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CHROMATIN IMMUNOPRECIPITATION

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Recipes for all solutions (highlighted) in **bold** are included at the end of the protocol.

Day 1

ChIP & Cell Line Samples:

For suspension cells, ensure the appropriate number are resuspended in 10 ml of fresh media to which 37% of formaldehyde solution is subsequently added until a final concentration of 1% is reached. Vortex for a few seconds to displace from the bottom and incubate for 10 minutes at room temperature in agitation.

ChIP & Tissue Samples:

Grind frozen tissue into powder with a pestle and mortar. Pour the resulting powder into a 15 or 50 ml falcon. Add 10 ml PBS + 270 µl formaldehyde 37% (final concentration 1%) to the frozen powder. Vortex for a few seconds to displace from the bottom, next incubate for 10 minutes at room temperature in agitation.

a. Block the reaction with 500 µl Glycine 2.5 M (final concentration 0.125 M). Incubate for 5 minutes at room temperature.

b. Transfer the cells to a 50ml falcon and centrifuge at 2500 rpm for 5 minutes at 4°C.

c. Discard the supernatant and wash twice with ice-cold **PBS** ph 7.4 and centrifuge at 2500 rpm for 5 minutes at 4°C after each washing.

d. Resuspend cells in 5 ml **Cell Lysis Buffer** supplemented with protease inhibitor. To facilitate the cell membrane breaking, pass the lysate 3 times to a douncer. Incubate for 15 minutes at 4°C.

e. Centrifuge at 4000 rpm for 5 minutes at 4°C. Discard the supernatant.

f. Resuspend nuclei in **Nuclear Lysis Buffer**.

Tip 1

Pipette with cut tips to homogenize better.

g. Divide the sample into small aliquots and sonicate for 15 minutes (high power; 30 seconds sonication, 30 seconds rest).

Tip 2

Put ice into the sonicator to avoid sample overheat.

h. Centrifuge at 12000 rpm for 10 minutes at room temperature to remove nuclear debris. Discard the pellet. Repeat this passage the pellet cannot be detected. Store the samples at -20°C.

Tip 3

If SDS precipitates, dissolve it prior to centrifuge.

Tip 4

Take an aliquot of chromatin to quantify (2–3 µl) and to assess the size (30–40 µl).

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Day 1

DNA Fragment Testing:

a. De-crosslink chromatin by incubating samples at 65ºC for 4 hours (results may improve with an overnight incubation).
b. Incubate for 30 minutes with Proteinase K 50 µg/ml final concentration at 42ºC.
c. Add 1 volume of Phenol:Chloroform:Isoamyl Alcohol (25:24:1). Mix with vortex and let samples stand at room temperature for 2–3 minutes.
d. Centrifuge at 12000 rpm for 5 minutes and transfer the aqueous phase to a new tube.

Tip 5

If the interphase is dirty, repeat steps c and d.

e. Add 1/5 volume of AcNH4 10 M and 2.5 volumes of EtOH 100%. Mix and let the DNA precipitate for at least 30 minutes at -20ºC.
f. Centrifuge at max speed for 15 minutes at 4ºC. Discard the supernatant.
g. Wash with 70% ethanol and centrifuge at max speed for 15 minutes at 4ºC. (Try to discard as much supernatant as you can without touching the pellet.)
h. Resuspend with **TE Buffer** and pipette until complete dissolution.
i. Incubate for 30 minutes at 37ºC with RNAse A at a final concentration of 50 µg/µl.
j. Prepare a 1.5% agarose gel.

Day 2

a. Take 70 µl of Magnetic Beads for each sample to be immunoprecipitated. (Take the extra volume in excess: 0.5–1 times more.)
b. After precipitation with a magnet, discard the supernatant and wash twice with 600 µl at 5% BSA/PBS.
c. After the second wash reconstitute the initial volume (70*Nº of samples µl). (Take the extra volume in excess: 0.5–1 times more.)
d. Take 20 µg (dependant on the tissue/cell type) for each sample, dilute the chromatin 1:10, and bring to a final volume of 1 ml with **Dilution Buffer**.
e. Take 25 µl of beads for each sample and add them to the chromatin for the pre-clearing step.
f. Divide the remaining beads into 45 µl aliquots. Add the corresponding antibody to each tube, plus a negative control (specific IgG). Incubate overnight at 4ºC in a rotating wheel.

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Day 3

Please Note: From this stage it is better to work with siliconized tubes.

a. Discard the beads from the chromatin samples by putting the tubes in the magnet.
b. Wash the Ab-Bead complexes twice with ice-cold 300 µl BSA/PBS 5%. Spin after second wash to displace the sample from the tube walls. Remove as much of the supernatant as possible.
c. Add 1 ml of the chromatin to each sample and resuspend with the tip. Incubate for 2 hours at 4°C in a rotating wheel.

Tip 6

Store the excess chromatin from the input sample.

d. Spin the samples and put them on the magnet.
e. Wash twice with 1 ml **Low Salt Buffer**.
f. Wash twice with 1 ml **High Salt Buffer**.
g. Wash twice with 1 ml **LiCl Buffer**.
h. Wash twice with 1 ml **TE** when adding the second washes. Change tubes for new ones.
i. Remove last wash almost completely with the pipette.
j. Prepare **Elution Buffer (EB)** and set the thermomixer to 65°C.
k. Add 100 µl of **Elution Buffer** to each sample. Incubate for 10 minutes at 65°C in the thermomixer.
l. Put the supernatant in a new tube and repeat the step k to reach a 200 µl final volume.
m. Take 50 µl of the exceeded chromatin from the pre-clearing as a 5% input. Add 150 µl **Elution Buffer** to reach a 200 µl final volume.
n. Incubate samples and inputs at 65°C overnight to de-crosslink.

Day 4

a. Add 1 µl of Proteinase K to reach a 50 µg/ml final concentration. Incubate at 42°C for 1 hour.
b. Elute samples twice with 30 µl of TE/EB/water until a final volume of 60 µl is reached.

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Buffer Solutions

1X PBS
10 mM Na_2HPO_4
1.8 mM KH_2PO_4
137 mM NaCl
2.7 mM KCl
Adjust pH to 7.4
Add ddH_2O to the final volume

Cell Lysis Buffer
5 mM HEPES
85 mM KCl
0.5% NP40, pH 8.0

Nuclear Lysis Buffer
50 mM Tris•HCl
10 mM EDTA
1% SDS, pH 8.1

Dilution Buffer
0.1% SDS (protein interaction depend)
1.1% Triton X-100
1.2 mM EDTA
165 mM NaCl
16.7 mM Tris•HCl, pH 8.1

Low Salt Buffer
Tris•HCl 50 mM, pH 8.0
150 mM NaCl
0.1% SDS
1% NP40
1 mM EDTA
0.5% Deoxycholate Na

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Buffer Solutions

High Salt Buffer
Tris•HCl 50 mM, pH 8.0
500 mM NaCl
0.1% SDS
1% NP40
1 mM EDTA
0.5% Deoxycholate Na

LiCl Buffer
Tris•HCl 50 mM, pH 8.0
250 mM LiCl
0.1% SDS
1% NP40
1 mM EDTA
0.5% Deoxycholate Na

TE Buffer
Tris•HCl 10 mM, pH 8.0
0.25 mM EDTA

Elution Buffer
100 mM $NaHCO_3$
1% SDS

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