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ChIP-qPCR in human cells

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ABSTRACT

Protocol to perform ChIP-qPCR in human cells.

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Day 1 10m

1 10m

1. Split the human cells in a 100 mm dish for a ~80% confluence on Day 2.

Day 2 3h 35m

2 2h 45m

Chromatin preparation

1. Add 1% formaldehyde to the cells and mix 10 minutes on a shaker at 20-25 rpm at room temperature (270.3 µl 37% formaldehyde for 10 ml of medium).
2. Add 125 mM Glycine and mix 5 minutes on a shaker at 20-25 rpm at room temperature (625 µl 2M Glycine for 10 ml of medium).
3. Put the cells on ice and wash twice with 5 ml of ice-cold PBS.
4. Scrap the cells in 1.2 ml of ice-cold PBS and transfer to a chilled 1.5 ml Eppendorf tube.
5. Centrifuge 10 minutes at 1,500 rpm at 4°C.
6. Remove supernatant and resuspend the pellet in 1 ml of chilled ChIP Lysis buffer (10 mM Tris-HCl pH 8.0, 0.25% Triton X-100, 1% SDS, 10 mM EDTA, protease inhibitor cocktail and phosphatase inhibitor to be added fresh).
7. Incubate 10 minutes on ice.
8. Centrifuge 5 minutes at 1,500 g at 4°C.
9. Remove supernatant and resuspend each 1.5 ml tube with 1 ml of chilled ChIP Wash buffer (10 mM Tris-HCl pH 8.0, 200 mM NaCl, 1 mM EDTA, protease inhibitor cocktail and phosphatase inhibitor to be added fresh).
10. Centrifuge 5 minutes at 1,500 g at 4°C.
11. Remove supernatant and resuspend each 1.5 ml tube with 600 µl of chilled ChIP Sonication buffer (10 mM Tris-HCl pH 8.0, 100 mM NaCl, 0.1% SDS, 1 mM EDTA, protease inhibitor cocktail and phosphatase inhibitor to be added

fresh).

12. Incubate on a rotating wheel at 16 rpm in the cold room for 10 minutes.
13. For each tube, transfer in two new ice-cold sonication tubes (2 x 300 µl).
14. Sonicate to shear the chromatin to ~ 200-1000 bp fragments (time and amplitude depends on the cell line, sonicator, and amount of material so it needs to be determined experimentally in advance).
15. Merge the two tubes for each plate in a single ice-cold 1.5 ml tube.
16. Centrifuge 20 minutes at 13,300 rpm at 4°C.
17. Transfer supernatant in a new ice-cold 1.5 ml Eppendorf tube.

3 Immunoprecipitation

45m

1. In an ice-cold 1.5 ml Eppendorf tube, add 10 µl of Dynabeads protein G (or 10 µl of Dynabeads protein A if needed). Wash with 100 µl of ice-cold RIPA buffer (10 mM Tris-HCl pH 8.0, 150 mM NaCl, 1 mM EDTA, 0.1% SDS, 1% Triton X-100, 0.1% Sodium deoxycholate) (add the RIPA buffer, vortex a few seconds at low strength, add the tube on a magnetic rack, and remove the solution when it is cleared). For pipetting beads, cut the bottom of the tip.
2. Add the sonicated chromatin extract to the washed beads and incubate on a rotating wheel at 16 rpm for 30 minutes in the cold room.
3. Centrifuge briefly at < 1,000 rpm, put the tubes on a magnetic rack, and transfer the supernatant to a new ice-cold 1.5 ml Eppendorf tube.
4. Nanodrop for DNA each sample.
5. Prepare a new 1.5 ml Eppendorf tube for each IP and for the IgG/Input. For the amount of chromatin and antibody, follow the recommendation from the company. If not available, use for each tube 70-100 µg of human chromatin and 1-5 µg of antibody.
6. Incubate overnight on a rotating wheel at 16 rpm in the cold room.

4 Beads preparation

5m

1. For each IgG/IP tube, prepare 15 µl of Dynabeads protein G (or 15 µl of Dynabeads protein A). Wash with ice-cold 100 µl of RIPA buffer. Add ice-cold 15 µl of RIPA containing 4 mg/ml of BSA (prepare only one tube with the beads for all the samples).
2. Incubate overnight on a rotating wheel at 16 rpm in the cold room.

