Western Blot (BLOT CYLCER) Protocol

Cells Line (Lysate): GM12878, K562, HepG2, Liver

Gel Electrophoresis

1) Denature protein samples at 90 ^{O}C for 5 minutes.

2) Fill gel box with 700 ml 1x Running buffer.

3) Load 2ug of each nuclear protein sample and 5 µL marker (Bio Rad dual color) on 4-15% gradient SDS-PAGE precast gel (Bio-Rad).

4) Attach leads to the gel box. Run the gel at 80 volts. It takes about 1 to 1.5 hours.

Transferring

5) Discard the stacking gel (Trick: Put the paper tower on top of the stacking gel, then lift it. The gel will come off very easily). Transfer the separated proteins to nitrocellulose paper for **1-1.5 hours** at 250 mA (Bio-Rad power supply). (Keep cold in ice bucket)

NOTE: The nitrocellulose paper may be stored at this stage. For short term storage, up to 5 days, wrap NC paper in seran-wrap and store @ 4 ^{O}C. For long term storage the NC paper should be placed in a seal-a-meal bag with 2-3 mL 0.1% thimersol and stored @ 4 ^{O}C. The 0.1% thimersol MUST be thoroughly rinsed off before using the NC paper.

Blocking and Detection

6) After transfer, take out nitrocellulose membrane and put it in blocking buffer (5% milk).

7) Turn on Blotcycler and listen for click to make sure vent are closed.

8) Add Wash Buffer (PBS/0.1% Tween-20) to main compartment, till max line ~3liter.

9) Add 15ml Blocking Buffer (5% milk/PBS-0.1%Tween 20 (PBST)) to each tray. Place membrane in respective trays. Cover trays with lid.

10) Prepare Primary Antibody. Dilute the testing antibodies in 15ml 5% milk in PBS-T. Add to column labeled P1-P6. ** Make sure that PA and trays are matched.

*Save the primary antibody (add 0.02% sodium azide), and store at 4 ^{0}C for reuse, use collection vial. Collection vials should be matched to proper tubing.

11) Prepare Secondary Antibody. Dilute the IgG antibodies in 15ml 5% milk in PBS-T. Add to column labeled S1-S6

Secondary Antibody dilution:
Mouse (1:5000)
Rabbit (1:5000)
Goat (1:1000)

12) Start BlotCycler.

Analysis

13) Place the blot on saran wrap. Add 750 μL of PICO per strip of NC paper. Incubate at room temperature for 5 minutes.

14) Tap off the solution. Place the blot inside a new sheet of saran wrap. Tape it securely inside a cassette.

15) When inside the dark room, place a sheet of film over the blot for the desired length of time. Then feed it into the developing machine.

*Redo with PICO (80%) and FEMTO (20%) when any of the bands was faint. Add 750 μL of the substrate per strip of NC paper. Incubate at room temperature for **5 minutes**.