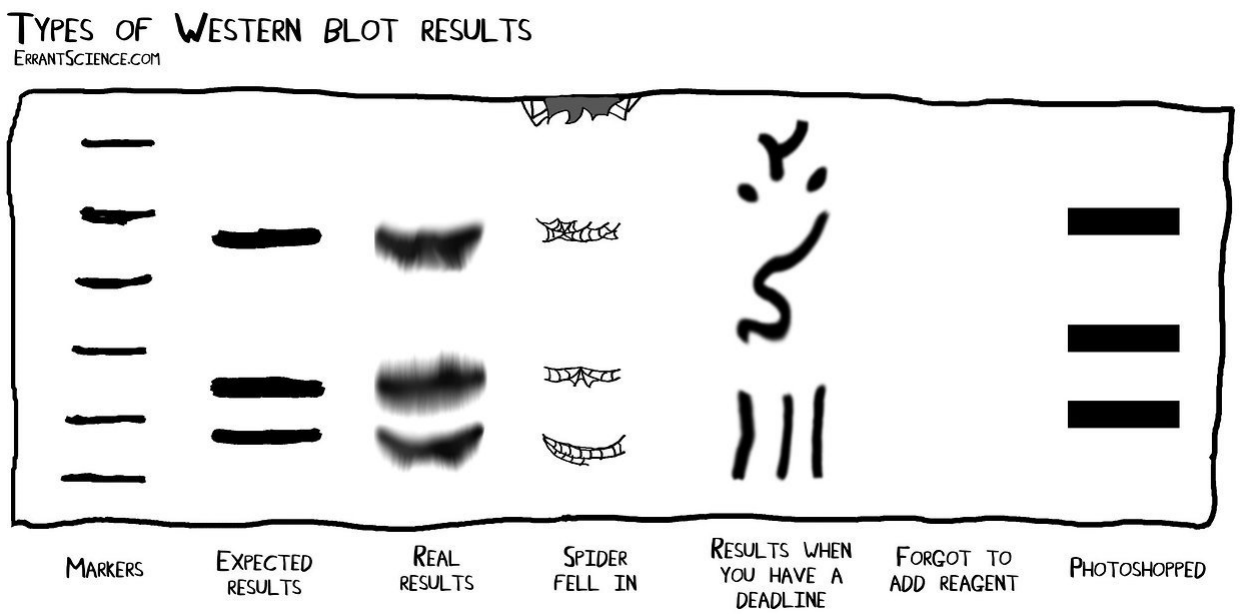
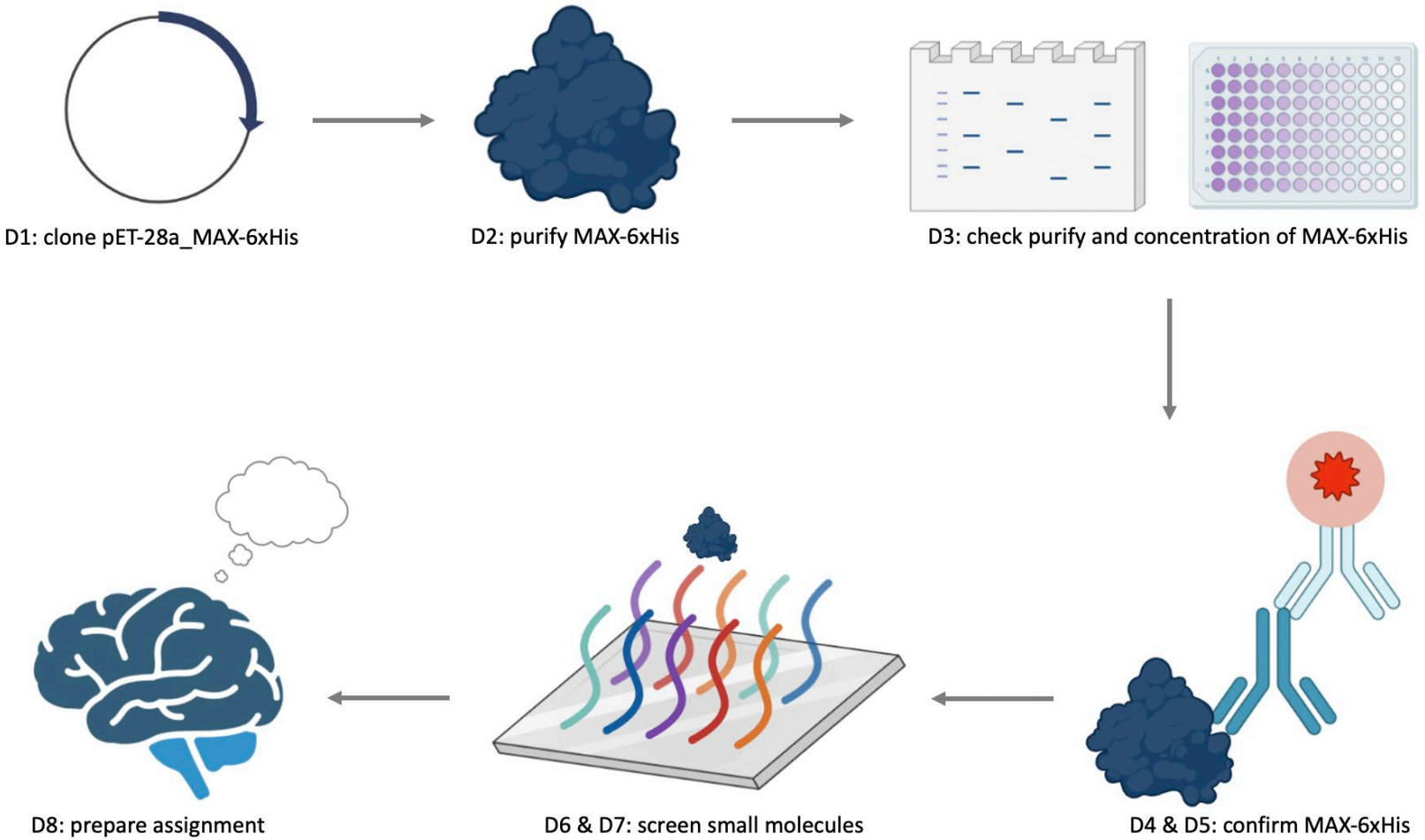
M1D4:
Confirm purified protein using Western blot

