BISHOP LAB (Modified from Red Book Protocol and Lab Protocols)
WESTERN BLOTS

Setting up Transfers

1. Items needed per transfer: 1 gel transfer cassette, 2 scouring sponges, 6 pieces of Whatman filter paper (cut to size of scouring sponges), 1 piece Immobilon P PVDF membrane from Millipore (cut to size of SDS-PAGE gel without the stacker)
2. Make up 1L 1x transfer buffer for each gel transfer tank (two transfer cassettes per tank)
3. Pre-soak sponges and filter paper in a tray with 1x transfer buffer
4. Pre-soak membrane in small tray in 100% methanol
5. After gels are done running, set up transfer "sandwich" as written below, being very careful to remove any air bubbles in between layers

Clear Side (+) **Black Side (-)**

Scouring pad 3 pieces Whatman Membrane Gel 3 pieces Whatman Scouring pad

Note: *Air bubbles can be prevented by rolling a cut-off pipet over the layers of the sandwich to squeeze out any air. Dipping the SDS-PAGE gel into transfer buffer before placing it on the membrane makes it easier to position the gel on the membrane and also removes any excess SDS from the gel running buffer.*

6. Insert transfer cassettes into transfer apparatus and place into gel buffer tank
7. Add ice pack to tank and fill with 1x transfer buffer (you can also use the buffer in which the filter paper and sponges were soaking)
8. Transfer proteins (for Dmc1 gels, transfer at 100V for 1hr at 4ºC or overnight at 10V at 4ºC)

Western Blotting

1. Remove blot from transfer sandwich and rinse briefly two times in TBST to remove residual transfer buffer
2. In a small, shallow plastic tray, incubate blot in blocking buffer and place on rocker for 1 hour at room temperature
   Note: Depending on the size of the tray, you will need about 5mL blocking buffer per blot. Also note that blots can be blocked for a few days at 4ºC. Be sure NOT to do this at room temperature, because the milk will spoil!
3. Incubate blot in primary antibody diluted in blocking buffer and place on rocker for 1 hour at room temperature (For Dmc1 blots: 1:8000 dilution of Rabbit Dmc1 antibody and 1:800 dilution of Goat Arp7 antibody, which is used as a loading control)
4. Transfer blots into larger plastic tray for washes to allow for large wash volume and faster shaking
5. Wash blot 10 times in 10-20mL TBST on a shaker for 5-10 minutes per wash at room temperature
6. Incubate blot in secondary antibody diluted 1:3000 in blocking buffer and place on rocker for 1 hour at room temperature
7. Wash blot 3 times in 10-20mL TBST on a shaker for 10 minutes per wash
8. Mix equal volume of each ECL kit chemiluminescence reagent in a small plastic tray
9. Immerse blot into chemiluminescence solution for 30 seconds
10. Shake off excess liquid from blot and wrap in plastic wrap
11. Image blot on film in dark room

10X Transfer Buffer
30.3g Tris Base
144g Glycine

Dissolve into 1L ddH_20
Store at room temperature

1X Transfer Buffer
1X transfer buffer + 20% methanol

20X TBS
160g NaCl
4g KCl
60g Tris Base

Dissolve in 900mL ddH_20
Adjust pH to 8.0 with HCl (will need 30-35mL)
Bring volume to 1L with ddH_20

TBST
1X TBS + 0.1% Tween

Blocking Buffer
5% dried milk in TBST