Chromatin Immuno Precipitation PCR (ChIP-PCR) for Yeast

8/28/2014 (Ikui)

As used for analysis of ARS region for Cdc6 or Mcm3 binding

NOTE 1: This is a Buratowski lab protocol, modified by Ikui.

SAFETY:
phenol and ultracentrifugation – be careful.
Protocol published in: Keogh & Buratowski (2004) Using chromatin immunoprecipitation to map cotranscriptional mRNA processing in Saccharomyces cerevisiae. *Methods Mol Biol* **257:**1-16 (**manuscript included in this protocol**).

PROTOCOL
1. To the **40ml culture** (OD600 ≈ 0.5; ≈ 107 cells/ml) add **4ml 11% HCHO** (freshly made from commercial 37% solution) so that the final [HCHO] = 1%. Make the 11% HCHO by adding 7.5 ml of 37% HCHO to 17.5 ml diluent (final concentration 0.1M NaCl, 1mM EDTA, 50mM HEPES-KOH, pH 7.5). Incubate 20 min at RT, swirling briefly every 5 min.

Diluent (500 ml): 0.143 M NaCl 14.3 ml 5M NaCl
1.43 mM EDTA 1.43 ml 0.5M EDTA
71.43 mM HEPES-KOH 8.51 g HEPES
Adjust pH with KOH
Water to 500 ml

2. Add **6 ml of 3M glycine, 20 mM Tris (not pH-ed)**. Mix and incubate for another 5 min. You may have to dissolve the glycine in a rotating water bath at 50 oC prior to use.

3. Pellet the cells, wash 2x with 45 ml cold TBS (20 mM Tris-HCl, pH 7.5, 150 mM NaCl), once with 5 ml ice cold FA lysis buffer/**0.1% SDS**. At this point pelleted cells can be frozen at –70 oC.

2x FA lysis buffer (200ml): 100 mM Hepes-KOH, pH 7.5 4.77 g HEPES
300 mM NaCl 12 ml 5M NaCl
2 mM EDTA 0.8 ml 0.5M EDTA
2% Triton X-100 4 ml 100% Triton
0.2% Na Deoxycholate 4 ml 10% Na Deox.

4. Resuspend the pellet in 0.4ml ice cold FA lysis buffer / **0.5% SDS**

5. Add 2 scoop glass beads (**Sigma G8772**) and vortex in screw cap plastic tube (*eg.* 2ml) tube for 2 cycles: 45" with #6 speed, 45" intevals.

6. Collect lysate by centrifugation at 4oC @1000 rpm for 5 min.
7. Add 400ul of FA lysis buffer / 0.1% SDS and transfer the lysate to an ultracentrifuge bottle.

8. < THIS IS YOUR RESOLUTION STEP > Place on ice and sonicate to shear the chromatin : 3 pulses, 20" on, 20" off with a peak to level 5. (you will have to determine this for other sonicators empirically).

9. Spin at 10,000 rpm for 20 min.

10. Sheared chromatin is now in the supernatant. You can freeze it at –70 oC.

11. To test chromatin sonication yield and quality, take 100µl of the chromatin supernatant, perform decrosslinking (see below), extract with phenol-chloroform, resuspend in 200µl TE and run 20µl on an agarose gel.

IMMUNOPRECIPITATION AND DECROSSLINKING
12. Thaw chromatin solution and add 5M NaCl until the final concentration is 275 mM NaCl; this corresponds to 20 µl for 800 µl. Add 20 µl of IgG beads (as a control) and your favorite antibody-conjugated beads (anti-MYC etc). Save remainder as an INPUT sample.

13. Incubate on a rotator overnight @ 4 oC

14. Pellet beads (microfuge 1100rpm, 4min, RT) and wash once with 1ml of each of the following:
i) FA lysis buffer/**0.1% SDS/275 mM NaCl**, 4 min @ RT, rotator (250µl 5M in 10 ml)
ii) FA lysis buffer/**0.1% SDS/500 mM NaCl**, 4 min @ RT, rotator (700µl 5M in 10 ml)
iii) 10 mM Tris-HCl, pH 8.0, 0.25 M LiCl, 1 mM EDTA, 0.5% NP-40, 0.5% Na Deoxycholate, 4 min @ RT, rotator
iv) TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA), 4 min @ RT, rotator

15. Elute IPed stuff by heating for 10min @ 65 oC in 50 µl of 50 mM Tris-HCl, pH 7.5, 10mM EDTA, 1% SDS (Elution buffer)

16. Pellet beads and transfer the supernatant to a fresh tube. Wash the beads with 50µl TE and add that to the supernatant. To reverse the crosslinks add 5 µl of 10mg/ml RNase per 100 µl of solution and incubate for 4 - 5 hrs @ 65 oC.

17. Add 10 µl 4M LiCl and vortex.

18. Use ChIP DNA clean up kit to purify gDNA (Zymolase #D5205).

19. Resuspend it in 50ul elution buffer. Store @ -20 oC.

PCR ANALYSIS
20.

0.5ul KOD polymerase	4
5ul dNTP	40
12.5ul 2xbuffer	100
2.5ul primer-Fwd (10uM)	20
2.5ul primer-Rev (10uM)	20
2ul gDNA	--- (2ul each)
H2O	20

total 25ul

Program PLTPFX (Tm for ARS305 primers=62, Tm for ARS501 primers=58)

qPCR anaysis

use Qiagen sbgr qPCR kit

1. Design Primer: 17-mer primers to amplify 50-100bp genomic DNA. Primers should contain about 55% AT content (if it is more than 70% it does not work) Primers for Cln3 is your control.

2. Qiagen 2x rtPCR master mix (including polymerase, buffer and syber green)

12.5ul master mix
2ul primer (Forward) 100uM
2ul primer (Reverse) 100uM
2ul of gDNA after ChIP
6.5ul H2O

Total 25ul

3. PCR conditions:
Pre-heat 95 degree 15 minutes
94 degree for 15 seconds
53 degree for 30 seconds
72 degree for ??
Read

40 cycles

*For the first time to use your new primers set, you need to optimize the annealing temp by PCR gradient from 50-65 degree.