

Mononucleosome Chromatin Immunoprecipitation

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Yeast culture. Grow 450 mL of yeast to an A_{600} OD of 0.9 in 2 L flasks shaking at 200 rpm in a 28°C water bath. Add 37% formaldehyde to a 1% final concentration, and incubate cells for 15 minutes at 25°C, shaking, at 90 rpm. Add 2.5 M glycine to a final concentration of 125 mM to quench the formaldehyde. Invert cells and then let stand at 25°C for 5 minutes. Spin cells down at 3000 × g for 5 minutes at 4°C and wash twice, each time with an equal volume of ice cold sterile water.

Micrococcal nuclease digestion. Resuspend cell pellets in 39 ml Buffer Z (1 M sorbitol, 50 mM Tris-Cl pH 7.4), add 28 µl of β-ME (14.3 M, final conc. 10 mM), and vortex cells resuspend. Add 1 ml of zymolyase solution (10 mg/ml in Buffer Z; Seikagaku America), and incubate at 28°C shaking at 200 rpm, in 50 ml conical tubes, to digest cell walls. Spin spheroplasts at 3000 × g, 10 min, 4°C. Resuspend spheroplast pellets and split into aliquots of 600 µl of NP-S buffer (0.5 mM spermidine, 1 mM β-ME, 0.075% NP-40, 50 mM NaCl, 10 mM Tris pH 7.4, 5 mM MgCl₂, 1 mM CaCl₂) per 90 ml cell culture equivalent. Add 40 units of micrococcal nuclease (Worthington Biochemical), and incubate spheroplasts at 37°C for 20 minutes – this was determined in initial titrations to yield >80% mononucleosomal DNA, but for any given yeast strain/growth condition an independent titration should be carried out as a preliminary study. Digestion is halted by shifting the reactions to 4°C and adding 0.5 M EDTA to a final concentration of 10 mM.

Chromatin immunoprecipitation. All steps are done at 4°C unless otherwise indicated. For each aliquot, add Buffer L (50 mM Hepes-KOH pH 7.5, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate) components from concentrated stocks (10-20X) for a total volume of 800 µl per aliquot. Incubate each aliquot with 80-100 µl 50% Sepharose Protein A Fast-Flow bead slurry (Sigma) equilibrated in Buffer L for 1 hour on a tube rotisserie rotator. Pellet beads with a 1 minute spin at 3000 × g, and set aside approximately 2.5-5% of the supernatant as CHIP input material. With the

remainder, add antibodies (amount of antibody to be added depends on the antibody being used) to each aliquot (20% of a 450 ml cell culture).

Incubate samples, rotating, overnight (~16 hours), after which the sample should be transferred to a tube containing 80-100 μ l of 50% Protein A bead slurry. Incubate sample with the beads for 1 hour for the IP, then pellet the beads by a 1 minute spin at 3,000 \times g. After removal of the supernatant, wash the beads with a series of buffers in the following manner: add 1 ml of the buffer, and rotate the sample on the tube rotisserie for 5 minutes, then pellet the beads in a 30 second spin at 3,000 \times g and remove the supernatant. Perform washes twice for each buffer in the following order: Buffer L, Buffer W1 (Buffer L with 500 mM NaCl), Buffer W2 (10 mM Tris-HCl pH 8.0, 250 mM LiCl, 0.5% NP-40, 0.5% sodium deoxycholate, 1mM EDTA), and 1X TE (10 mM Tris, 1 mM EDTA pH 8.0). After the last wash, add 125 μ l of elution buffer (TE pH 8.0 with 1% SDS, 150 mM NaCl, and 5 mM dithiothreitol) to each sample, and incubate the beads at 65°C for 10 minutes with frequent mixing. Spin beads for 2 minutes at 10,000 \times g, and remove and save the supernatant. Repeat the elution process once for a total volume of 250 μ l of eluate. For the ChIP input material set aside, add elution buffer for a total volume of 250 μ l. After overlaying the samples with mineral oil, incubate the samples overnight at 65°C to reverse crosslinks.

Protein degradation and DNA purification. After cooling the samples down to room temperature, incubate each sample with an equal volume of proteinase K solution (1X TE with 0.4 mg/ml glycogen, and 1 mg/ml proteinase K) at 37°C for 2 hours. Extract each sample twice with an equal volume of phenol and once with an equal volume of 25:1 chloroform:isoamyl alcohol. Use phase lock gel tubes (Eppendorf) to separate the phases (light gel for phenol, heavy gel for chloroform:isoamyl alcohol). Afterwards, add 0.1 volume 3.0 M sodium acetate pH 5.3 and 2.5 volumes of 100% ice cold ethanol, and precipitate the DNA overnight at -20°C. Pellet DNA by centrifugation at 14,000 \times g for 15 minutes at 4°C, wash once with cold 70% ethanol, and spin at 14,000 \times g for 5 minutes at 4°C. After removing the supernatant, allow the pellets to dry and then resuspend them in 20 μ l 10 mM Tris-Cl, 1 mM EDTA pH 8.0, and add 0.5 μ g of RNase A. Incubate samples at 37°C for one hour and then treat with 7.5 units of calf intestinal

alkaline phosphatase in a 30 μ l volume supplemented with NEB Buffer 3 (10X concentration of 100 mM NaCl, 50 mM Tris-HCl pH 7.9, 10 mM MgCl₂, 1 mM dithiothreitol (DTT)). Incubate samples for a further 1 hour at 37°C and then clean up with the Qiagen MinElute Reaction Cleanup Kit, following manufacturer's directions, except with an elution volume of 20 μ l.