.5ml

5% Na-deoxycholate (DOC) (Store at RT):

	Stock	Final	For 100ml	For 250ml
Na-Deoxycholate	powder	5% (weight /volume)	5gr	25gr
H2O			100ml (final)	250ml (final)

RIPA-HS (store at 4C):

	Stock	Final	For 100ml	For 250ml
Tris-HCl, pH 8.0	1M	10mM	1ml of 100xTE	2.5ml of 100xTE
EDTA, pH 8.0	100mM	1mM		
NaCl	5M	500mM	10ml	25ml
Triton x-100	10%	1%	10ml	25ml
SDS	10%	0.1%	1ml	2.5ml
DOC	5%	0.1%	2ml	5ml
H2O			76ml	190ml

RIPA-LiCl (store at 4C):

	Stock	Final	For 100ml	For 250ml
Tris-HCl, pH 8.0	1M	10mM	1ml of 100xTE	2.5ml of 100xTE
EDTA, pH 8.0	100mM	1mM		
LiCl	8M	250mM	3.125ml	7.81ml
NP-40	100%	0.5%	0.5ml	1.25ml
DOC	5%	0.5%	10ml	25ml
H2O			85.37ml	213.4ml

ChIP elution buffer (store at room temp):

	Stock	Final	For 100ml	For 250ml
Tris-HCl pH 8.0	1M	10mM	1ml of 100xTE	2.5ml of 100xTE
EDTA pH 8.0	0.5M	5mM	0.8ml	2ml
NaCl	5M	300mM	6ml	15ml
SDS	10%	0.4%	4 ml	12.5ml
H2O			87.2ml	218ml

Nextera custom primers (from Buenrostro et al 2013 Nature Methods, can be ordered “desalted”):

Ad1_noMX:	AATGATACGGCGACCACCGAGATCTACACTCGTCGGCAGCGTCAGATGTG
Ad2.1_TAAGGCGA	CAAGCAGAAGACGGCATACGAGATTCGCCTTAGTCTCGTGGGCTCGGAGATGT
Ad2.2_CGTACTAG	CAAGCAGAAGACGGCATACGAGATCTAGTACGGTCTCGTGGGCTCGGAGATGT
Ad2.3_AGGCAGAA	CAAGCAGAAGACGGCATACGAGATTTCTGCCTGTCTCGTGGGCTCGGAGATGT
Ad2.4_TCCTGAGC	CAAGCAGAAGACGGCATACGAGATGCTCAGGAGTCTCGTGGGCTCGGAGATGT
Ad2.5_GGACTCCT	CAAGCAGAAGACGGCATACGAGATAGGAGTCCGTCTCGTGGGCTCGGAGATGT
Ad2.6_TAGGCATG	CAAGCAGAAGACGGCATACGAGATCATGCCTAGTCTCGTGGGCTCGGAGATGT
Ad2.7_CTCTCTAC	CAAGCAGAAGACGGCATACGAGATGTAGAGAGGTCTCGTGGGCTCGGAGATGT
Ad2.8_CAGAGAGG	CAAGCAGAAGACGGCATACGAGATCCTCTCTGGTCTCGTGGGCTCGGAGATGT
Ad2.9_GCTACGCT	CAAGCAGAAGACGGCATACGAGATAGCGTAGCGTCTCGTGGGCTCGGAGATGT
Ad2.10_CGAGGCTG	CAAGCAGAAGACGGCATACGAGATCAGCCTCGGTCTCGTGGGCTCGGAGATGT
Ad2.11_AAGAGGCA	CAAGCAGAAGACGGCATACGAGATTGCCTCTTGTCTCGTGGGCTCGGAGATGT
Ad2.12_GTAGAGGA	CAAGCAGAAGACGGCATACGAGATTCCTCTACGTCTCGTGGGCTCGGAGATGT
Ad2.13_GTCGTGAT	CAAGCAGAAGACGGCATACGAGATATCACGACGTCTCGTGGGCTCGGAGATGT
Ad2.14_ACCACTGT	CAAGCAGAAGACGGCATACGAGATACAGTGGTGTCTCGTGGGCTCGGAGATGT
Ad2.15_TGGATCTG	CAAGCAGAAGACGGCATACGAGATCAGATCCAGTCTCGTGGGCTCGGAGATGT
Ad2.16_CCGTTTGT	CAAGCAGAAGACGGCATACGAGATACAAACGGGTCTCGTGGGCTCGGAGATGT
Ad2.17_TGCTGGGT	CAAGCAGAAGACGGCATACGAGATACCCAGCAGTCTCGTGGGCTCGGAGATGT
Ad2.18_GAGGGGTT	CAAGCAGAAGACGGCATACGAGATAACCCCTCGTCTCGTGGGCTCGGAGATGT
Ad2.19_AGGTTGGG	CAAGCAGAAGACGGCATACGAGATCCCAACCTGTCTCGTGGGCTCGGAGATGT
Ad2.20_GTGTGGTG	CAAGCAGAAGACGGCATACGAGATCACCACACGTCTCGTGGGCTCGGAGATGT
Ad2.21_TGGGTTTC	CAAGCAGAAGACGGCATACGAGATGAAACCCAGTCTCGTGGGCTCGGAGATGT
Ad2.22_TGGTCACA	CAAGCAGAAGACGGCATACGAGATTGTGACCAGTCTCGTGGGCTCGGAGATGT
Ad2.23_TTGACCCT	CAAGCAGAAGACGGCATACGAGATAGGGTCAAGTCTCGTGGGCTCGGAGATGT
Ad2.24_CCACTCCT	CAAGCAGAAGACGGCATACGAGATAGGAGTGGGTCTCGTGGGCTCGGAGATGT

