CeMM- ChIPmentation protocol v1.1 (2015/10/14)

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Paper website: http://chipmentation.computational-epigenetics.org

Improvements from version v1.0 to v1.1 (highlighted in red in the "Experimental procedure" part)

- Tagmentation time is reduced, which improves results for difficult antibodies, but also works well for good antibodies.
- Tagmentation buffer is changed to a buffer containing dimethylformamide, which can increase tagmentation efficiency.
- Two "tube transfers" are included (one before and one after the tagmentation reaction) to decrease background.

Experimental procedure

ChIPmentation is compatible with various protocols for ChIP, which makes it easy to apply ChIPmentation to antibodies that work best with a certain ChIP protocol. In the original manuscript, we used 3 slightly different protocols. To use ChIPmentation:

1. Follow the ChIP protocol of choice until the beads carrying immunoprecipitated chromatin were washed with LiCl-containing wash buffer (WBIII for ChIP version 1, RIPA-LiCl for ChIP version 2, and TF-WBIII for ChIP version 3, as described below in **Chromatin immunoprecipitation protocols;** it is marked with * where to start with the ChIPmentation procedure).
2. Wash beads once with cold Tris-Cl pH 8.0 to remove detergent, salts, and EDTA.
3. Wash beads once more with cold Tris-Cl pH 8.0 but do not place the reaction on a magnet to discard supernatant immediately. Instead, transfer the whole reaction including beads to a new tube, then place on a magnet to remove supernatant. This will decrease tagmentation of unspecific chromatin fragments sticking to the tube wall.
4. Resuspend beads carefully in 25 µl of the tagmentation reaction mix (10 mM Tris pH 8.0, 5 mM $MgCl_2$, 10 % v/v dimethylformamide) containing 1 µl Tagment DNA Enzyme from the Nextera DNA Sample Prep Kit (Illumina) and incubate at 37°C for 1 minute in a thermocycler.
5. Place tagmentation reaction on a cold magnet, discard the supernatant and wash beads twice with WBI (ChIP version 1), RIPA (ChIP version 2), or TF-WBI (ChIP version 3).
6. Continue with the ChIP protocol, but again transfer the reaction in a new tube when washing for the second time with WBIV (ChIP version 1), TE (ChIP version 2), or TET (ChIP version 3). This will decrease carry-over of tagmented unspecific fragments sticking to the tube wall.

Consideration using your own ChIP protocol

It is recommended to wash beads with a buffer containing SDS/EDTA to inactivate and strip the transposase from DNA after tagmentation.

Improvements of ChIPmentation

Although the ChIPmentation procedure described in the paper is very robust across many antibodies and applications, optimizations might be beneficial for difficult antibodies or when using very low input amounts.

We will share our experience using ChIPmentation with many more samples and antibodies- please check our website for updated ChIPmentation protocols (http://chipmentation.computational-epigenetics.org). In addition, if you have any suggestions, improvements or questions regarding ChIPmentation please feel free to contact us!

Chromatin immunoprecipitation protocols

ChIPmentation was tested and validated in combination with three different chromatin immunoprecipitation (ChIP) protocols that are described below.

ChIP version 1 (used for H3K4me3 and H3K27me3)

