Chromatin Immunoprecipitation (ChIPs) Protocol (Stanadard)

Prepare Chromatin

1. Cross-link chromatin with formaldehyde (1% final concentration) for 10 minutes at 37ºC.

2. Remove media and wash twice with cold 1X PBS buffer including protease inhibitors.

3. Remove cells into a conical tube and pellet cells for 4 minutes at 2,000 rpm and 4ºC.

4. Resuspend cell pellet to $1x10^6$ cells per 200 µl of SDS Lysis Buffer with protease inhibitors and incubate for 10 minutes on ice. Each ChIP requires $1x10^6$ cells; scale accordingly.

5. Sonicate lysate to shear DNA to an average length between 200 and 1000 base pairs. Keep samples on ice throughout the procedure.

6. Centrifuge samples for 10 minutes at 13,000 rpm at 4°C and transfer the supernatant to a new tube. Discard pellet.

7. Dilute the sonicated cell supernatant 10-fold in ChIP Dilution Buffer, including protease inhibitors.

8. Pre-clear the 2 ml diluted cell supernatant with 75 µl of Salmon Sperm DNA/Protein A-Agarose (50% Slurry) for 30 minutes at 4°C with agitation. Pellet agarose by brief centrifugation and transfer the supernatant to a new tube.

Immunoprecipitate

1. Add the primary antibody to the pre-cleared 2 ml supernatant and incubate from 4 hours to overnight at 4°C with constant rotation.

2. Add 60 µl of Salmon Sperm DNA/Protein A-Agarose Slurry for one hour at 4°C with rotation to collect the antibody/histone complex.

3. Pellet agarose by gentle centrifugation (1,000 X g for 1 min). Carefully remove the supernatant that contains unbound chromatin.

Wash

Wash the protein A agarose/antibody/chromatin complex for 5 minutes with rotation, pellet agarose (1,000 X g for 1 min) and discard the wash buffer between steps:

1. Low Salt Wash Buffer, one wash (at 4ºC) – 1 ml

2. High Salt Wash Buffer, one wash (at 4ºC)– 1 ml

3. LiCl Wash Buffer, one wash (at 4ºC) – 1 ml

4. TE Buffer, two washes (at room temp.) – 1 ml each

Elute

1. Remove TE wash buffer and resuspend the protein A-agarose/antibody/chromatin complex in 250 µl of fresh elution buffer. Mix and incubate at room temperature for 15 minutes with rotation.

2. Spin down agarose beads and transfer the supernatant (with eluted chromatin) to another tube.

3. Repeat elution and combine eluates (total volume = ~500 µl).

4. Reverse cross-links by adding 20 µl of 5 M NaCl to the combined eluates (500 µl) and heat at 65°C for 4 hours.

5. Add 10 µl of 0.5 M EDTA, 20 µl of 1 M Tris-HCl, pH 6.5 and 2 µl of 10 mg/ml Proteinase K to the combined eluates and incubate for one hour at 45°C.

6. Recover DNA by phenol/chloroform extraction and ethanol precipitation.

7. Wash pellets with 70% ethanol and air dry.

8. Proceed with detection step (PCR, dot blot, etc.).

ChIP Solutions

SDS Lysis Buffer

1ml 10 % SDS

200 µl 0.5 M EDTA

500 µl 1 M Tris HCl pH 8.0

8.3 ml ddH_20

Total Volume: 10 ml

ChIP Dilution Buffer

50 µl 10 % SDS

0.5 5 ml Triton-X 100

120 µl 0.5 M EDTA

835 µl 1 M Tris HCl pH 8.0

1.67 ml 5 M NaCl

46.8mL ddH_20

Total Volume: 50ml

Lo Salt

0.5 ml 10% SDS

0.5 ml Triton-X 100

200 µl 0.5 M EDTA

1 ml 1 M Tris-HCl pH 8.0

1.5 ml 5 M NaCl

46.3 ml ddH_20

Total Volume 50 ml

Hi Salt

0.5 ml 10% SDS

0.5 ml Triton-X 100

200 µl 0.5M EDTA

1 ml 1 M Tris-HCl pH 8.0

5 ml 5 M NaCl

42.8 ml ddH_20

Total Volume: 50 ml

LiCl

2.5 ml 5 M LiCl

0.5 ml NP-40

0.5g Deoxychloric Acid

100 µl 0.5 M EDTA

0.5 ml 1 M Tris-HCl pH 8.0

46.4 ml ddH_20

Total Volume: 50 ml

1x TE pH 8.0

1 ml Tris-HCl

0.2 ml 0.5 M EDTA

98.8 ml ddH_20

Total Volume: 100ml

Elution buffer (Freshly prepared)

1 ml 10 % SDS

2 ml 0.5 M $NaHCO_3$

8 ml ddH_20

Total Volume: 10ml

Tips

Cross link chromatin

- Use high quality formaldehyde or make your own from para-formaldehyde
- Be consistent with your fixation conditions.
- Use formaldehyde in a laminar flow cabinet.

Cell Lysis

- To insure high efficiency of lysis, use the correct amount of buffer for the number of cells you are lysing.

Sonication

- Keep cells on ice throughout the procedure – even during sonication.
- Be sure that you don't sonicate for too long (no more than 30 sec.),which could cause sample overheating and denaturation.

IP procedure

- Don't spin agarose beads at high rpm; use gentle centrifugation –700-1000 x G for 1 minute in a microfuge.
- It may be possible to reduce the primary incubation time of the IP (depending on the antibody).

PCR detection

- Design primers adhering as closely as possible to the following parameters:
    1. Length: 24 nucleotides
    2. Tm: 60°C (+/- 2.0ºC)
    3. % GC: 50% (+/- 4%)
- Do not use more than 20-25 cycles to keep dNTPs in excess.

Controls

- Negative control: (1) PCR amplification using chromatin from your experimental antibody IP with PCR primers specific for a DNA region at which your protein or modification is not present; (2) No primary antibody IP control or normal rabbit IgG

- PCR control on DNA from cross-link reversed chromatin Freezing

- Samples can be frozen after step 1, part B (please refer to Certificate of Analysis), snap freeze cells and thaw on ice.