Appendix

Inhibitors

Proteaseinhibitors should be added to all adequate buffers (sonication buffer, equilibration buffer, RIPA-LS buffer to bring IP to desired volume) freshly. Proteaseinhibitors can also be added to the IP wash buffers. We also add 1µM PMSF to PBS when washing collected cells.

Consider adding deacetylase/phosphatase inhibitors when ChIPmenting for acetylated/phosphorylated histones or transcription factors (e.g. add 5 mM Na-butyrate to all steps when ChIPmenting acetylated proteins) to all buffers including PBS for cell wash and IP wash buffers.

Sonication buffer

For most of the cells we used so far 0.25% SDS in the sonication buffer was sufficient for good sonication results. If you do not manage to sonicate most fragments to 200-700 bp consider rising SDS concentration to 0.5% or even 1% in the sonication buffer. Please consider that you will need to adjust the sonication equilibration buffer to bring down SDS concentration to 0.1% for the IP, but at the same time equilibrate to RIPA-LS conditions.

Detergent concentration in the IP

It is recommended to have 0.1% SDS final concentration in the IP. However, some antibodies can profit from higher detergent concentrations to reduce background.

Tagmentation time/enzyme amount

We changed the tagmentation time from 10 minutes to 1 minute in the updated version of the protocol. This improved the signal for 500k cells for the antibodies used as indicated on the website. However, we noted decreasing library complexity when only tagmenting for 1 minute in low cell number samples (100k or less). Hence, cell numbers and antibody efficiency both should be considered when deciding on a tagmentation time. As a guideline:

- ChIPmentation reactions using very good antibodies or IgG control should be tagmented for 10 minutes.
- Sensitive antibodies using higher cell numbers (500,000 or more) can be tagmented with less time to increase signal-to-noise. 3-5 minutes is a good starting range, but tagmentation time might be decreased to 1 minute. For very short tagmentation times add ~10 seconds to ensure thorough heating of the whole reaction!
- Enzyme amount: For IPs with extremely low or high DNA recovery it might be beneficial to adjust the Tn5 enzyme amount in the tagmentation reaction.

Input libraries

When ChIPmentation is used as a fast and convenient alternative to ChIP-seq on higher cell numbers it is very easy to prepare a control library by running along an IgG control with the other antibodies and sequence it.

For low cell numbers this is also possible, but has the potential pitfall of reduced library complexity that result in high PCR duplicates upon sequencing. In this case it is possible to tagment reverse-crosslinked DNA according to the ChIP-Tagmentation procedure described in the paper.

As guidance: around 2.5 ng of reverse-crosslinked input DNA can tagmented for 5 minutes at 55°C in a 5 µl tagmentation reaction containing 1 µl of 1:10 diluted transposase. The reaction is then stopped by adding 1 µl of 0.6% SDS, incubated at room temperature for 5 minutes and then topped up with PCR reagents to a 50 µl PCR reaction. PCR setup and cycling should be done as for the normal ChIPmentation reactions, amplifying for ~12 cycles.

PCR reagents

It is important to use the PCR reagents, oligos and cycling parameters indicated here. Always preheat hot-start enzymes to activate them, as the initial nick translation at 72°C is critical for amplifications!

MNase digestion

We have not tried MNase digestion instead of sonication and would be grateful if you'd share your insights with us if you tried it!