ChIP Assay for Cell Culture

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[Abstract] Chromatin immunoprecipitation (ChIP) is a method used to determine the location of DNA binding sites on the genome for a particular protein of interest. Following crosslinking, cells are lysed and the DNA is broken into pieces 0.2-1.0 kb in length by sonication. At this point immunoprecipitation is performed resulting in the purification of protein–DNA complexes. The identity and quantity of the isolated DNA fragments can then be determined by PCR. This protocol describes how to perform a ChIP experiment and can be applied to different types of cell culture.

Materials and Reagents

1. Salmon Sperm DNA (Life Technologies, Invitrogen™, catalog number: AM9680)
2. Protein inhibitors
   a. Phenylmethylsulfonyl fluoride (PMSF) (Sigma-Aldrich, catalog number: 78830-5G)
   b. Aprotinin (Sigma-Aldrich, catalog number: A3428-10MG)
   c. Pepstatin A (Sigma-Aldrich, catalog number: P5318-5MG)
3. Formaldehyde (Thermo Fisher Scientific, catalog number: F75F-1GAL)
4. Protein A agarose beads (Upstate, Millipore, catalog number: 16-157)
5. General chemicals (Sigma-Aldrich)
6. DPBS (Life Technologies, Invitrogen™, catalog number: 14190-250)
7. QIAGEN PCR purification kit (QIAGEN, catalog number: 28104)
8. ChIP lysis buffer (see Recipes)
9. Low salt wash buffer (see Recipes)
10. High salt wash buffer (see Recipes)
11. LiCl buffer (see Recipes)
12. TE buffer (see Recipes)
13. Elution buffer (see Recipes)

Equipment

1. Standard bench-top refrigerated centrifuge that can reach at least 13,000 rpm

2. Conical tube
3. Rotating platform

Procedure

1. Grow about 1-2 x 10^7 cells in a 100 mm petri dish, and then crosslink by adding formaldehyde to a final concentration of 1%. Incubate at 37 °C for 10 min.
2. Wash cells twice using ice cold DPBS containing protease inhibitors [1 mM PMSF, 1 µg/ml aprotinin and 1 µg/ml pepstatin A].
   Note: Add protease inhibitors to the DPBS just prior to use. PMSF has a half-life of approximately 30 min in aqueous solutions.
3. Scrape cells in 2 ml ice cold DPBS containing the protease inhibitors and transfer into a conical tube, then pellet cells for 4 min at 2,000 rpm at 4 °C.
4. Remove the PBS and resuspend cells in ChIP lysis buffer with protease inhibitors (inhibitors: 1 mM PMSF, 1 µg/ml aprotinin and 1 µg/ml pepstatin A), then incubate for 10 min on ice.
5. Sonicate the cells to shear DNA to lengths between 200 and 1,000 bp; bubbles will be produced, so spin down to get rid of bubbles and then repeat the sonication step 5 times, 1 min each time, being sure to keep samples ice cold.
6. Centrifuge the samples for 10 min at 13,000 rpm at 4 °C, and determine the OD_{260} of the lysates.
7. Dilute the sonicated lysates to OD_{260} 2 with ChIP dilution buffer. Add 60 µl Protein A agarose beads to the sonicated lysates and rotate at 4 °C for 1 h to reduce non-specific binding.
8. Pellet agarose by brief centrifugation and collect the supernatant fraction, 20 µl of lysates should be taken out as input control.
9. Add the immunoprecipitating antibody (the amount will vary per antibody) to the 2 ml supernatant fraction and incubate for 2 h at 4 °C with rotation. For negative control, perform a no-antibody immunoprecipitation by incubating the supernatant fraction with 60 µl mix of 0.1 µg/µl salmon sperm DNA and Protein A agarose beads for one hour at 4 °C with rotation.
10. Pellet agarose by gentle centrifugation (700 to 1,000 rpm at 4 °C, ~1 min). Carefully remove the supernatant that contains unbound, non-specific DNA. Wash the Protein A agarose/antibody/histone complex for 3-5 min on a rotating platform with 1 ml of each of the buffers listed in the order as given: Low salt wash buffer; high salt wash buffer; LiCl buffer; TE buffer.

11. Elute the histone complex from the antibody by adding 250 µl elution buffer to the pelleted Protein A agarose/antibody/histone complex from step 10 above. Vortex briefly to mix and incubate at room temperature for 15 min with rotation. Spin down agarose, and carefully transfer the supernatant fraction (eluate) to a separate clean tube and repeat elution. Combine eluates (total volume = approximately 500 µl).
12. Add 20 µl 5 M NaCl to the combined eluates (500 µl) and reverse histone-DNA crosslinks by heating at 65 °C for 4 h.
    Note: Include the input DNA from this step.
13. Dilute with 2 ml TE buffer, add 5 µl of ice acetic acid, purify the DNA by using the QIAGEN PCR purification kit, then recover DNA in 50 µl elution buffer.
14. Perform PCR and standard agarose gel electrophoresis. PCR conditions must be determined empirically.

Recipes

1. ChIP lysis buffer
   1% SDS
   10 mM EDTA
   50 mM Tris-HCl (pH 8.0)
2. Low salt wash buffer
   0.1% SDS
   1% Triton X-100
   2 mM EDTA
   20 mM Tris-HCl (pH 8.0)
   150 mM NaCl
3. High salt wash buffer
   0.1% SDS
   1% Triton X-100
   2 mM EDTA
   20 mM Tris-HCl (pH 8.0)
   500 mM NaCl
4. LiCl buffer
   0.25 M LiCl
   1% NP-40
   1% SDC
   1 mM EDTA
   10 mM Tris-HCl (pH 8.0)

5. TE buffer
   20 mM Tris-HCl (pH 8.0)
   1 mM EDTA (pH 8.0)
6. Elution buffer
   1% SDS
   0.1 M $NaHCO_3$

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References

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