Chromatin Immunoprecipitation in mouse ES cells

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Method Article

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Abstract

This protocol describes how the binding of transcription factors like TFIID to gene promoters in embryonic stem cells can be tested using formaldehyde cross linking, immunoprecipitation, and qPCR analysis.

Procedure

Cell Growth and harvest: - Grow mouse ES cells to two 70% confluent 15 cm culture dishes \(a total of 50 · 10e6 cells). - Crosslink cells by adding 1/37 volume of Formaldehyde 37% \(Merck) to each plate. - Incubate on shaking table for 20 min at RT. - Quench Formaldehyde by adding 1/7 volume of freshly prepared Glycine 1M. - Wash cells 2x with 10 ml cold PBS \(Lonza). - Add 10 ml cold PBS and scrape off the cells. - Spin down cells at 2000g for 10 min at 4°C. - Discard supernatant, flash freeze pellet in liquid nitrogen, store at -80°C. Chromatin Extraction: - Wash pellet with 15 ml buffer A for 10 min at 4°C. - Spin down at 1300 rpm for 10 min at 4°C. - Wash pellet with 15 ml buffer B for 10 min at 4°C. - Spin down at 1300 rpm for 10 min at 4°C. - Resuspend nuclei in 1 ml buffer C in a 15 ml tube. Sonication: - Sonicate samples \(Biorupter, Diagenode) 20 times on high power, 30 sec on/ 30 sec off. Keep samples at 4°C. - Run 10 µl sample \(de-crosslink by adding 1µl ProtK \(5 mg/ml) and 1µl RNAse \(10 mg/ml), 1hr at 37°C) on a 1% agarose gel to check sonication-efficiency \(product sizes must be mostly between 400 and 1000 bp). - Spin down samples at max speed for 10 min. at 4°C. - Flash-freeze supernatant in dry ice. Preparation of Beads \(Prot A/G PLUS-Agarose beads, Santa Cruz, sc-2003): - Take 40 µl beads per IP \(25% solution). - Spin down for 1 min at 1000g in 4°C \(check for presence of pellet). - Wash in 750 µl 1X incubation buffer \(1X PIC, 0,1% Bovine Serum Albumin \(Merck)) at 4°C. - Repeat previous step 2 times. - Resuspend beads in 20 µl 1X incubation buffer \(1X PIC, 0,1% BSA) at 4°C to get a 50% suspension. - Incubate and rotate at 4°C overnight. ChIP Incubation: - Prepare the following mix \(per ChIP) at 4°C: o 280 µl total volume: o 107 µl H2O o 60 µl 5X incubation buffer o 3 µl 10% BSA o 10 µl 50X PIC o 100 µl chromatin suspension - Add 4 µg antibody, fill to 300 µl with H2O . - Incubate with rotation at 4°C overnight. We have used this protocol with the following antibodies: TAF1: ab51540 \(Abcam) TBP: ab818 \(Abcam) Nanog: A300-397A \(Bethyl) Oct4: Sc-8628 \(Santa Cruz) H3: ab1791 \(Abcam) H3K4Me3: ab8580 \(Abcam) RNA polII: 8WG16 HA: 12CA5 ChIP Wash and pull-down: - Add 20 µl beads suspension \(50%) to each sample. - Rotate at 4°C for 2 hrs. - Pellet beads at 1000g for 1 min at 4°C. - Wash beads 2x with buffer 1, each time with a 5 min incubation at 4°C whilst rotating. - Wash beads 1x with buffer 2, with a 5 min incubation at 4°C whilst rotating. - Wash beads 1x with buffer 3, with a 5 min incubation at 4°C whilst rotating. - Wash beads 2x with buffer 4, each time with a 5 min incubation at 4°C whilst rotating. - After last wash, take out as much supernatant as possible, and elute by adding 400 µl elution buffer. Rotate for 30 min at RT. - Spin down sample at maximum speed for 1 min. - Sample is now in supernatant. Take off supernatant without taking any of the beads\! Transfer to new tube. - Get 50 µl input chromatin sample and add elution buffer to same level as other samples. - Add 16 µl of a 5M NaCl \(Sigma) solution to the ChIP eluates and the input chromatin and incubate overnight at 65°C for decrosslinking. DNA purification: - Add 1µl RNAse \(10 mg/ml) and incubate for 2 hrs at 37°C. - Add 150

