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ChIP-seq protocol

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Protocol status: Working
We use this protocol and it's working

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Abstract

ChIP-seq protocol for ESC-DE differentiation

ChIP-seq protocol

1 For each sample, around 30 million cells were crosslinked with 1% formaldehyde and quenched with 0.125M glycine.

2 Fixed cells were then lysed in 700uL SDS buffer (1% SDS, 10 mM EDTA, 50 mM Tris–HCl, pH 8), and incubated for 10min on ice.

3 Sonication was performed on a Branson Sonifier 150 set at 30% amplitude for 5min30s total on (10s on/off pulsing).

4 Clear supernatant was collected for antibody binding overnight, followed by Dynabeads (Thermo Fisher Scientific;10004D) incubation for 6 hours at 4°C.

5 Then the beads were pelleted and washed twice with low salt (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris–HCl, pH 8, 150 mM NaCl), high salt (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris–HCl, pH 8, 500 mM NaCl), and TE buffer (10 mM Tris–HCl, pH 8, 1 mM EDTA) respectively.

6 The DNA was eluted from the beads by incubating in elution buffer (1% SDS, 0.1 M NaHCO3) at 65°C for 15min and decrosslinked with 5M NaCl at 65°C overnight.

7 A total of 10 µl 0.5 M EDTA, 20 µl 1 M Tris–HCl, pH 6.5, and 1 µl Proteinase K (20 mg ml−1) were added to decrosslinked product and incubated for 1 h at 45°C.

8 DNA was isolated by using QIAquick PCR purification kit (Qiagen, 28104; QIAGEN).

9 Then the sequencing library was generated by using the NEBNext® Ultra II DNA Library Prep Kit (New England Biolabs; E7103S) and NEBNext® Multiplex Oligos for Illumina® (New England Biolabs Index Primers Set 1; NEB, E7335S).

10 Samples were pooled and submitted to MSKCC Integrated Genomics Operation core for quality control and sequencing on Illumina HiSeq 4000 platform.