

M3D4: Complete antibody staining for Western blot analysis

1. Prelab
2. Incubate blots with primary antibody
3. Wash
4. Incubate blots with secondary antibody
5. Discuss research proposal with other group partner during incubations



Upcoming Assignments and Deadlines

- **Tuesday, Nov. 26**

- Research proposal pitch during lecture
 - Take the detailed outline you did for M3D4 homework and modify it based on feedback
- Turn in a hard copy of your updated detailed outline at the pitch lecture

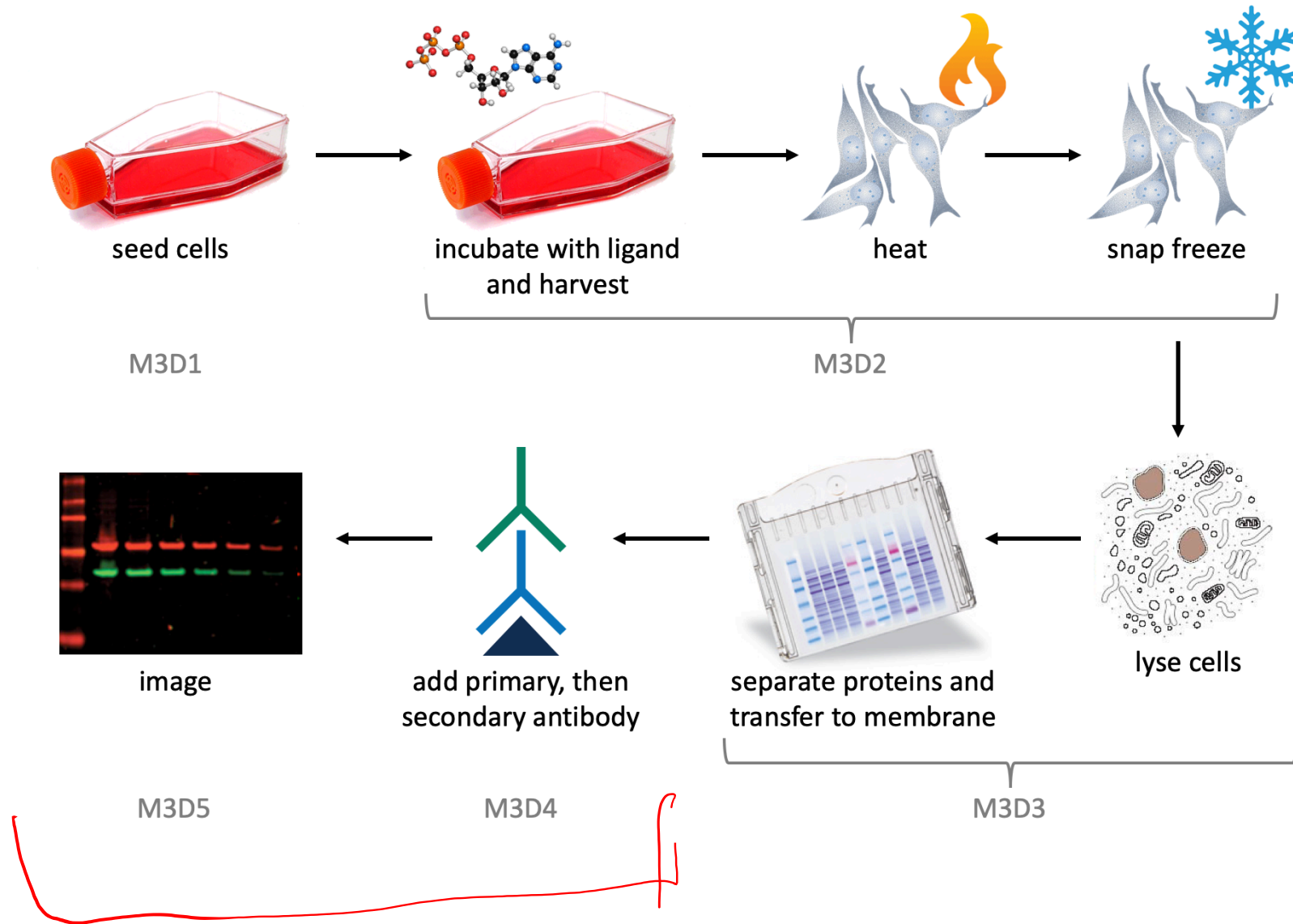
- **Friday, Dec. 6**

