M3D3: Run SDS-Page gel and begin Western blot analysis

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- 1. Quiz
- 2. Prelab
- 3. Finish lysing/centrifuge cells
- 4. Run SDS-Page gel
- 5. Transfer to Nitrocellulose blot
- 6. Leave NC blot in Block at 4C



Prepare cell lysate by removing protein aggregates

- In the previous class
 - Cells were treated with ligand
 - Samples were heated to denature protein
 - Samples went through snap-freeze/thaw cycles
 - Weakens/fractures cell membranes to lyse cells
- Today
 - Lysate will be centrifuged to pellet protein aggregates and cell debris
 - Soluble protein will be used for CETSA



Denature soluble proteins for CETSA analysis

- Laemmli buffer added to prepare proteins for electrophoresis
 - Tris Dalts, buffer pt
 - · SDS coats proting to produce net negative
 - glycerol adds weight to Sample
 BME Brevcapteethand weaks disulfide bonds

 - bromophenol blue

tracking sample through get

Separate proteins using SDS-PAGE

- <u>S</u>odium-<u>d</u>odecyl <u>s</u>ulfate <u>polya</u>crylamide <u>g</u>el <u>e</u>lectrophoresis
- SDS-PAGE separates proteins according to size
 - Charge and secondary structure are alleviated
- As with agarose electrophoresis, molecules migrate toward positive electrode (anode)



iBlot2 Transfer of proteins from gel to blot

- Move protein bands from polyacrylamide gel to a nitrocellulose membrane for further assessment
- Again, net negatively charged proteins are migrated using a current from the gel to a membrane
- Membrane can be immunoblotted to determine presence of protein of interest



CETSA visualized via Western blot

- Membrane with proteins is 'blotted' using antibodies to probe for specific for protein of interest
- Why do we need to probe for our protein of interest? FKRP12 -Ab to identify endogeness protein



• Why do we probe for tubulin? Loading Cantol

For today...

• Use downtime to work on your Research proposal idea!

For M3D4...

- As a group, create a Research page to organize your ideas / thoughts

 See prompts on wiki!
- As a group, outline Background / Approach section of Mini-report
 - Review Mini-presentation assignment page on wiki