ChIP (Chromatin Immunoprecipitation)

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Abstract

Identification of DNA binding proteins by chromatin immunoprecipitation adapted for yeast cells.

Reagents

REAGENTS - Formaldehyde solution (F8775-500ML, SIGMA ALDRICH) - TBS buffer: 20 mM Tris-HCl pH 8.0, 0.5 M NaCl - Lysis buffer : 50 mM HEPES-KOH pH 7.9, 40 mM NaCl, 1 mM EDTA, 1% (v/v) Triton X-100, 0.1% (w/v) sodium desoxicholate, 1 mM PMSF, 1 mM benzamidine and Complete Mini protease inhibitor (Roche Diagnostics) - Dynabeads Protein G (10004D, invitrogen) - HA-probe (F-7) antibody (SC-7392, Santa Cruz Biotechnology Inc.) - Monoclonal GFP antibody (11814460001, Roche Diagnostics). - PBS : 150 mM NaCl, 40 mM Na₂PO₄, 10 mM NaH₂PO₄ - Tween 20 (P7949-100ML, SIGMA ALDRICH) - Elution buffer: 50 mM Tris-HCl pH 8.0, 10 mM EDTA, 1% (w/v) SDS - Proteinase K (03115879001, Roche Diagnostics) - High Pure PCR product purification kit (11732676001, Roche Diagnostics). - SYBR® Premix Ex Taq™ Tli RNase H Plus Green with ROX (RR420A, Takara).

Equipment

EQUIPMENTS - Genie-2 shaker (Scientific Industries) - BioRuptor Diagenode sonicator - DNA Engine Peltier Thermal Cycler (Bio Rad)

Procedure

Chromatin Immunoprecipitation assay
Crosslinking and sample collection
1. Collect approximately 5×10^8 cells from an exponentially growing yeast culture
2. Crosslink the cells by adding formaldehyde 1% (v/v) to the growth medium and incubate 15 min
3. Stop crosslinking adding glycine 125 mM and incubate during 5 minutes
4. Collect the cells by centrifugation at 4000xg and washed four times with 25 mL of cold TBS buffer at 4°C
5. Cells can be stored at -20°C
Immunoprecipitation
6. Resuspend the cell pellet in 300 µL of Lysis buffer
7. Lyse the cells by vortexing with glass beads for 30 min at 4°C with Genie-2 (Scientific Ind.) and supplement with lysis buffer to a final volume of 600 µL
8. Fragment the chromatin by sonication using a BioRuptor Diagenode with discontinuous pulses during 10 min
9. Centrifuge the sample at 13400xg for 15 min at 4°C
10. Keep 20 µL from the supernatant as a control of whole-cell extract (Input)
11. Incubate the remaining volume with orbital rotation for 2 h at 4°C with Dynabeads Protein G (Invitrogen) previously bound to a specific antibody
12. Wash the beads 4 times in PBS containing 0.02% (v/v) Tween 20
13. Elute the bound protein with two times 40 µL of Elution buffer by heating at 65°C for 8 min
Deproteinization, DNA purification and qRT-PCR
14. Revert the cross-linking by overnight incubation at 65°C shaking
15. Digest the eluted sample for 90 min at 37°C with 0.33 mg/mL proteinase K (Roche Diagnostics)
16. Purify the DNA using the High Pure PCR product purification kit (Roche Diagnostics)
17. Analyse the co-immunoprecipitated DNA in triplicate by quantitative PCR in a DNA Engine Peltier Thermal Cycler (Bio Rad) using the SYBR® Premix Ex Taq™ Tli RNase H Plus Green with ROX (Takara)
18. Binding values indicates the specific enrichment of the

analysed promoter region in the immunoprecipitated sample compared to the whole cell extract \ (Input) using the intergenic region as a control, calculated with the $\Delta\Delta CT$ method. Values are relative to the no tag control strain \ (value of 1 equivalent to no specific enrichment of the analysed region) Schmittgen, T.D. and Litvak, K.J. \ (2008) Analyzing real-time PCR data by the comparative CT method. Nat. Protoc. 3, 1101–1108doi:10.1038/nprot.2008.73 GEN PROMOTER ANALYSED REGION CLN2 –753 to –428 RNR1 –725 to –442 FKS1 –556 to –304 MNM1 –755 to –456