- Research Proposal Presentations due
- Blog post due 10pm

- **Tuesday, Dec. 10**

- Mini-report due by 10pm

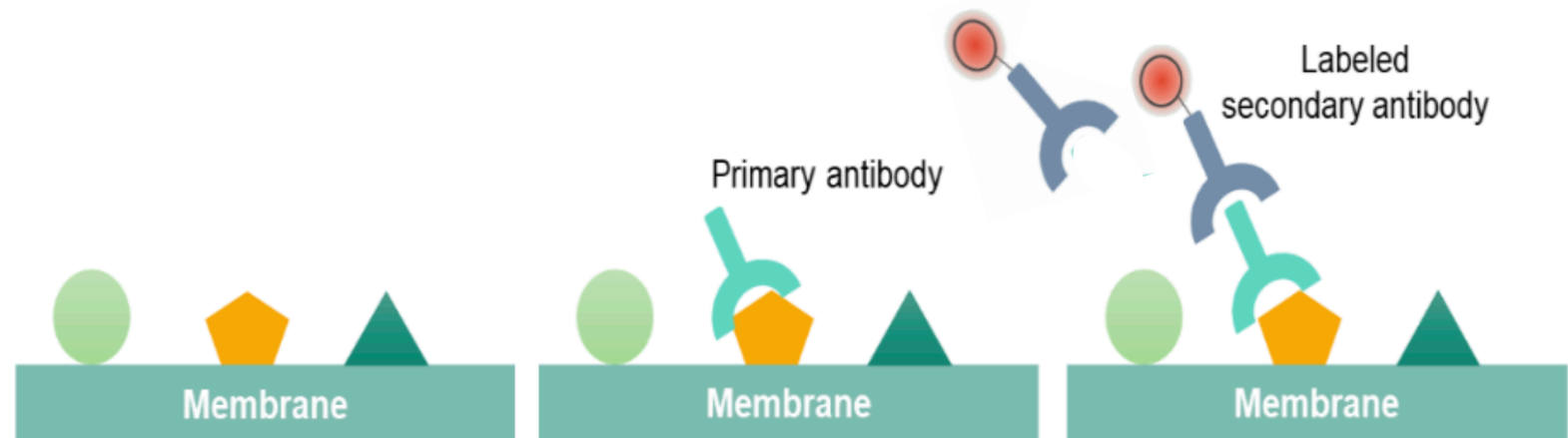
Mod3 Overview



Western blotting

- AKA: immunoblotting
- Uses Primary antibody raised against proteins of interest to identify protein bands on the blot
- Uses Secondary antibody raised against the species of the primary antibody to visualize primary antibody binding to the protein of interest
- Semi-quantitative

Bonus point: Why is it called the Western blot?

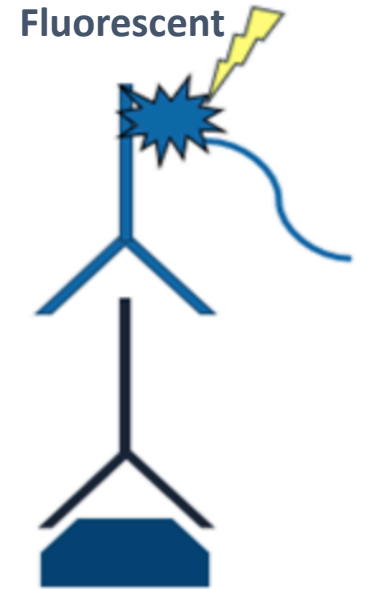
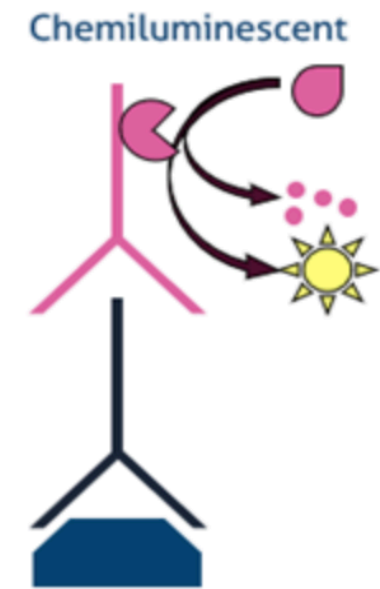


