Western Blotting

Protocol for Western Blotting proteins carrying a his-tag

Day 0

- Inoculate the desired cultures in 5 mL LB medium with appropriate antibiotic (AMP or CAM, depending on the resistance marker) and leave overnight at 37°C.

Day 1

- Inoculate 15 mL LB medium with 500 uL of each overnight culture.
- Add appropriate antibiotic
- Leave to grow until OD600 ≈ 0.6.
- Add _____ uL 1M IPTG for a final concentration of _____ mM.
    - Use concentrations of 0.1, 0.5 or 1.0 mM
- Leave at 37 °C overnight.

Day 2

N.B.: Keep everything on ice until loaded on the gel.

- Transfer 2 mL from each sample into a 2 mL centrifuge tube and spin for 5 min at 13.000 rpm and 4°C.
- Remove supernatant.
- Re-suspend the pellets in 500 uL Lysis Buffer.
- Sonicate each sample for ~10-15 sec.
- Spin each sample for 5 min at 13.000 rpm and 4°C.
- For each sample mix 10 uL loading dye with 50 uL sample.
- Fill gel tub with MOBS.
- Load gels into pre-cast SDS-gel (Criterion XT Bis-Tris Precast Gel).
- Run for ~15-30 min at 250 V.
- Take out the gel and place/wash it in PBS-T so it won't dry out.

- Prepare the nitrocellulose membrane and transfer the gel to it (Bio-Rad Trans-Blot Turbo Transfer System)
- Mix 2.5 g skimmed milk powder with 50 mL PBS-T buffer.
- Place the membrane in the 50 mL milk mixture and leave it shaking at 88 rpm. for 1 hour.
- Discard the milk and wash with PBS-T 3 times (5 min on shaker per wash, renew PBS-T between washes).
- Mix 1.5 g skimmed milk powder with 30 mL PBS-T and _____ uL _____ M primary chicken antibodies.
- Place the membrane in the antibody mixture and leave overnight at 5ºC.

Day 3

- Take membrane out of cold room.
- Collect the antibody mixture the membrane was placed in for later re-use.
- Wash the membrane 3 times with PBS-T (10 min on shaker per wash; 88 rpm).
- Mix 1.5 g skimmed milk powder with 30 mL PBS-T and _____ uL _____ M secondary chicken antibodies.
- Remove the PBS-T and add the 30 mL secondary AB mixture, leave shaking for 1 hour.
- Remove the secondary AB mixture and was 2 times at 10 min with 30 mL PBS-T.
- Wash once for 5 min with 30 mL PBS.
- Place the membrane on a transparent plastic sheet.
- Mix Immobilon Western Chemiluminescent HRP Substrate for a final volume of 2 mL.
- Spread the mixture on the membrane and place a piece of transparent plastic above.
- Image the western using Bio-Rad Universal Hood iii and Image Lab software.

Buffers

Lysis buffer

- 5.0 mM Imidazole
- 0.5 M NaCl
- 20 mM Tris-HCl pH 7.9

- 1.0 mM PMSF

For 15 mL mix:

- 18.85 uL 4M Imidazole
- 1.5 mL 5 M NaCl
- 0.6 mL 0.5 M Tris-HCl pH 7.9
- 150 uL 0.1 M PMSF