Chromatin Immunoprecipitation (ChRIP) Assay

1. Add 1% formaldehyde to the medium, 10 min at room temperature on a rocker
2. Add 125 mM glycine (5 min at RT)
3. Rinse cells twice with PBS
4. Scrape cells of the plates on ice with cold PBS,
5. Sediment cells (4°C, 3.000 rpm, 3 min)
6. Resuspend about 5 x10^6 cells in 1ml Buffer A, incubate 15 min on ice followed by 15 min at 30°C
7. Spin down cells (4°C, 5.000 rpm, 3 min), resuspend pellet in 1ml Buffer B, incubate 5 min on ice
8. Spin down cells, resuspend pellet in 1ml Buffer C, incubate for 5 min on ice
9. Spin down cells, resuspend pellet in 600 ul Buffer D
10. Shear chromatin to fragments of 300-500 bp by sonication (we use a Bioruptor from Diagenode with two times 10 cycles, 0.5 min on/ 0.5min off, high power)
11. Centrifuge (12.000 rpm, 10 min), dilute the supernatant with 5 volumes IP buffer, add 100 U/ml RNAsin RNase inhibitors (Promega, cat. no. N2511)
12. Add 10 µl blocked Protein A/G Sepharose and incubate for 1-2 h at 4°C (= preclearing)
13. Spin down beads and incubate 1ml lysate with appropriate antibody (1-3ug) overnight at 4°C; take 200 µl supernatant as input (20%),
14. Add 300 mM NaCl (20%) and de-crosslink for 2 h at 65°C
15. Spin IP sample briefly, add 10 µl blocked Protein A/G Sepharose and incubate for 1-2 hours
16. Wash beads twice (5 min, RT) with 1 ml of :
    Low Salt Washing Buffer
    High Salt Washing Buffer
    LiCl Washing Buffer
    TE Buffer
17. Add 60 µl Elution buffer to beads and incubate for 10 min at RT (shaking), sediment beads and transfer supernatant to a new tube
18. Repeat step 15 twice
19. Pool supernatants ($\sum$ 180 µl), add 40 µg Proteinase K and digest for 2 h at 52°C
20. Add 12 µl 5M NaCl, and de-crosslink for 2 h at 65°C
21. Purify DNA (PCR Purification Kit, Qiagen) from one aliquot to assay ChIP efficiency
22. Isolate RNA with TRI Reagent (Sigma, cat. no. 93289) from the other aliquot
23. Analyse precipitated RNA by RT-PCR

Buffer A

100 mM Tris-HCl (pH 8.0)
10 mM DTT

Buffer B

10 mM HEPES (pH 7.5)
10 mM EDTA

0.5 mM EGTA
0.25% (w/v) Triton X-100

Buffer C
10 mM HEPES (pH 7.5)
10 mM EDTA
0.5 mM EGTA
200mM NaCl

Buffer D (Sonication Buffer)
50 mM Tris-HCl (pH 8.0)
10 mM EDTA
1% SDS
1x complete protease inhibitors (Roche, cat. no. 04693132001)

IP buffer
15 mM Tris-HCl (pH 8.0)
1.2 mM EDTA
180 mM NaCl
1.2% (w/v) Triton X-100

Low Salt Wash Buffer
20 mM Tris-HCl (pH 8.0)
2 mM EDTA
150 mM NaCl
0.1 (w/v) SDS
1% (w/v) Triton X-100

High Salt Wash Buffer
20 mM Tris-HCl (pH 8.0)
2 mM EDTA
500 mM NaCl
0.1 (w/v) SDS
1% (w/v) Triton X-100

LiCl Wash Buffer
10 mM Tris-HCl (pH 8.0)
1 mM EDTA
250 mM LiCl
1% (w/v) NP40
1% (w/v) sodium deoxycholate

Elution Buffer
0.1 M $NaHCO_3$
1% SDS