Visualizing Western blots

- Once you have antibodies bound to your protein of interest, you need to visualize it

- Most common ways:
 - Chemiluminescence
 - HRP
 - Film

Chemical /
Enzymatic
RXN



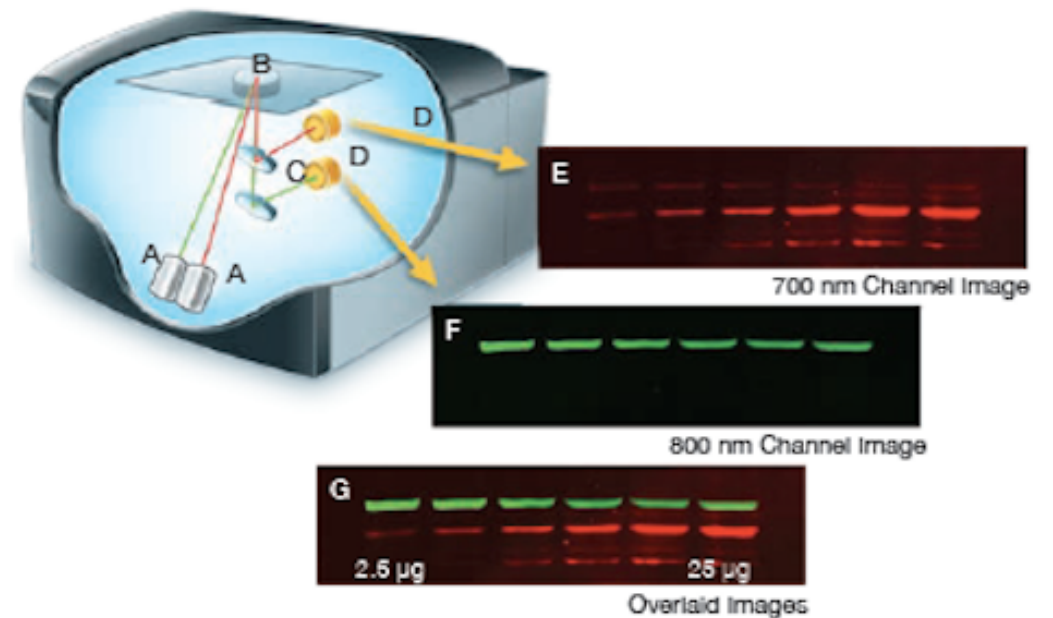
- ★ • Fluorescence

- IR → Infrared tag
- Digital scan

680 channel
800 channel

Visualizing your Western blot

- Licor System
- Uses infrared conjugated secondary antibodies
- Lasers inside Licor box allows excitation of 700 and 800nm wavelengths
- Produce overlaid image from both channels to identify protein of interest and loading control on the same blot



Points to note for your experiment today

- Primary antibodies:
 - FKBP12 @ 1:1000
 - Tubulin @ 1:5000
- Secondary antibodies:
LIGHT SENSITIVE!!
- Keep your membranes covered during all incubations
- We will scan your membranes so you can analyze your western blot data after Thanksgiving

During incubations:

- Discuss your Research proposal details (from homework) with classmates
 - Use prompts on Wiki to guide discussion

Group 1: Daniel & Haley

Group 2: Wilson & Yara

Group 3: Sarah & Christy

Group 4: Jose & Kylie

Group 5: Pranav & Apolonia

Group 6: Courtney & Fidelia

Due M3D5 (December 3)

- Submit specific aims for your research proposal
 - Each aim must include a bulleted list of experiments to accomplish each aim