Chromatin immunoprecipitation for Plant materials

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1. Materials

1.1 Tissues

Seedling, inflorescence, rosetta leaves before bolting, roots, etc. We have used both fresh and frozen tissue and gotten results from both tissues whereas fresh tissues work slightly better.

1.2 Crosslinking

Buffer A

0.4 M sucrose, 10 mM Tris pH8, 1 mM EDTA, 1 mM PMSF, 1% Formaldehyde

Note: PMSF and Formaldehyde are added just before use

Glycine 2 M glycine

1.3 Tissue extraction

Lysis buffer

50 mM HEPES pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% deoxycholate, 0.1% SDS, 10 mM Na butyrate, 1 mM PMSF, 1X plant proteinase inhibitor cocktail (sigma)

Note: PMSF and proteinase inhibitor are added just before use

1.4 Wash/Elution

Lysis buffer see above

LNDET

0.25 M LiCl, 1% NP40, 1% deoxycholate, 1 mM EDTA

Elution buffer

1% SDS, 0.1 M $NaHCO_3$, 1 mg/ml proteinase K

2. Methods

Note: ChIP results strongly depend on the antibody quality. We use histone antibodies as control.

1. 240 mg of tissue is being immersed in 10 ml of buffer A in a 50 ml falcon tube under vacuum for 20 min.

Note: In general we use approximately 240 mg tissue for three precipitations plus input (IgG negative control, histone H3, antibody to test and input fraction). We have used 15 to 300 mg tissue/ precipitation.

2. Add 2 M glycine to a final concentration of 0.1 M and continue incubation for 10 min.

3. Wash the tissue with excess amount of distilled water and remove as much water as possible. You can store tissue at - 80˚C.

4. Grind tissue in liquid nitrogen and resuspend in 400 µl of lysis buffer.

Note: The quality of grinding is very critical!

5. Shear DNA by sonication to approximately 100-1000 bp within an eppendorf tube. (See figure 1).

Note: Sonication is the most important step for ChIP experiment. Conditions depend on sonicator. You should choose the optimal conditions prior to the ChIP experiment. Conditions we use: 10% amplitude, 15 sec, 5 repeats using Vibra cell sonicator (Sonics&Materials). To avoid bubbles, lower output and longer sonication is recommended.

Note: Try to avoid bubbles. Bubbles cause a significant reduction in sonication efficiency.

6. Centrifuge at 13000rpm for 10 min at 4˚C.

7. Pre-clear supernatant with 30 µl of salmon sperm / protein A agarose (Upstate) for at least 60 min with rotation.

8. Centrifuge at 3000rpm for 1 min at 4˚C.

9. Transfer 100 µl of supernatant into three new tubes and add the antibody. Keep a small amount (approx. 30 µl) of extract as input fraction.

Note: Antibody amounts are variable. We use 2 µg of IgG, 1 µg of histone H3 (abcam), 1 µl of GFP antibody (abcam) for 100 µl extract.

10. Incubate overnight with rotation at 4˚C.

11. Add 30 µl of salmon sperm / protein A agarose, continue incubation for at least 2 hours.
12. Centrifuge at 3000 rpm for 1 min at 4˚C.
13. Wash in cold room:
    Add 0.5 ml of Lysis buffer, inverse 6 times, centrifuge, and discard supernatant.
    Add 0.5 ml of Lysis buffer, rotate for 5 min, centrifuge, and discard sup.
    Add 0.5 ml of LNDET, inverse 6 times, centrifuge, and discard sup.
    Add 0.5 ml of LNDET, rotate for 5 min, centrifuge, and discard sup.
    Add 0.5 ml of TE, inverse 6 times, centrifuge, and discard sup.
    Add 0.5 ml of TE, rotate for 5 min, centrifuge, and discard sup.
14. Add 40 µl of Elution buffer and incubate at 65˚C for 10 min.
15. Centrifuge and transfer supernatant to new tube.
16. Repeat steps 13-14 and recover elution. Final elution volume is 80 µl. In parallel, add 70 µl of elution buffer into 10 µl of input fraction for the 10% input control.
17. Incubate all samples over night at 65˚C.

Note: The use of a thermocycler is recommended for prevention of evaporation.

18. Extract DNA by using PCR purification kit (QIAGEN). Elute in 30 µl of EB buffer (Tris, pH 8.5).

Note: Elution volume depends on the amount of tissue used.

19. Use 0.1 - 4 ul for PCR

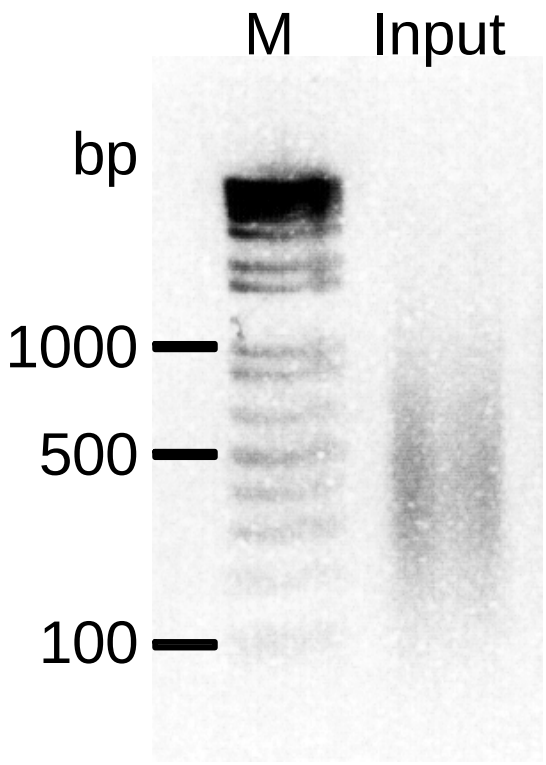

Figure 1. 1% agarose gel stained with EtBr. Input: 5 μl of input fraction. M: 100 bp ladder marker.