Native Chromatin immunoprecipitation(ChIP) Protocol

-Instruction

ChIP is a technique for studying the interactions between proteins with DNA as it is in nature. It depends on the specific antibody reactions with antigen, so which can truly reflect the combination of protein factors and genomic DNA in vivo. The target protein was cross-linked together with DNA. Then DNA is broken into small fragments by sonication or enzymatic hydrolysis. So we can pull down the predicted DNA fractions by the specific reactions between antibody and antigen. This process specifically enriched DNA fragments bound by target proteins. Finally, we can purify the DNA from the complex and validate the DNA following PCR or qPCR protocol.

-Solution and reagents

Reaction buffer: 1mM $CaCl_2$, 0.2% Triton X-100 or NP-40, 50mM Tris-HCl (pH 7.6)

RIPA buffer: 0.1% SDS, 0.1%NaDOC, 1% Triton X-100, 1mM EDTA, 10mM Tris-HCl (pH 7.6)

LiCl buffer: 0.25M LiCl, 0.5% NP-40, 0.5% NaDOC, 10mM Tris-HCl (pH 7.6)

TE buffer: 1mM EDTA, 10mM Tris-HCl (pH 7.6)

-Sample preparation

Cells culture in 10cm culture dishes with 20mL culture medium. When the density of cells reaches to 80%-90%, it can be used to a ChIP assay. The number of cells differs from the target protein which you are research on. You can refer to the form as follow:

Protein	Number of cells(for a ChIP reaction)	Protein target abundance
Histone protein RNA polymerase II	10^4	high
Transcription factor	10^5-10^6	medium
Cofactor	10^7 or more	low

To ensure a better result, we recommend more than 4×10^6 cells for histone protein and RNA polymerase II, while transcription factor or cofactor need more cells.

1. Take out the culture dishes and discard the culture medium. The number of cells is about 2×10^7.
2. Wash the cells by ice PBS for 3 times.
3. Add 1ml PBS and collect the cells by scraping the cells from culture dishes.

4. Centrifuge for 3min, 4℃ at 2500rmp to collect the cells.
5. Re-suspend the cells by adding 500μl reaction buffer (add fresh protease inhibitors) to lysate the cells on ice for 10min.
6. Centrifuge for 3min, 4℃ at 2500rmp to collect the cells.
7. Re-suspend the pellet by adding 1ml reaction buffer and microccal nuclease to react at 37℃ for 20min. The enzymolysis condition should refer to the instructions of the microccal nuclease. The processing time and the concentrate of microccal nuclease should be optimized by a preliminary experiment.
8. 5mM EDTA can be added to terminate the enzymatic hydrolysis.
9. Sonicate to completely broken the DNA. 6×5'.
10. Centrifuge for 2min, 4℃ at 2500rmp. Separate the supernatant into a new tube.
11. You can get 5μl supernatant for an agarose gel analysis to validate the effect of sonication.

-Immunoprecipitation

1. Take 500μl cell lysates which contains DNA from 1×10^7 cells into a tube.
2. Before the immunoprecipitation, you have to design four groups of experiments:
   a. Experience group: add 2-5μg specific antibody into 500μl cell lysates and place the tube on a rotary mixer at 4℃ overnight to form antigen-antibody complex.
   b. Input group: all process is same as experience group except for the immunoprecipitation reaction.
   c. Negative control: all process is same as experience group and instead the antibody with normal rabbit IgG.
   d. Positive control: all process is same as experience group and instead the antibody with a histone H3 or RNA polymerase II antibody
3. Add 50μl magnetic beads into the complex solution at 4℃ for 2-4h.
4. Attach the beads to magnet and remove the supernatant.

-Wash the beads

1. Wash the beads with filtered tips
   a. 2×1ml RIPA buffer
   b. 2×1ml RIPA buffer +0.3M NaCl
   c. 2×1ml LiCl buffer
   d. 2× 1ml TE buffer + 0.2%Triton X-100

e. 1× 1ml TE buffer. Mix 1min on rotator.

Wash the beads with the buffer above for twice in turn. Pipet 3-8 times by tips and rotate for 10min each.

2. Remove the wash buffer by magnet and collect the precipitation complex.
3. Re-suspend the precipitation complex in 100μl TE buffer. Add 3-5ul 10% SDS and 5ul of 20mg/ml proteinase K. Incubate at 65℃ overnight.
4. The second day, vortex briefly and transfer supernatant to a new tube using magnet.
5. Wash beads with 100μl TE buffer + 0.5M NaCl. Combine with the surpernatant in step4.

-DNA purification

You can extract DNA by a Reagent kit according to the instructions.

-Detection

Perform a PCR or Real time PCR assay to detect the predict DNA which may react with the target protein.