Santa Cruz Biotechnology, Inc.

Chromatin IP (ChIP) Protocol

The Power to Question

- Use 50 ml cells OD600nm = 0.7 - 1.0 per timepoint/sample.
- Add 1.35 ml 37 % Formaldehyde (end concentration = 1 %), incubate 15 minutes at 25 °C.
- Add 2.5 ml 2.5 M Glycine, incubate 5 minutes at 25 °C.
- Spin down, wash once with 20 ml PBS.
- Transfer to 2 ml Eppendorf tube, wash again with PBS and freeze cells or proceed on.
- Resuspend in 200 - 400 µl CHIP lysis buffer, add an equal volume of glass beads.
- Shake for 30 minutes at 4 °C on vortexer (maximum level).
- Pierce tube bottom with needle and spin liquid into fresh tube.
- Resuspend extracts and sonicate for 30 sec level 2 (Branson, microtip probe).
- Spin extract for 10 minutes, 10,000 rpm, at 4 °C.
- Take supernatant and measure protein concentration (BioRad assay).
- Use 1 - 5 mg protein per IP.
- Immunoprecipitate for 2 hours to over night, 4 °C.
- Load up to 10 µl of lysate per 1.0 mm of well width for gels of 0.75 mm thickness.
- Wash immunoprecipitations pelleting the beads each time:
    - 2 x 1 ml CHIP lysis buffer
    - 2 x 1 ml CHIP lysis buffer (high salt)
    - 2 x 1 ml CHIP wash buffer
    - 2 x 1 ml TE
- Elute immunoprecipitations: add 75 µl elution buffer.
- Incubate for 10 minutes at 65 °C.
- Spin, take supernatant, elute pellet again with 75 ul elution buffer.
- Combine supernatants, incubate at 65 °C 6 hours to overnight.
- Take 1/100 of the protein amount taken for the IP, add to 150 µl elution buffer (INPUT control).
- Incubate at 65 °C 6 hours to over night.
- Add 750 µl PB buffer (Qiagen PCR purification kit).
- Purify DNA through Qiaquick column.
- Elute DNA into 50 µl H_2O.
- Use 0.5 - 1 µl per 25 µl PCR reaction.
- PCR reactions:
    - 1 x 95 °C, 2 minutes
    - 21 x 95 °C, 30 sec; 60 °C, 30 sec; 72 °C, 1 minutes
    - 1 x 72 °C, 3 minutes
- Primers (1 µM): 20 - 24 bp, 50 % GC, producing a 200 - 500 bp fragment.
- PCR products (10 - 15 µl) are separated on 2 % agarose gels or 5 -6 % non denaturing PAA gels