# **Western Blot** – Developing

**Day 1:**

- Transfer to nitrocellulose membrane at 400mA for 3-4 hours
  - (3 if fresh transfer buffer or 4 if used twice)
  - Put white sponge in back and run to Red
- Mark ladder with pencil and bottom right corner of membrane
- Cut membrane if blotting for different Antibodies
- Put each piece in a sealable sleeve with 3-10mL 3% Milk or 3% BSA
  - 3mL for small pieces, 5mL for half blot, 10mL for full blot
  - 3% BSA for phospho antibodies and 3% Milk for rest
- Seal and place on rocker at room temp for 1hr
- Add 1° Ab, reseal and place on 4°C rocker overnight or over weekend

**Day 2:**

- 3x5min washes in TBST
- While washing make 2°Ab in 15mL conical tube
  - Used at 1:1000 in either 3% Milk or 3% BSA which ever was used for 1°Ab
- Put each piece in sealable sleeve with 2°Ab
- Seal and place on rocker at room temp for 1hr
- 3x5min washes in TBST
- Incubate 3min in Lumi-phos
  - Either directly in the wash tray or onto a piece of cling wrap on the bench
- Transfer blots to cassette with transparency sleeve and smooth out any bubbles
- Expose and develop film
  - Usually start with 30sec exposure and then do shorter or longer exposures depending on how film looks
  - REMEMBER: the blots always scan in darker so better to have lighter exposure
- If needing to blot for another Antibody at this step you would strip 30min in stripping buffer in the hybridization oven at 50°C
  - Stripping buffer can be used 3x and then fresh should be made
  - Let warm up in hybridization oven as it comes to temperature
  - The stock is in the 4°C deli fridge. Add 50mL to each stripping bottle and add 390µL BME to each
- 3x15min washes in TBST
- Block each piece in sealable sleeve with 3% Milk or 3% BSA at room temp for 1hr
- Add 1°Ab, reseal and place on 4°C rocker overnight

**Day 3:**

- Repeat Day 2 stopping after developing if don't need to blot for another Antibody
  - Store blots in sealable sleeve with TBST if want to keep, otherwise toss