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H3K27Ac High-Throughput ChIPmentation protocol for 10k cells compatible with 96-well format

PFA fixation of cells (after FACS antibody and viability dye staining)

1. Suspend cells in PBS+2%FCS to 10mil cells/ml.
2. Add 1 volume of 2% PFA (formaldehyde) freshly diluted in PBS.
3. Mix well by inverting tube, incubate 10min RT rotating dark (to avoid fluorochrome bleaching).
4. Add 0.1 volume of 1M glycine in PBS.
5. Mix well by inverting tube, incubate 10min RT rotating dark.
6. Spin down 2000g 10min 4°C.
7. Wash with 0.1M glycine in PBS (volume similar to fixation volume).
8. Spin down 2000g 10min 4°C.
9. Re-suspend cells in PBS+2%FCS and FACS sort if desired, otherwise store pelleted cells in -80°C until use in ChIP experiment. 10k cells can be sorted directly into 100µl SDS lysis buffer with 2 µL of 50x protease inhibitors and stored in -80°C.

Wash and pre-load beads with antibodies

1. Prepare PBS with 0.5% BSA (*prepare in advance to solve)*. Filter through a 0.22 µm filter.
2. Vortex Protein G-coupled Dynabeads for 1min.
3. For each 10k cell H3K27Ac ChIP, take out 2µL dynabeads and add the total volume to one eppendorf tube. If needed (volume > 50ul) place tube on magnet for 1min to collect beads and discard supernatant with pipette.
4. Add 1mL PBS with 0.5% BSA. Wash by rotating for 3min at RT.
5. Place tubes on magnet for 1min to collect beads and discard supernatant with pipette.
6. Wash once more with 1mL PBS with 0.5% BSA (as above, step 4-5).
7. Resuspend beads in 200µL (or 200µl per up to 50µl of beads) PBS with 0.5% BSA for antibody binding.
8. Per 10k ChIP, add 0.2µl (0.6µg) anti H3K27Ac antibody to the resuspended beads*.
9. Incubate rotating at 4°C for 4h.

Sonication of cells

1. If pelleted, resuspend cells in 100 µL of SDS Lysis Buffer and add 2 µL of 50x protease inhibitors.
2. Put tubes in fridge >15min (not on ice, to avoid precipitation of SDS).
3. Sonicate 12min x 30s ON/30s OFF on max power on a Bioruptor Plus.
4. Neutralize SDS by adding 6µl of 20% triton X100 (≈1% final concentration) and add 2µl 50x protease inhibitors.
5. When applicable, take out a 10µl aliquot of the sample (1000 cell equivalents) for input control preparation. To the 10% aliquot, add 2µl 50mM $MgCl_2$ (≈10mM final concentration in 10µl to inactivate EDTA before tagmentation), save input control at +4°C. Continue to the ChIP step with the rest of the sample.

ChIP:

1. Place tube with antibody coated beads (previously prepared) on magnet to collect beads and remove supernatant with pipette.
2. Add 1mL PBS/0.5% BSA, rotate at RT for 3min, put in magnet for 1min to collect beads and remove supernatant. Repeat for a total of two washes.
3. Resuspend antibody coated beads in 50µl ChIP dilution buffer per ChIP reaction (containing 2µl original bead volume) and add 50µl to sonicated cell-lysate from 10k cells.
4. Incubate antibody coated beads and sonicated cell-lysate rotating over night at 4°C.

* If using more than 10k cells, use 10µl dynabeads and 3µg (1.1µl) antibody per reaction. This is also a good rule of thumb for testing other antibodies.

