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ChIP-seq guidelines for histone marks on skin tissue sample

CONTENT

Required materials

Reagents

iDeal ChIP-seq kit for Histones (Diagenode Cat.# C01010051)

H3K4me1 polyclonal antibody - Premium (Diagenode Cat.# C15410194)
H3K4me3 polyclonal antibody - Premium (Diagenode Cat.# C15410003)
H3K27me3 polyclonal antibody - Premium (Diagenode Cat.# C15410195)
H3K27ac polyclonal antibody - Premium (Diagenode Cat.# C15410196)
MicroChIP Diapure columns (Diagenode Cat.# C03040001)

MicroPlex Library Preparation Kit v2 (Diagenode Cat# C05010012)

Shearing buffer from HighCell ChIP kit (Diagenode Cat.# C01019021)
LowCell Buffer A (Diagenode Cat.# C01019016)

Gloves to wear at all steps
Formaldehyde, 37%, Molecular Grade
Phosphate buffered saline (PBS) buffer
RNase/DNase-free 1.5/2 ml tubes
qPCR SYBR® Green Mastermix
Quant-IT dsDNA HS assay kit (Invitrogen)

Equipment

Diagenode Bioruptor® Pico sonication device (Cat.# B01060001)
1.5 ml Bioruptor® Microtubes with Caps (Cat.# C30010016)
IP Star® Compact Automated system (Cat.# B03000002)

Refrigerated centrifuge for 1.5 ml, 15 ml and 50 ml tubes
Rotating wheel
Vortex
Thermomixer
Qubit system (Invitrogen) qPCR cycler
Scalpel blades - Petri dishes
Dounce homogenizers
Tissue Lyser II (Qiagen)

Tissue disaggregation and DNA-protein cross-linking (for skin frozen tissues)

1. Weigh approximately 120 mg of frozen skin tissue in a petri dish. Keep samples on dry ice at all times and minimize the time of manipulation to prevent sample degradation.
2. Chop tissue into small pieces (between 1-3 mm3) using a scalpel blade.
3. Transfer the chopped tissue in a 2ml tube with 2 metal beads. Homogenize on a Tissue Lyser II (Qiagen) for 2min at 30 strockes/sec.
4. Resuspend the powdered tissue in 2 ml of PBS containing 1% of formaldehyde at RT.
5. Fix for a total of 9min by homogenizing in a dounce homogenizer and by rotating.
6. Stop the cross-linking reaction by adding 200 µl of glycine. Continue to rotate at RT for 5 min.
7. Centrifuge samples at 850g for 5min at 4°C.
8. Wash the pellet twice with 10ml cold PBS.

Cell lysis and chromatin shearing (derived from skin tissue samples)

9. Add 400 µl of lyis/sonication buffer (HighCell ChIP buffer + Protease Inhibitor Cocktail).
10. Homogenize using Tissue Lyser II (Qiagen) for 3 minutes at 25 strockes/sec.
11. Split the samples into 200 µl aliquots in 1.5 ml sonication tubes and incubate on ice for 10 min. Please use only recommended tubes: For Bioruptor® Pico, 1.5 ml Bioruptor® Microtubes with Caps (Cat. No. C30010016)
12. Shear chromatin by sonication using the Bioruptor®. An initial time course experiment is highly recommended.
13. For Bioruptor® Pico, sonicate samples for 8 cycles (30 sec ON/30 sec OFF).
14. Transfer samples to new 1.5 ml tubes and centrifuge at 16000g for 10 min at 4°C.
15. Collect the supernatant which contains the sheared chromatin.
16. Take an aliquot of 20-50 µl for assessment of chromatin shearing.

Magnetic immunoprecipitation

17. Set aside 1% of the sheared chromatin to use as input sample and keep at 4°C.
18. Prepare the following ChIP reaction mix (1 IP):

- x µl of 200x protease inhibitor cocktail
- x µl of 5% BSA (50x concentrated)
- x µl of LowCell buffer A (9x the volume of the chromatin used for each ChIP. Ex: 180µl for 20µl of chromatin)
- x µl of sheared chromatin. Start with the same amount of chromatin for each condition and equilibrate with lysis/sonication buffer.
- z µl ChIP-seq grade antibody (0.5 µg-1 µg)

19. Rotate at 40 rpm for 16 hours at 4°C.
20. Take the required amount of DiaMag Protein A-coated magnetic beads (20 µl/IP).
21. Add 110µl of Beads Wash Buffer (Low cell buffer A + PIC + BSA) to 22µl of beads and resuspend. Rotate at 4°C for 5min. Place the beads in the magnetic rack. Discard the supernatant and keep bead pellet. Repeat this wash four times; during the last wash extend the incubation time to 45min to block non-specific sites.
22. Resuspend bead pellet in 22 µl Beads Wash Buffer per IP.

23. Add 20 µl of washed beads to each IP and rotate at 40 rpm for 2 hours at 4°C.
24. Place IP tubes in the magnetic rack. Keep the bead captured and remove the supernatant. Add 350 µl of Wash Buffer W1 and rotate at 40 rpm for 5 minutes at 4°C.
25. Repeat Step 24 once with Wash Buffers W2, W3 and W4, respectively.

Elution, decross-linking and DNA purification

26. Briefly spin the tubes and place them into the Diagenode magnetic rack. Remove the supernatant and add 100µl of buffer iE1.
27. Rotate at 40 rpm for 30 minutes at RT.
28. Briefly spin the tubes and place them into the Diagenode magnetic rack. Transfer the supernatant to a new tube and add 4 µl of Elution buffer iE2. Also add 100 - x µl buffer iE1 and 4 µl of buffer iE2 to the x µl input sample. Incubate for 4 hours at 65°C at 900 rpm.
29. Purify the DNA using the Diapure columns.
30. Purified DNA was quantified using the Qubit ds DNA HS kit.
31. Store ChIP DNA, and Input DNA at -20C°.

Library preparation for sequencing

Libraries were prepared using IP-Star® Compact Automated System (Diagenode Cat# B03000002) from input and ChIP'd DNA using MicroPlex Library Preparation Kit v2 (Diagenode Cat# C05010013). Optimal library amplification was assessed by qPCR using KAPA SYBR® FAST (Sigma-Aldrich) on LightCycler® 96 System (Roche) and by using High Sensitivity NGS Fragment Analysis Kit (DNF-474) on a Fragment Analyzer™ (Agilent). Libraries were then purified (double size-selected) using Agencourt® AMPure® XP (Beckman Coulter) and quantified using Qubit™ dsDNA HS Assay Kit (Thermo Fisher Scientific, Q32854). Finally their fragment size was analysed by High Sensitivity NGS Fragment Analysis Kit (DNF-474) on a Fragment Analyzer™ (Agilent).