

ChIP Assay

CHROMATIN IMMUNO-PRECIPIATION (ChIP)

Materials/Reagents:

Buffer C

20 mM HEPES pH7.9
25% glycerol
420 mM NaCl
1.5 mM Mg Cl₂
0.2 mM EDTA

Triton Buffer

50 mM Tris-HCl pH8.0
1 mM EDTA
150 mM NaCl
0.1% Triton X-100

Breaking Buffer

50 mM Tris-HCl pH8.0
1 mM EDTA
150 mM NaCl
1% SDS
2% Triton X-100

SDS-NaCl-DTT Buffer

62.5 mM Tris HCl pH6.8
200 mM NaCl
2% SDS
10 mM DTT

Primers:

Hsp70 HSE (183 bp)

F: 5'- GAAGACTCTGGAGAGTTCTG-3'

R: 5'- CCCTGGGCTTTTATAAGTCG-3'

DHFR promoter (400 bp)

F: 5'-GGCCTCGCCTGCACAAATAGGG

R: 5'-GGGCAGAAATCAGCAACTGGGC

β-actine (253 bp)

F: 5'-CAGGGCGTGATGGTGGGCA

R: 5'-CAAACATGATCTGGGTCATCTTCTC

Procedure (For HSF1 binding to Hsp70 promoter):

DAY1

1. HeLa cells growing in 10 cm dish (80% confluent) or HeLa S3 (2x10⁵ cells) in 10 ml of DME+10% FBS.
2. Add 280 μl of Formaldehyde (37% concentrated) and incubate 2 min at RT.
3. Add 1 ml of 1M Glycine to stop the X-linking.
4. Remove the media and wash with 1xPBS twice. Scrape cells out, collect them into 1.5 ml tube, spin 13,000 rpm 1 min, take pellet, and freeze with dry ice.
5. Thaw the cells by adding Buffer C with 1 mM PMSF and pipetting. Place tube on ice for 20 min.
6. Spin down nuclei at 4°C, 13,000 rpm 10 min. Remove supernatant.

7. Resuspend in 100 μ l of Breaking Buffer, sonicate with bath sonicator at least 1min each sample. Spin down and make sure there is no or very little visible white pellet.
8. Add 1 ml of Triton buffer and 20 μ l of ProteinA Sepharose (1:1 slurry). Shake tubes over night in a cold room.

DAY2

1. Spin 13k rpm, 10 min at 4°C. Take 950 μ l supernatant into new tube (leave more than 100 μ l to prevent contamination by non-specific precipitant.).
2. Aliquot 400 μ l each into two tubes for no-antibody or preimmune control and HSF1 IP and 40 μ l for control of 10% total DNA. Add 6 μ l of HSF1 polyclonal antibody and 40 μ l of Protein A Sepharose. Incubate 6 hours in cold room with continuous shaking. (The incubation time is not crucial. Principally, 1 hour incubation is fine, even though I have never tried. Overnight incubation was no problem.)
3. Wash the beads with triton buffer twice and add 10 mM Tris HCl pH8.0 (1xPBS also OK), move all solution into new tube. (Changing tube is crucial. If you do not change the tube here, you may get higher non-specific DNA amplification.)
4. Spin down the Sepharose beads and remove supernatant carefully. (use gel loading tip to remove small amount of supernatant.)
5. Add 200 μ l of SDS-NaCl-DTT Buffer, vortex well and incubate at 65°C over- night. (This step is for reverse cross-linking.)

DAY3

1. Add 200 μ l Phenol-Chloroform, vortex very well and spin 13,000 rpm, 10 min at RT.
2. Take upper aqueous phase into new tube and do phenol-chloroform extraction again. (Sepharose beads are precipitated on the surface of organic phase. DO NOT take it.)
3. After second phenol-chloroform extraction, change tube and do chloroform extraction.
4. Take aqueous phase into new tube, add about 15 μ l of 3 M NaOAc (final concentration is 0.3 M). Add 600 μ l of ethanol, incubate for 30 min at RT. Spin down, 13K rpm, 10 min at 4°C (RT is OK).
5. After washing with 70% EtOH, dry , and re-suspend in 20 μ l of dH₂O
6. Mix 5 μ l of sample, 20 pmol of forward and reverse PCR primer in 20 μ l of water and one Ready-to-GO PCR bead.
7. PCR 95°C 5min, 95°C 30 sec – 55°C 30sec – 72°C 1 min (26-30 cycles).
8. Load 10 μ l of PCR products on 2% agarose gel.

References:

1. *Current Protocols in Molecular Biology* (1999) 21.3.1-21.3.12
2. Boyd, K.E., Wells, J., Gutman, J., Bartley, S.M. and Farnham, P.J. (1998) *Proc. Natl. Acad. Sci. USA.* **95**, 13887-13892

3. <http://mcardle.oncology.wisc.edu/farnham/protocols/chips.html>

Submitted by: Gen Matsumoto