1. Comm Lab workshop
2. Prelab discussion
3. Electrophorese and transfer purified protein
4. Participate in paper discussion

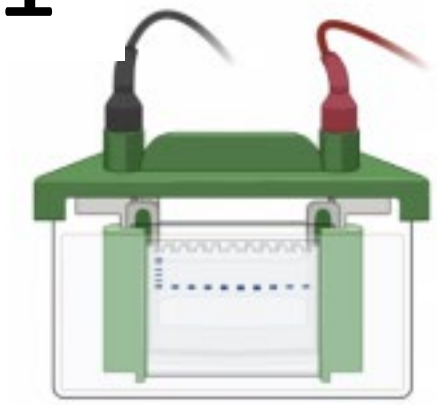


Overview of Mod 1 experiments:



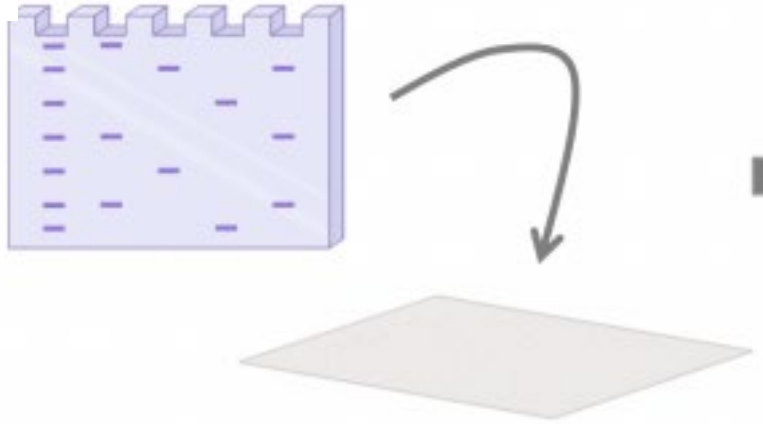
Western blots probe for specific proteins

