Torres-Padilla et al., http://www.jcb.org/cgi/content/full/jcb.200603146/DC1

Materials and methods

ChIP cloning protocol for mouse zygotes

A total of 417 zygotes were used for this experiment, which were distributed in eight tubes from step 10. Collect embryos in M2 medium and add formaldehyde to 1% final concentration. Rotate 20 min in the wheel at room temperature. Add 1 ml of ice-cold PBS and spin down at 3,000 rpm. Repeat the wash twice and remove supernatant. Add cell lysis buffer and incubate 10 min on ice. Centrifuge at 5,000 rpm for 5 min at 4°C. Resuspend the pellet (which consists of the pronuclei) in nuclei lysis buffer and incubate on ice for 10 min. Sonicate pronuclei at 40% with small (Stepped) probe. Centrifuge at 14,000 rpm for 1 min at 4° and transfer the supernatant to a fresh tube. Complete the volume per tube up to 1.5 ml with IP buffer. Add 20 μl of preblocked A/G beads and incubate 1 h at 4°C in the wheel. Spin down at 6,000 rpm and transfer supernatant to a fresh tube. Perform IP with 5 μl of TIF1α antibody (1 μg) overnight at 4°C. Add 25 μl of A/G beads and rotate 2 h at 4°C. Centrifuge at 8,000 rpm for 1 min and discard the supernatant. Wash the pellet three times with 1 ml of IP buffer. Pool samples in three tubes and elute with 400 μl of elution buffer 15 min in the wheel. Vortex for 30 s. Centrifuge for 2 min at 8,000 rpm at room temperature. Recover the supernatant and incubate with 200 μg/ml proteinase K at 65°C for 5 h. Extract DNA with phenol/chloroform and precipitate with glycogen at −20°C for 2 d. Centrifuge 30 min at 14,000 rpm and wash with 70% ethanol. Air dry pellet. Resuspend in 30 μl of water and pool the three samples. Blunt end the DNA with T4 DNA Polymerase for 30 min at 37°C. Perform a phenol/chloroform purification and precipitate with glycogene at −20°C for 2 d. Centrifuge 30 min at 14,000 rpm and wash with 70% ethanol. Ligate the DNA to annealed JW102 and JW103 oligos. Purify by removing nucleotides using the PCR purification kit (QIAGEN). Amplify the DNA using the JW102 oligo in the presence of 1.3 M Betaine. Cycling conditions are as follows: 1 cycle (55°C for 2 min, 72°C for 5 min, and 95°C for 2 min); 20 cycles (95°C for 30 s, 55°C for 30 s, and 72°C for 1 min); 1 cycle (72°C for 5 min). Clone PCR products into a TA vector (e.g., pGEM-T easy), pick colonies, and sequence.

Solutions

The following solutions were used: cell lysis buffer (5 mM Pipes, pH 8.0, 85 mM KCl, 0.5% NP-40, and protease inhibitors), nuclei lysis buffer (50 mM Tris, pH 8.0, 10 mM EDTA, 1% SDA, and protease inhibitors), IP buffer (150 mM NaCl, 1 mM EDTA, 20 mM Tris, pH 8.0, 0.5% NP-40, 0.01% Triton X-100, and 5% glycerol), and elution buffer (50 mM $NaHCO_3$ and 1% SDS).