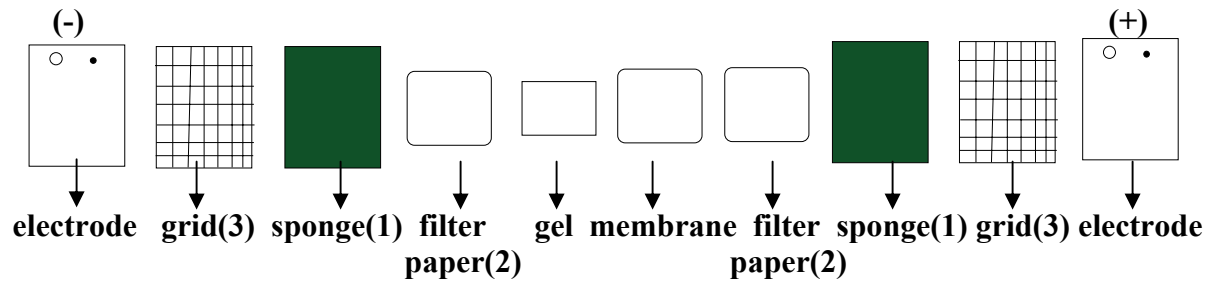


Sugden lab.

## Western blot analysis



- Transfer for 45 min at 25 volts at room temperature.
- Use transfer buffer:
  - 100 mL of 10xTris Glycine
  - 5 mL of 20% SDS
  - 700 mL of Water
  - 200 mL of Methanol
- Incubate the membrane with Milk Solution overnight
  - 5% Milk solution:
    - 500 mL of 1xPBS
    - 25 g of milk powder
    - 1.25 mL of 20 %Tween-20
- Next day, incubate the membrane with primary antibody in milk solution at room temperature for 1 hour.
- Wash 3 times for 20-30 min with milk solution. (3<sup>rd</sup> time, I like to flip the membrane).
- Incubate with secondary antibody in milk solution at room temperature for 1 hour.
- Wash 3 times for 20-30 min with milk solution.
- Wash with water.
- Add stain:
  - 45 mL of water
  - 333ul of BCIP
  - 333ul of NBT
  - 5 mL of 10xAP Buffer
  - 250 ul of MgCl<sub>2</sub>.