1



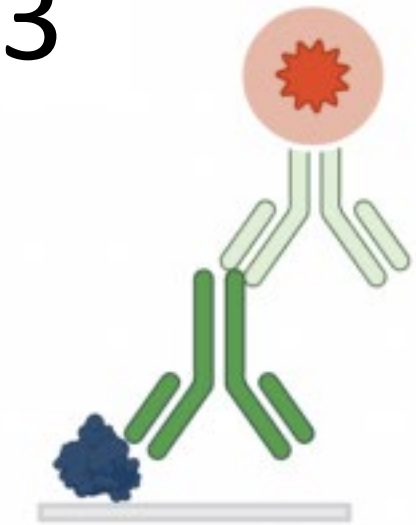
separate proteins using electrophoresis

2



transfer proteins onto nitrocellulose membrane

3



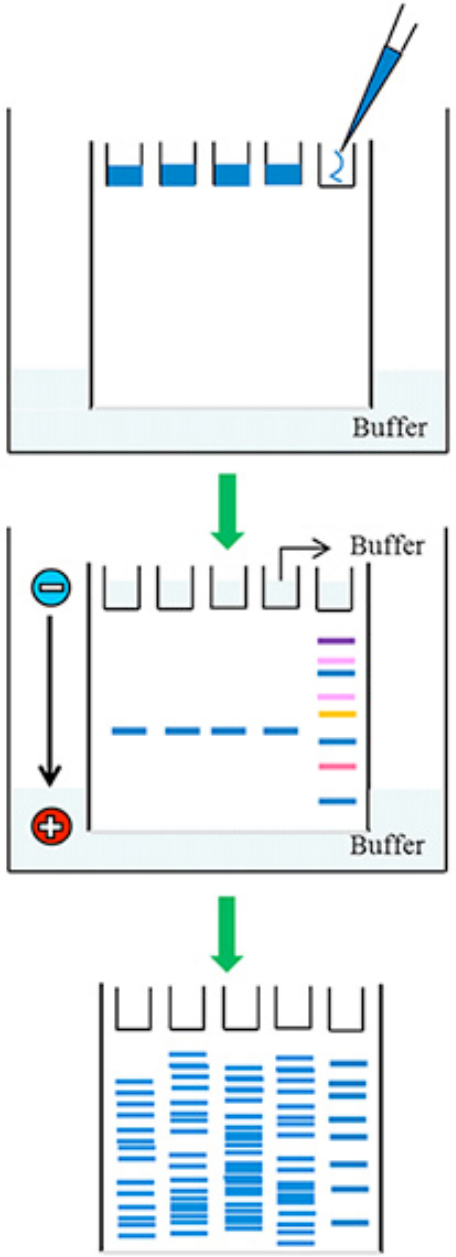
probe membrane using antibodies

Pro and con of the Western blot vs Coomassie staining?

Step 1: separate proteins using electrophoresis

- SDS-PAGE used to separate proteins

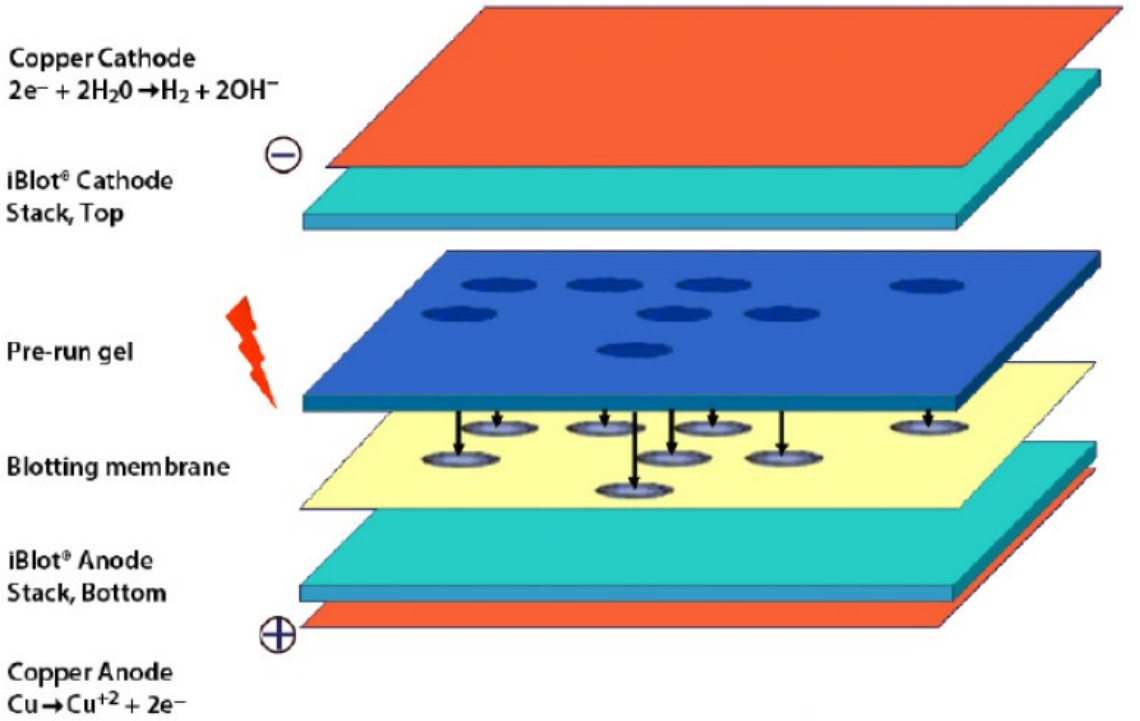
- How does adding Laemmli buffer and boiling change protein structure?
- What determines how far a protein migrates in a polyacrylamide gel?