Day 3 8h 20m

5 ⚠

2h

Beads washes

1. Centrifuge briefly at < 1,000 rpm. Make sure that the beads are still well mixed.
2. Transfer 15 µl of Dynabeads protein G (or 5 µl of Dynabeads protein A) in new 1.5 ml Eppendorf tubes. Put the tubes on a magnetic rack and remove the supernatant.
3. Transfer each chromatin extract incubated with antibody to a tube containing the Dynabeads protein G. Mix by inverting the tubes several times.
4. Incubate for one hour on a rotating wheel at 16 rpm in the cold room.
5. Centrifuge briefly at < 1,000 rpm and put the tubes on a magnetic rack.
6. Keep the IgG supernatant as the total Input. Discard the supernatant of the IP tubes.
7. Wash the beads three times with 300 µl of ice-cold RIPA buffer (add the buffer, vortex a few seconds at low speed, put back the tubes on the magnetic rack, wait ~ one minute, invert the magnetic rack a few times to recover the beads at the top of the tubes, remove supernatant).
8. Wash the beads three times with 300 µl of ice-cold High Salt Wash buffer (10 mM Tris-HCl pH 8.0, 500 mM NaCl, 1 mM EDTA, 0.1% SDS, 1% Triton X-100, 0.1% Sodium deoxycholate).
9. Wash the beads twice times with 300 µl of ice-cold LiCl Wash buffer (10 mM Tris-HCl pH 8.0, 250 mM LiCl, 1 mM EDTA, 1% NP-40, 1% Sodium deoxycholate).
10. Wash the beads twice times with 300 µl of ice-cold TE buffer (10 mM Tris-HCl pH 7.5, 1 mM EDTA).

6 Elution

40m

1. *For the IP samples:* add 50 µl of Elution buffer (100 mM $NaHCO_3$, 1% SDS, 10 mM DTT (DTT to be added fresh)), resuspend the beads by flicking the tubes, centrifuge briefly at < 1,000 rpm, and put the tubes on a Thermomixer at 1,400 rpm at 37°C for 15 minutes.
2. Put the tubes on a magnetic rack and transfer the supernatant to a new 1.5 ml Eppendorf tube.
3. Repeat the elution from step 6.1 to 6.2 from the beads one more time with 50 µl of Elution buffer and combine both elutes (final volume: 100 µl).
4. *For the Input samples:* add 90 µl of Elution buffer to 10 µl of Input (one tube for each Input, $1/10^{th}$ dilution of the

Input)

7 RNase treatment and reverse crosslink

5h 40m

1. Add 0.6 µl of RNase A (10 mg/ml) to each tube and incubate 30 minutes at 37°C.
2. Add 4 µl of 5 M NaCl (final concentration: 200 mM) and incubate five hours at 65°C to reverse crosslink (possible to do it overnight).
3. Add 300 µl (or 2.5X volume) of 100% ethanol, vortex a few seconds at low speed, and precipitate overnight at -20°C.

Day 4 3h 30m

8 DNA purification and qPCR

3h 30m

1. Centrifuge 20 minutes at 13,300 rpm at 4°C. Remove most of the supernatant with a 1 ml pipette.
2. Centrifuge two minutes at 13,300 rpm at 4°C. Remove the remaining supernatant with a 10 µl pipette.
3. Air dry for one-two minutes.
4. Add 100 µl of TE and 25 µl of 5X Proteinase K buffer (50 mM Tris-HCl pH 7.5, 25 mM EDTA, 1.25% SDS). Dissolve the pellets by pipetting up and down or wait a few minutes for the pellet to dissolve.
5. Add 1.5 µl of Proteinase K (20 mg/ml) to each sample.
6. Incubate two hours at 45°C to degrade the proteins.
7. Purify the DNA with the PCR purification kit (QIAGEN). Add 625 µl (or 5 volumes) of PB + pH indicator I buffer (if the colour is not yellow: add 5-15 µl of 3M AcoNa pH 5.2 or until the colour becomes yellow).
8. Prepare a PCR purification column for each Input, IgG, and IP samples.
9. Load the PCR purification column and centrifuge one minute at 5,000 rpm. Discard the flowthrough.
10. Add 750 µl of PE buffer and centrifuge one minute at 5,000 rpm. Discard the flowthrough.
11. Centrifuge one minute at 5,000 rpm to remove the residual PE buffer.
12. Transfer the PCR purification columns into clean 1.5 ml Eppendorf tubes (or DNA LoBind).
13. Add 50 µl of EB buffer into each column, wait one minute at room temperature, and centrifuge one minute at max speed.
14. Discard the PCR purification columns and keep the 1.5 ml Eppendorf/DNA LoBind tubes at -20°C.
15. Perform qPCR for example with the QuantiTect SYBR Green PCR kit (QIAGEN). IgG and IP samples are measured in triplicates while four Input dilutions are measured (1/5, 1/25, 1/125, and 1/625).
16. For each reaction, the following components were included: 1 µl of template, 1 µl of primer pair mix (10 µM), 3 µl of water and 5 µl of SYBR Green Mix (2x).
17. The thermo-cycling parameters were: 95°C for 15 min followed by 40 cycles of 94°C for 15 s, 57°C for 20 s and 72°C for 25 s.
18. The RotorGene Q Series Software was used to calculate the threshold cycle (Ct) value. Signals are presented as a percentage of Input DNA after removal of the IgG background signal.