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Innovating Epigenetic Solutions

ChIP-seq guidelines for histone marks on Bone tissue

CONTENT

Required materials

True-microchip-kit (Diagenode Cat.# C01010130)
Universal Plant ChIP-seq kit (Diagenode Cat.# C01010152)

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)

Gloves to wear at all steps
Formaldehyde, 37%, Molecular Grade
Phosphate buffered saline (PBS) buffer
RNase/DNase-free 1.5 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
Tissue Lyser II (Qiagen)
Mortar and pestle

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

1. Weigh 600 mg of frozen Sesamoid or MC3 bone tissue in a petri dish. Keep samples on dry ice at all times and minimize the time of manipulation to prevent sample degradation.
2. Crush tissue into small pieces (between 1-3 mm3) using mortar and pestle in liquid nitrogen.
3. Reduce the pieces in powder using Tissue Lyser II (Qiagen) for 4 minutes at 30 strokes/sec.
4. Resuspend the chopped tissue in 1,5 ml of PBS containing 1% of formaldehyde at room temperature (RT).
5. Rotate/agitate tube for 10 min at RT.
6. Stop the cross-linking reaction by adding 150 µl of glycine. Continue to rotate at RT for 5 min.
7. Centrifuge samples at low speed (1,300 rpm) at 4°C.
8. Wash the pellet with 1 ml of PBS plus protease inhibitors.
9. Centrifuge at low speed (1,300 rpm) for 10 min at 4°C and discard the supernatant.
10. Wash the pellet with 1 ml of PBS plus protease inhibitors.
11. Centrifuge at low speed (1,300 rpm) for 10 min at 4°C and discard the supernatant.

Cell lysis and chromatin shearing (derived from tissue samples)

12. Add 600 µl of lysis/sonication buffer from True-Microchip kit (600 µl of tL1 buffer + Protease Inhibitor Cocktail).
13. Homogenize using Tissue Lyser II (Qiagen) for 3 minutes at 25 strokes/sec.
14. Split the samples into 200 µl aliquots in 1.5 ml sonication tubes. Please use only recommended tubes:
    For Bioruptor® Pico, 1.5 ml Bioruptor® Microtubes with Caps (Cat. No. C30010016)
15. Shear chromatin sequentially by sonication using the Bioruptor®. An initial time course experiment is highly recommended.

For Bioruptor® Pico, shear samples using 3 sequential sonication of 10 cycles (30 sec ON/30 sec OFF):
After the first round of 10 cycles sonication (30 sec ON/30 sec OFF) at 10°c, transfer samples to new 1.5 ml tubes and centrifuge at 13,000 rpm for 10 min at 10°c. Collect the supernatant which contains the sheared chromatin (fraction 1).

Add another 600 µl of lysis/sonication buffer to the pellet which is leftover and transfer it to a new sonication tube. Repeat the sonication for another 10 cycles (30 sec ON/30 sec OFF) at 10°c. Transfer samples to new 1.5 ml tubes and centrifuge at 13,000 rpm for 10 min at 10°c. Collect the supernatant which contains the sheared chromatin (fraction 2).

Repeat the steps described above in order to collect 3 different chromatin fractions for each sample.

16. Take an aliquot of 20-50 µl for assessment of chromatin shearing for each fraction of each sample.

Magnetic immunoprecipitation

17. Take the required amount of DiaMag Protein A-coated magnetic beads (10-30 µl/IP).
18. Set aside 10% of the sheared chromatin to use as input sample and keep at 4°C.

19. Prepare the following ChIP reaction mix (1 IP) :

    x µl of 200x protease inhibitor cocktail

    9y µl of ChIP Dilution Buffer Plant (9 volumes of the chromatin volume used for each ChIP). Meaning that the chromatin is diluted 10X before starting the immunoprecipitation (ex : 90µl of ChIP Dilution Buffer Plant are added to 10 µl of chromatin).

    y µl of sheared chromatin. Start with the same amount of chromatin (250-1500 ng) for each condition and equilibrate with (tL1).

    z µl ChIP-seq grade antibody (0.5 µg-1 µg)

20. Rotate at 40 rpm for 16 hours at 4°C.
21. Wash the beads. Add 110 µl of ChIP dilution buffer plant to 22 µl of beads (per IP) and resuspend. Place the beads in the magnetic rack. Discard the supernatant and keep bead pellet. Repeat this wash twice. Then resuspend bead pellet in 22 µl ChIP dilution buffer plant per IP.
22. Add 20 µl of washed beads to each IP and rotate at 40 rpm for 2 hours at 4°C.
23. Place IP tubes in the magnetic rack. Keep the bead captured and remove the supernatant. Add 100 µl of Wash Buffer tW1 and rotate at 40 rpm for 4 minutes at 4°C.
24. Repeat Step 23 once with Wash Buffer tW2, tW3 and tW4, respectively.

Elution, decross-linking and DNA purification

25. Place IP tubes in the magnetic rack. Keep the beads captured and remove the supernatant.
26. Add 100 µl of Elution Buffer tE1 and rotate at 40 rpm for 45 minutes at room temperature.
    Also add 100-x µl of Elution Buffer tE1 to x µl of input (corresponding to 10% of the chromatin engaged in the ChIP). Work with both input and IP sample in parallel for remaining steps.
27. Place tubes in the magnetic rack. Transfer the supernatant into new tubes.
28. Add 4 µl of Elution buffer tE2. Incubate for 4 hours at 65°C.
29. Purify the DNA using the Diapure columns.
30. Purified DNA was quantified using the Qubit ds DNA HS kit
31. Store ChIPed DNA, and Input DNA at -20C°.

Library preparation for sequencing

After the ChIP, library preparation is performed using the MicroPlex Library PreparationTM kit v2 (Diagenode Cat.# C05010012) protocol for each ChIP sample and for the inputs. Library preparation was performed on IP Star Compact. According to the protocol, 10-12 cycles of amplification were performed to amplify the libraries. After amplification, 1 µl of each library was loaded on Fragment Analyser to check if enough material was generated. If not, additional cycles were performed until having enough material. The additional PCR cycles were performed progressively until reaching enough amount of library for each sample.