Cells were washed once with PBS and fixed with 1% paraformaldehyde in up to 1 ml PBS for 5 minutes at room temperature. Glycine was added to stop the reaction. Cells were collected at 500 x g for 10 minutes at 4°C (subsequent work was performed on ice and used cool buffers and solutions unless otherwise specified) and washed twice with up to 1 ml ice-cold PBS supplemented with 1 µM PMSF. The pellet was lysed in Cell Lysis Buffer (50 mM HEPES/KOH pH 7.4, 140 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 10% Glycerol, 0.5% NP-40, 0.25% Triton X-100, 1x protease inhibitors (Sigma)) for 10 minutes on ice. Nuclei were isolated by spinning the lysed cells for 10 minutes at 1,000 x g at 4°C, the supernatant was discarded, and the pellet was resuspended in Sonication Buffer (10 mM Tris-HCl pH 7.6, 1 mM EDTA, 0.1% SDS) and sonicated in a 130 µl microTUBE (for up to 3 x 10^6 cells) on a Covaris S220 for 12 minutes until most of the fragments were 200-700 base pairs long (settings: duty cycle 2%, peak incident power 105 Watts, cycles per burst 200). Lysates were centrifuged at full speed for 5 minutes at 4°C and the supernatant was transferred to a new tube. The lysate was adjusted to 200 µl per IP with a buffer composition of 20 mM HEPES, 0.1% SDS, 1%Triton X-100, 150 mM NaCl, 1 mM EDTA, 0.5 mM EGTA and incubated with an antibody against H3K4me3 (1 µg/IP, Diagenode pAb-003-050) or H3K27me3 (1 µg/IP, Diagenode pAb-195-050) overnight at 4°C on a rotator. 20 µl of Protein A (or Protein G, dependent on the antibody) magnetic beads were blocked overnight with 0.1% BSA in PBS and added to the IP the next day for 2 hours on a rotator at 4°C to capture the immunoprecipitated fragments. The immunoprecipitated chromatin was washed subsequently with WBI (20 mM HEPES, 150 mM NaCl, 0.1% SDS, 0.1% DOC, 1% Triton X-100, 1 mM EDTA, 0.5 mM EGTA) (twice), WBII (20 mM HEPES, 500 mM NaCl, 0.1% SDS, 0.1% DOC, 1% Triton X-100, 1 mM EDTA, 0.5 mM EGTA) (once), WBIII (20 mM HEPES, 250 mM LiCl, 0.5% DOC, 0.5% NP-40, 1 mM EDTA, 0.5 mM EGTA) (once), ***(start ChIPmentation part here)**, and WBIV (20 mM HEPES, 1 mM EDTA, 0.5 mM EGTA) (twice). Beads were then incubated with 70 µl elution buffer (0.5% SDS, 300 mM NaCl, 5 mM EDTA, 10 mM Tris-HCl pH 8.0) containing 2 µl of Proteinase K (NEB) for 1 hour at 55°C and 8 hours at 65°C to revert formaldehyde crosslinking, and supernatant was transferred to a new tube. Another 30 µl of elution buffer was added to the beads for 1 minute, and eluates were combined and incubated with another 1 µl of Proteinase K for 1 hour at 55°C. Finally, DNA was purified with SPRI AMPure XP beads (sample-to-beads ratio 1:2) or Qiagen MinElute columns.

ChIP version 2 (used for H3K4me1, H3K36me3, and REST)

Cells were washed once with PBS and fixed with 1% paraformaldehyde in up to 1.5 ml PBS for 10 minutes at room temperature. Glycine was added to stop the reaction. Cells were collected at 500 x g for 10 minutes at 4°C (subsequent work was performed on ice and used cool buffers and solutions unless otherwise specified) and washed twice with up to 1 ml ice-cold PBS supplemented with 1 µM PMSF. The pellet was lysed in RIPA buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0, 140 mM NaCl, 1% Triton x-100, 0.1% SDS, 0.1% DOC, 1x protease inhibitors (Sigma)) and sonicated in a 1 ml milliTUBE in a Covaris S220 for 30 minutes until most of the fragments were 200-700 base pairs long (settings: duty cycle 5%, peak incident power 140 Watts, cycles per burst 200). Lysates were centrifuged at full speed for 5 minutes at 4°C, and the supernatant containing the sonicated chromatin was transferred to a new tube. In parallel, 50 µl (10 µl for low-input ChIPmentation) magnetic Protein A (or Protein G, dependent on the antibody) were blocked and conjugated to an antibody by washing and resuspending twice in PBS, 0.5% BSA, 0.5% Tween-20. The antibody was added and bound to the beads by rotating >1 hour at room temperature. Used antibodies were H3K4me1 (1 µg/IP, Diagenode pAb-194-050), H3K36me3 (1 µg/IP, Diagenode pAb-192-050), and REST (10 µg/IP, Millipore 07-579). Blocked antibody-conjugated beads were then placed on a magnet, supernatant was removed, and the sonicated lysate was added to the beads followed by incubation for 3 hours at 4°C on a rotator. Beads were washed subsequently with RIPA (twice), RIPA-500 (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0, 500 mM NaCl, 1% Triton x-100, 0.1% SDS, 0.1% DOC,) (twice), RIPA-LiCl (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0, 250 mM LiCl, 1% Triton X-100, 0.5% DOC, 0.5% NP40), ***(start ChIPmentation part here)**, and TE pH 8.0 (twice). Beads were then incubated with 70 µl elution buffer (0.5% SDS, 300 mM NaCl, 5 mM EDTA, 10 mM Tris-HCl pH 8.0) containing 2 µl of Proteinase K (NEB) for 1 hour at 55°C and 8 hours at 65°C to revert formaldehyde crosslinking, and supernatant was transferred to a new tube. Finally, DNA was purified with SPRI AMPure XP beads (sample-to-beads ratio 1:2) or Qiagen MinElute columns.

