Future Western Blot Functionality

3/18/14

Additional Western Blotting Functionality

Add functionality to perform an immunoprecipitation (IP) and co-immunoprecipation (co-IP) to the western blotting experimental technique.

Sample Prep page
The two assays that we will be adding will affect the Sample Prep table in the following ways:
Sample Prep Table Columns: Select, Samples, Lysate Type, Western Blot Analysis, Conditions

Lysate Type

- When only one option is available, then the only available option will be listed in a text format.
- If there is more than one option for lysate type, then there will be a drop down menu in some assignments. The options would potentially be: whole cell, cytoplasmic, and nuclear

Western Blot Analysis

- When only one option is available (for example "Western blot"), then nothing will appear here or in the "Conditions" column.
- When there is more than one option, then there will be a drop down menu. The options are potentially: Western, IP-Western, and Co-IP-Western

Conditions

1. Western Blot - no conditions needed
2. IP-Western - There would be radial buttons with the available antibodies to perform an IP
3. Co-IP-Western - There would be radial buttons with the available antibodies to perform an IP

Note:
Like in the microscopy technique, a student can select more than one type of western blotting analysis for each sample. There are two options for how a user can select more than one type of western blotting analysis.

1. If there is more than one available lysate type and/or assay type, then multiple "sub-rows" appear for each row in the Sample Prep table.
2. Use copy and trash icons to duplicate/delete a row. A user can then select the conditions for one type of western blotting analysis, click "copy" to duplicate it, and then edit the duplicated version for a second type of analysis.

We will have to be careful about the limit of 15 samples per gel. This may mean that option two is better than option 1.

Load Gel Step
Each row from the Sample prep table will then become it's own row in the Samples window (when a user is re-ordering the samples to load onto the gel). A user can run IP and "regular" samples side by side on the same gel.

Depending on the length of the sample name, we may need to abbreviate the sample's name. The sample should be listed as follows:

- Western blot: Sample name - Lysate Type. For example: Sample name - WCL

(WCL = whole cell lysate)

- IP: Sample name - WCL - IP Protein X
- Co-IP: Sample name - WCL - Co-IP Protein X

New Experimental Technique: Kinase assays

This type of assay is very similar to a western blotting procedure, but it is different enough that it should become its own experimental technique.

Here are the necessary changes that would need to be made for each of the steps of a Kinase assay
1. Sample Prep

Columns: Select, Sample, Lysate type, Kinase assay, Conditions

Things to think about:

- *in vitro* vs *in vivo* kinase assay
- IP-kinase assay
- adding substrate

2. Prepare Gel
Same as standard western blotting procedure.
Depending on the assignment, specific % of gels may be available to the user. These % of gels will behave in the same manner as a standard western blotting procedure.

3. Load Gel
Same as standard western blotting procedure
Users can re-order the samples and add a marker. Then they will click "Load". The samples will appear the same way on the gel (with the blue dye in each well)

4. Run & 5. Transfer
Same as before - click "Run Gel & Transfer"
A square will appear that represents the membrane with the transferred proteins on it.

Need to check:

- What kind of marker is used? Would it have colorful bands on it at this step?

6. Develop

- Remove the "Blotting conditions", Primary antibody and secondary antibody information from this page
- Move up "Analysis Tools" and exposure time slider up to the top of the right side of the small tabbed window
- Remove the "Re-probe" button

- Modify the times on the exposure slider to be much longer: 0, 1 min, 10 min, 30 min, 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 24 hours, 48 hours