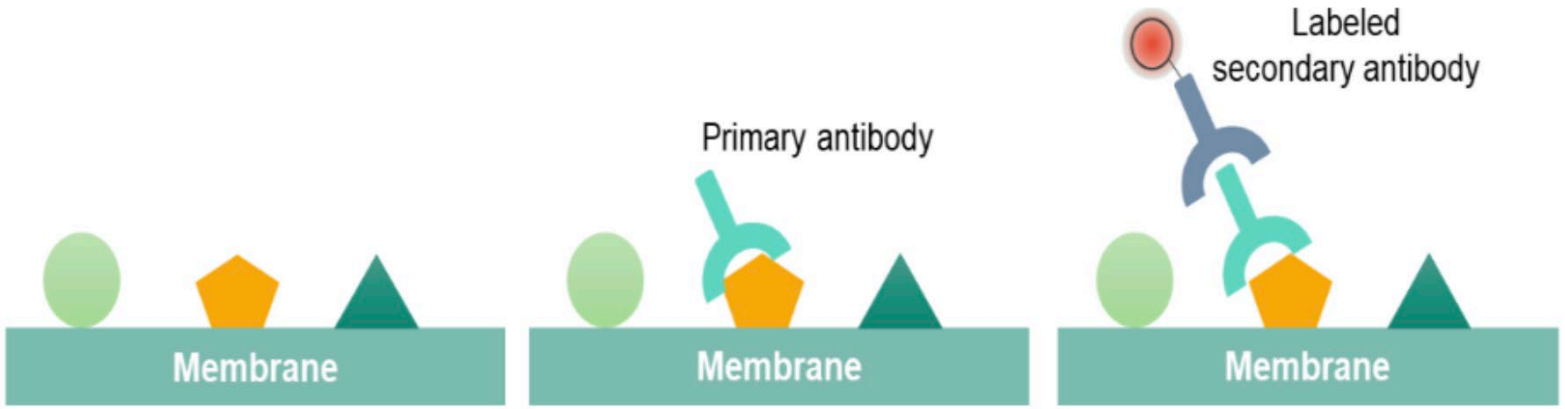
Step 2: transfer proteins onto nitrocellulose membrane

- Protein bands from polyacrylamide gel transferred to a nitrocellulose membrane via applying a current

- Why is it necessary to transfer proteins onto a membrane?



Step 3: probe membrane using antibodies



Primary – Mouse IgG anti-MAX

Secondary – Goat anti-Mouse IgG

- Primary antibody raised against protein of interest to identify band that corresponds to specific protein on the blot
- Secondary antibody raised against the species of the primary antibody to visualize band that corresponds to specific protein of interest

- Why use a secondary antibody (rather than a labeled primary antibody)?

For today…

- Class divided into two groups
  - Pink Yellow Blue will start on Western blot
  - Green, Red, Orange, Purple will start with paper discussion

For M1D5…

- Revise due M1D3 homework using feedback and workshop materials
- Draft outline of script for Research talk

Mini-presentation due Saturday, March 4

- Prepare a video of you verbally discussing your research
  - Use any device or Zoom
  - No visuals / slides
  - Do not edit / splice the video
- Submit to Gmail account!
  - bioeng20.109@gmail.com
  - Remember to follow file name guidelines

Presentation should be 3 min (+/- 15 sec)

- Introduce yourself
- Provide important background information
- Describe key results
  - Briefly describe critical methods used to generate important data
  - Use quantitative descriptions when discussing results
- Highlight the take-home message

What data / results should be included?

- Protein purification

- Protein purity and concentration

- Western blot results **(Include as a placeholder in your next homework)**

Review assignment description on wiki

Category	Elements of a strong presentation	Weight
Introduction	• Introduce yourself and the research • Summarize the background information necessary to understand the research • State the research question	25%
Methods & Data	• Provide ONLY the method information necessary to understand the results • Give complete and concise explanations of the results • Relate the results to the central question	25%
Summary & Conclusions	• Highlight the key finding(s) relevant to the central question / hypothesis	25%
Organization	• Give a logical, easy-to-follow narrative • Include transition statements	15%
Delivery	• Show confidence / enthusiasm and speak clearly • Use appropriate language (technical or informal, as appropriate) • Be mindful of the time limit (3 minutes +/- 15 seconds!)	10%

The Research talk will be graded by Dr. Noreen Lyell with input from Dr. Becky Meyer and Jamie Zhan.