

mES Cell Extraction

1-12-22

Mouse ESCs

B6, P17

129S, P14

2mL RIPA Protein Extraction Buffer prepared (2mL RIPA, 1X Protease Inhibitors (Roche), 1X PMSF, 0.5mM EDTA, 1mM EDTA, Benzonuclease) and place on ice.

Add buffer to cells on ice at 100ul per 3 million cells (B6: 16million: 500ul and 129S: 8million: 250uL).

Immediately vortex cells.

Incubate cells on ice vortexing every 5m for 20m.

Spin extracts at 10,000 × g at 4°C for 10m.

Transfer supernatant to a new tube.

Determine total protein concentration by Bradford Assay.

Dilute mES cell extracts with standard loading buffer to a final concentration of 1mg/mL.

Heat gel samples at 95°C for 5m. Store at -20°C till use.

ES cell extracts were a gift from Dr. Laura Reinhold

Western Blots

1-13-22

Mouse ESCs

B6, P17 , 1.0 ug/ul

129S, P14, 1.0 ug/ul

2 Gels (Bio-Rad 4–20% Mini-PROTEAN TGX Precast Gel, 1.0mm, 10 well (#456-1094)) were loaded with 5ug and 25ug in suplicate of 129S and B6 mESC extracts

The gels were run at 150V for 1h 30m at room temperature in Tris-Glycine-SDS buffer

Gel transferred overnight at 4°C to 0.22um PVDF from Bio-Rad

The blot was removed from the gel and cut in half. One half of each blot was place into 2 separate boxes.

Block (3% BSA + 5% Dry Milk in TBST + 0.03% TWEEN-20) was added and the blots where shaken orbitally at room temperature for 1h.

One set of blots was placed in 5mL block with α CTCF at 1:1000 dilution.

One set of blots was placed in 10mL block with α RPB1 at 1:1000 dilution.

Both sets were left shaking at 4°C overnight.

The next morning the blots were each washed with TBS-T + 0.03% TWEEN-20 for 15m x 5m x 5m x 5m.

The blots were the placed in 10mL block with anti Rabbit-HRP (α CTCF) or anti Mouse-HRP (α RPB1) at 1:10000 dilution.

The blots were left shaking for 1h at room temperature.

The blots were then each washed with TBS-T + 0.03% TWEEN-20 for 15m x 5m x 5m x 5m.

The blots were then developed with Pico Plus reagents (ThermoFisher # PI-34087) and visualized with a Syngene G-Box.

The images are labeled (see below).

mES cells were a gift from Dr. Laura Reinhold

01-14-22

aRNA Polymerase II RPB1 Mouse (BioLegend #664906) 1:1000 in 1%BSA + 1%dry milk
129S and B6 mES cell total protein extract
Pico reagents (ThermoFisher #PI-34087)
RNA Pol II 220KDa