ChIP version 3 (used for H3K27ac, PU.1, CTCF, and GATA1)

Cells were washed once with PBS and fixed with 1% paraformaldehyde in up to 1.5 ml PBS for 5-10 minutes at room temperature. Glycine was added to stop the reaction. Cells were collected at 500 x g for 10 minutes at 4°C (subsequent work was performed on ice and used cool buffers and solutions unless otherwise specified) and washed twice with up to 1 ml ice-cold PBS supplemented with 1 µM PMSF. The pellet was lysed in buffer L3B (10 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 0.1% Na-Deoxycholate, 0.5% N-lauroylsarcosine, 1x protease inhibitors (Sigma)) and sonicated in a 1ml milliTUBE in a Covaris S220 for 20 minutes until most of the fragments were 200-700 base pairs long (settings: duty cycle 5%, peak incident power 140 Watts, cycles per burst 200). Lysates were supplemented with 1% Triton-X-100 and centrifuged at full speed for 5 minutes at 4°C, and the supernatant containing the sonicated chromatin was transferred to a new tube. In parallel, beads were blocked and conjugated to an antibody by washing them twice in PBS with 0.5% BSA and resuspending 50 µl (10 µl beads for low-input ChIPmentation) of magnetic Protein A (or Protein G, dependent on the antibody) per IP in 200 µl of PBS with 0.5% BSA. The antibody was added and bound to the beads by rotating >1 hour at room temperature. Used antibodies were H3K27ac (2 µg, Diagenode pAb-196-050), PU.1 (5 µg/IP, Santa Cruz sc-352), CTCF (10 µl/IP, Millipore 07-729), and GATA1 (4 µg/IP and 2 µg for low-input, Abcam ab11852). Blocked antibody conjugated magnetic beads were added to the tube containing the chromatin and incubated for 3 hours at 4°C. Beads were washed subsequently with TF-WBI (20 mM Tris-HCl/pH 7.4, 150 mM NaCl, 0.1% SDS, 1% Triton X-100, 2 mM EDTA) (twice), TF-WBIII (250 mM LiCl, 1% Triton X-100, 0.7% DOC, 10 mM Tris-HCl, 1 mM EDTA) (twice), ***(start ChIPmentation part here)**, and TET (0.2% Tween-20, 10 mM Tris-HCl/pH 8.0, 1 mM EDTA) (twice). Beads were then incubated with 70 µl elution buffer (0.5% SDS, 300 mM NaCl, 5 mM EDTA, 10 mM Tris HCl pH 8.0) containing 2 µl of Proteinase K (NEB) for 1 hour at 55°C and 8 hours at 65°C to revert formaldehyde crosslinking, and supernatant was transferred to a new tube. Another 30 µl of elution buffer was added to the beads for 1 minute and eluates were combined and incubated with another 1 µl of Proteinase K for 1 hour at 55°C. Finally, DNA was purified with SPRI AMPure XP beads (sample-to-beads ratio 1:2) or Qiagen MinElute columns.