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Chromatin Immunoprecipitation (ChIP)

Reagents

1) Non-biotinylated primary antibody against DNA-bound protein of interest
2) Biotinylated secondary antibody
3) Lysis buffer
4) Dilution buffer
5) Wash buffer
6) Chelating resin
7) 37% formaldehyde solution
8) 1M glycineLeupeptin
9) Aprotinin
10) Phenylmethylsulfonyl fluoride (PMSF)
11) Streptavidin agarose or magnetic beads
12) PCR kit
13) PBS
14) Dimethyl sulfoxide
15) DNA purification kit
16) Deionised water
17) Optional: MNase

Procedure

1) Cross-link protein-DNA complexes by incubating cells with 37% Formaldehyde, diluted to 1% final concentration. Shake for 15 mins at room temperature.
2) Quench the cross-linking by adding 1M glycine, diluted to a final concentration of 125 mM. Shake for 5 mins at room temperature.
3) Harvest the cells by centrifuging at 4 °C for 5 min at 1,000 x g.
4) Wash cell pellet twice with 10 ml of PBS 4 °C.

Note: Samples can be stored overnight at -70 °C, if required.

5) Add protease inhibitors to the Lysis buffer (10 µg/ml Leupeptin, 10 µg/ml Aprotinin, 1 mM PMSF).
6) Lyse the cells by re-suspending the pellet in 500 µl of Lysis buffer per 5 x 106. Pipette up and down to re-suspend the cells. Incubate on ice for 10 mins.

Note: From this step onwards, keep samples on ice.

7) Shear chromatin to an average fragment size of 200 – 1,000 b.p. by sonicating samples. Keep chromatin on ice at all times. Optimisation for this step will be required (different cell lines have different sonication requirements).

Note: Do not pulse for >30 s at one time to ensure proteins are not denatured. Alternatively, chromatin can be fragmented by enzymatic digestion using MNase.

8) Transfer 500 µl of each sample to a 1.5 mL microcentrifuge tube.
9) Centrifuge lysates for 10 mins using a refrigerated centrifuge at 12,000 x g. Collect the supernatant in a clean tube for immunoprecipitation. Discard pellet.
10) Use 50 µl of each sample to determine fragment size and DNA concentration.
11) Dilute supernatant with 1 ml of Dilution Buffer (containing 10 µg/ml Leupeptin, 10 µg/ml Aprotinin, 1 mM PMSF). Add Non-biotinylated primary antibody to the sample. Incubate for 15 mins at room temperature in an ultrasonic bath. Add 1 – 10 µg of antibody per 25 µg of DNA.

Note: If antigen is expected to have low level expression, incubate overnight at 2 to 8°C, with rotation.

12) Add 5 µg of Secondary antibody and incubate for 15 mins at room temp in an ultrasonic bath.
13) Add 50 µl of Streptavidin beads (agarose or magnetic) to the samples and rotate for 30 mins at 2 to 8°C.

Note: Save approx. 5% of your sample before adding the beads, to act as your input control.

14) If using agarose, collect the beads by centrifugation at 12,000 x g for 1 min. If using magnetic beads, collect the beads by leaving the tube in the magnet for 2 mins.
15) Wash 4 times with 1 ml Wash buffers pre-cooled to 2 to 8°C each time. Vortex samples to re-suspend beads between washes.
16) Reverse crosslinking by adding 100 µl of Chelating Resin Solution after the last wash directly to the beads and pipette up and down for 10 s. Boil sample for 10 mins in a temperature controlled water bath.
17) Microcentrifuge at 12,000 x g for 1 min at room temperature and transfer the supernatant to a clean microcentrifuge tube.

18) Add 120 ml of deionised water to beads. Pipette up and down for 10 s, centrifuge for 1 min and collect the supernatant. Pool with supernatant in step 17.

Note: Samples can be stored at -20 to -70 °C, if required.

19) Concentrate and purify the DNA preparation using a phenol-chloroform extraction or a spin column designed for DNA purification. Re-suspend DNA in 50 µl of deionised water.

Note: Samples can be stored at -20 to -70 °C, if required.

20) Use 2 – 10 µl of DNA sample to analyse using PCR or real-time PCR.