µl Glycogen solution \(20 mg/ml) and 50 µl ProtK 5 mg/ml \(Roche) and incubate for 2 hrs at 37°C. - Extract DNA with 600 µl Phenol/Chloroform \(Sigma Aldrich) by vortexing for 15 sec. - Incubate whilst shaking at RT for 5 min. - Spin down for 5 min at max speed. - Take off waterphase and transfer to new tube. - Repeat previous step once. - Add 600 µl Chloroform/Isoamylalcohol \(Sigma Aldrich) to the waterphase. - Incubate whilst shaking at RT for 5 min. - Spin down for 5 min at max speed. - Take off waterphase and transfer to a new 2 ml tube. - Add 1/10 volume of 3M NaAc pH 5.2, 2.5 vol. 96% EtOH and mix well. - Precipitate overnight at - 80°C. - Spin down for 30 min at 13,000 rpm at 4°C - Discard supernatant carefully, leaving the pellet untouched. - Wash pellet with 80% EtOH, spin down for 5 min at 13,000 rpm at 4°C. - Take off supernatant even more carefully, leaving the pellet untouched. - Dry pellet for a few min in the heat block at 37°C. - Resuspend in 100-200 µl 10 mM Tris, pH 8.0. Subsequently, samples can be measured by qPCR. Buffer compositions: Buffer A \(50 ml): - 1 ml 1M HEPES-KOH \(Sigma) buffer, pH 7.5 \(20 mM final) - 1 ml 0.5M EDTA pH 8.0 \(Merck) \(10 mM final) - 50 µl 0.5M EGTA pH 8.0 \(Sigma) \(0.5 mM final) - 625 µl Triton X-100 20% \(Merck) \(0.25% final) - 47.3 ml H2O Buffer B: - 2.5 ml 1M HEPES-KOH buffer, pH 7.5 \(50 mM final) - 1.5 ml 5M NaCl \(150 mM final) - 100 µl 0.5M EDTA pH 8.0 \(1 mM final) - 50 µl 0,5M EGTA pH 8.0 \(0.5 mM final) - 45.85 ml H2O Buffer C: Note: make this buffer just before use - 1 ml 1M HEPES-KOH buffer, pH 7.5 \(20 mM final) - 100 µl 0.5M EDTA pH 8.0 \(1 mM final) - 50 µl 0.5M EGTA pH 8.0 \(0.5 mM final) - 250 µl 10% SDS \(Merck) \(0.05% final) - 1% Protease Inhibitor Complete \(PIC) \(1 tablet, Roche) - 48.6 ml H2O 1X Incubation buffer \(10 ml): - 100 µl 1M Tris pH 8.0 \(10 mM final) - 300 µl 5M NaCl \(150 mM final) - 20 µl 0.5M EDTA \(1 mM final) - 10 µl 0.5M EGTA \(0.5 mM final) - 150 µl 10% SDS \(0.15% final) - 500 µl 20% Triton \(1% final)_ - 8.92 ml H2O Wash buffer 1 \(10 ml): - 100 µl 1M Tris pH 8.0 \(10 mM final) - 300 µl 5M NaCl \(150 mM final) - 20 µl 0.5M EDTA \(1 mM final) - 10 µl 0.5M EGTA \(0.5 mM final) - 100 µl 10% SDS \(0.1 % final) - 100 µl 10% sodium deoxycholate \(DOC) \(Sigma) \(0.1 % final) - 500 µl 20% Triton \(1% final)) - 8.87 ml H2O Wash buffer 2 \(10 ml): - 100 µl 1M Tris pH 8.0 \(10 mM final) - 1 ml 5M NaCl \(500 mM final) - 20 µl 0.5M EDTA \(1 mM final) - 10 µl 0.5M EGTA \(0.5 mM final) - 100 µl 10% SDS \(0.1 % final) - 100 µl 10% DOC \(Sigma) \(0.1 % final) - 500 µl 20% Triton \(1% final) - 8.17 ml H2O Wash buffer 3 \(10 ml): - 100 µl 1M Tris pH 8.0 \(10 mM final) - 20 µl 0.5M EDTA \(1 mM final) - 10 µl 0.5M EGTA \(0.5 mM final) - 500 µl 5M LiCl \(Sigma) \(0.25 M final) - 500 µl 10% DOC \(0.5 % final) - 500 µl 10% NP-40 \(Merck) \(0.5 % final) - 8.37 ml H2O Wash buffer 4 \(10 ml): - 100 µl 1M Tris pH 8.0 \(10 mM final) - 20 µl 0.5M EDTA \(1 mM final) - 10 µl 0.5M EGTA \(0.5 mM final) - 9.87 ml H2O Elution Buffer \(Prepare fresh): - 0.1 M NaHCO3 \(Sigma) - 1 ml 10% SDS \(1% final) - Fill to 10 ml with H2O

Timing

~2 days, excluding the qPCR analysis.

Anticipated Results

The protocol may be tested first for an abundant target, e.g. histone H3. An unrelated antibody such as 12CA5 should be tested to determine the non-specific DNA after immunoprecipitation. Another control

should be a gene-free genomic region. For transcription factors, the % of input DNA that may be recovered will range between 0.02-0.2%.

References

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