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Washing and tagmentation:

1. Place strips or plate with immunoprecipitated bead bound chromatin on magnet for 1min to collect beads and remove supernatant with pipette.
2. Wash bead bound chromatin once with WB I, WB II, WB III and twice with TE buffer as follows: Resuspend beads in 150µL of cold buffer off magnet, place back on magnet and let beads pass through buffer ten times by moving strips/plate in relation to magnetic parts. Remove supernatant with pipette and repeat with next buffer.
3. Wash twice with cold Tris-HCl (10mM, pH8) as described above (step 2). Take care to remove all supernatant after the second wash.
4. Resuspend bead bound chromatin in 30µl 1x Tagment DNA buffer (dilute 2x stock in water) with 1µl Tagment DNA enzyme (Illumina).
5. To input control chromatin (12µl), add 7µl water, 20µl 2x Tagment DNA buffer and 1µl Tagment DNA enzyme.
6. Incubate ChIP and input control samples at 37°C for 10 minutes.
7. Wash tagmented bead bound chromatin twice with WBI, as in step 2. Note that beads do not stick as well in the tagmentation buffer, so be careful and leave some buffer before adding WBI.
   Omit this step for input controls as chromatin is not bound to beads.
8. Resuspend bead bound chromatin in 20µl nuclease free water. Add 25µl PCR MM (NEB) +5µl primer mix** (1µM).
9. For input control, split the 40µl tagmented chromatin into two reactions, adding 25µl PCR MM and 5µl primer mix to each. Amplify ChIP and input libraries using the following PCR protocol:

   PCR protocol:
   72°C 5 min
   95°C 5 min
   98°C 30 sec
   11 cycles of:
   98°C 10 sec
   63°C 30 sec
   72°C 3 min
   ∞ 4°C

10. Place strips or plate on magnet for 1 min to collect Protein G-coupled beads used in ChIP, transfer supernatant to new strips/plate. Pool the two reactions per input ctrl into one single tube.
11. Run a SPRI bead cleanup at the ratio 1:1 x1 by adding 50µl SPRI beads (100ul for input ctrls), mix by pipetting and incubate at RT for 5 min. SPRI beads should be at RT before use (take from fridge 30 min prior to use).
12. Place samples on magnet for 5 min. Remove and discard 98µl (198 for input ctrls) of supernatant with pipette.
13. While still on magnet, add 200µl of 80% EtOH, wait 30s then remove and discard supernatant with pipette. Repeat once.
14. If available, spin strips or plate down quickly and put back on magnet.
15. Carefully remove any remaining EtOH with pipette, take samples off magnet and leave to dry with lid open for 3 minutes (do not over-dry the beads).
16. Elute chromatin from beads by adding 13ul of H2O and incubate for 2 minutes. Place samples on magnet for 3 minutes and carefully remove 10-12µl of final library while avoiding the beads.
17. Quality check samples with Qubit and tape station.

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Reagents and solutions:
16% Formaldehyde w/v, methanol free, Thermo Scientific, cat# 28906
Protein G-coupled Dynabeads, Invitrogen, cat#10003D
Anti-H3K27Ac antibody, conc 2.8µg/µl, Diagenode cat# C15410196 Lot#A1723-0041D
Illumina Tagment DNA TDE1 Enzyme and Buffer Kit, cat#20034197 or #20034198
NEBNext High-Fidelity 2X PCR Master Mix or similar non hot start enzyme, NEB, cat# M0541S
SPRI beads such as AMPure XP beads, Agencourt, cat#A63880
Bovine serum albumin (BSA) Sigma Aldrich cat#A9418

** Library amplification primers (adapted from Buenrostro et al. doi:10.1038/nmeth.2688, bought from Integrated DNA Technologies):
`Ad1_noIndex: AATGATACGGCGACCACCGAGATCTACACTCGTCGGCAGCGTCAGATGTG`
`Ad2_withIndex: CAAGCAGAAGACGGCATACGAGAT[index]GTCTCGTGGGCTCGGAGATGT`
Combine at 10µM stock solution: For 100µl add 10µl each of 100µM primer Ad1-noIndex and primer Ad2_withIndex to 80µl of ddH_2O. For working solution, dilute 1:10 in ddH_2O to 1µM and use 5µL per reaction.

	Index to use in sample sheet:	**Reverse complement index for use in primers:**
Ad2.1	TAAGGCGA	TCGCCTTA
Ad2.2	CGTACTAG	CTAGTACG
Ad2.3	AGGCAGAA	TTCTGCCT
Ad2.4	TCCTGAGC	GCTCAGGA
Ad2.5	GGACTCCT	AGGAGTCC
Ad2.6	TAGGCATG	CATGCCTA
Ad2.7	CTCTCTAC	GTAGAGAG
Ad2.8	CAGAGAGG	CCTCTCTG
Ad2.9	GCTACGCT	AGCGTAGC
Ad2.10	CGAGGCTG	CAGCCTCG
Ad2.11	AAGAGGCA	TGCCTCTT
Ad2.12	GTAGAGGA	TCCTCTAC
Ad2.13	GTCGTGAT	ATCACGAC
Ad2.14	ACCACTGT	ACAGTGGT
Ad2.15	TGGATCTG	CAGATCCA
Ad2.16	CCGTTTGT	ACAAACGG
Ad2.17	TGCTGGGT	ACCCAGCA
Ad2.18	GAGGGGTT	AACCCCTC
Ad2.19	AGGTTGGG	CCCAACCT
Ad2.20	GTGTGGTG	CACCACAC
Ad2.21	TGGGTTTC	GAAACCCA
Ad2.22	TGGTCACA	TGTGACCA
Ad2.23	TTGACCCT	AGGGTCAA
Ad2.24	CCACTCCT	AGGAGTGG

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Volume	(Final conc)	Stock solution
SDS Lysis Buffer 50ml		
2.5 ml	50 mM	Tris/HCl (1 M), pH 8.0 @20°
2.5 ml	0.5 %	SDS (10 %)
1 ml	10 mM	EDTA (0.5 M), pH 8.0
44 ml		*ddH_2O*
ChIP Dilution Buffer 50ml		
2.5 ml	50 mM	Tris/HCl (1 M), pH 8.0 @20°C
2.25 ml	225 mM	NaCl (5 M)
750 ul	0.15 %	NaDOC (10 %)
7.5 ml	1.5 %	Triton X-100 (10 %)
37.0 ml		*ddH_2O*
Wash Buffer I (WB I) 50ml		
2.5 ml	50 mM	Tris/HCl (1 M), pH 8.0 @20°C
1.5 ml	150 mM	NaCl (5 M)
500 µl	0.1 %	SDS (10 %)
500 ul	0.1 %	NaDOC (10 %)
5 ml	1 %	Triton X-100 (10 %)
100 µl	1 mM	EDTA (0.5 M, pH 8.0)
39.9 ml		*ddH_2O*
Wash Buffer II (WB II) 50ml		
2.5 ml	50 mM	Tris/HCl (1 M), pH 8.0 @20°C
5 ml	500 mM	NaCl (5 M)
500 µl	0.1 %	SDS (10 %)
500 ul	0.1 %	NaDOC (10 %)
5 ml	1 %	Triton X-100 (10 %)
100 µl	1 mM	EDTA (0.5 M, pH 8.0)
36.4 ml		*ddH_2O*
Wash Buffer III (WB III) 50ml		
500 µl	10 mM	Tris/HCl (1 M), pH 8.0 @20°C
6.25 ml	250 mM	LiCl (2 M)
2.5 ml	0.5 %	NP-40 (10 %)
2.5 ml	0.5 %	NaDOC (10 %)
100 µl	1 mM	EDTA (0.5 M, pH 8.0)
38.15 ml		*ddH_2O*
Tris-EDTA (TE) buffer 50ml		
500 µl	10 mM	Tris/HCl (1 M), pH 8.0 @20°C
100 µl	1 mM	EDTA (0.5 M, pH 8.0)
49.4 ml		*ddH_2O*

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