WESTERN BLOTTING

Notes: First the instructor will define all of the components of the first few stages of a western blot experimental technique.

Sample Prep

1. What lysate types are available?

- [X] Whole Cell Lysate
- [X] Nuclear fractionation
- [X] Cytoplasmic fractionation

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SAVE AND CONTINUE

Prepare Gel

2. What percentages of acrylamide are available?

- [x] 10%
- [ ] 12%
- [ ] 15%

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SAVE AND CONTINUE

Blotting

3. Name the primary antibodies available and their corresponding secondary antibody.

	Primary Antibody	Corresponding Secondary Antibody	
1	Primary Antibody 1	Secondary Antibody 1	
2	Primary Antibody 2	Secondary Antibody 2	x
3	Primary Antibody 3	Secondary Antibody 2	x

Add

Notes: The first row cannot be deleted, but others can be deleted.

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SAVE AND CONTINUE

5. Define the size of the protein bands in kilodaltons (kDa) detected by each primary and secondary antibody combination.
Note: When multiple bands are detected by a particular antibody combination, separate the sizes of the bands by a comma. For example: 13, 45, 60. Do not include the units in the text box.

Primary Antibody	Corresponding Secondary Antibody	Size(s) by Lysate Type (kDa)		
		WCL	Nuc	Cyto
Primary Antibody 1	Secondary Antibody 1	13, 45	13	45
Primary Antibody 2	Secondary Antibody 2	25	25	25
Primary Antibody 3	Secondary Antibody 2	60	60	60

Notes: The table is automatically populated based on the answer to the preceding question. The instructor will fill in the text boxes with each of the bands that a particular antibody combination gives. If multiple bands should appear on the blot, then the instructor will separate them by commas in the text box. There will be text boxes for each lysate type that was selected by the instructor in question 1. In the future, we should think of a way for the instructor to copy particular entires within one text box to other text boxes in the same row.

The size text box only allows numbers that are comma separated. The instructor cannot type in the units.

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SAVE AND CONTINUE

6. Define which bands will appear and their relative intensity for each of your samples in your experiment setup.

SEE THE NEXT PAGE FOR NOTES ABOUT THIS PAGE.

A. Primary Antibody 1, Secondary Antibody 1

Sample	Lysate Type	Size (kDa)	Relative band intensity	Copy to other samples?
1. Strain A, Treatment A, 100 ng/mL, 30 C	WCL	13		[X]
	WCL	45		
	Nuc	13		
	Cyto	45		
2. Strain A, Treatment A, 100 ng/mL, 37 C	WCL	13		[ ]
	WCL	45		
	Nuc	13		
	Cyto	45		
3. Strain A, Treatment B, 200 ng/mL, 30 C	WCL	13		[ ]
	WCL	45		
	Nuc	13		
	Cyto	45		
4. Strain A, Treatment B, 200 ng/mL, 37 C	WCL	13		[ ]
	WCL	45		
	Nuc	13		
	Cyto	45		

This list continues for as many samples as the instructor has defined in the experiment setup

Copy to: X

- [ ] 1. Strain A, Treatment A, 100 ng/mL, 30 C
- [ ] 2. Strain A, Treatment A, 100 ng/mL, 37 C
- [X] 3. Strain A, Treatment B, 200 ng/mL, 30 C
- [X] 4. Strain A, Treatment B, 200 ng/mL, 37 C
- [ ] 5. Strain B, Treatment A, 100 ng/mL, 30 C
- [ ] 6. Strain B, Treatment A, 100 ng/mL, 37 C

OK

If the instructor selects “copy to other samples” then a pop up window appears (shown) and the instructor can check the names of other samples to which the band intensity should be applied.

Preview Film

The next antibody combination will appear directly below this one (NOT SHOWN).

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SAVE AND CONTINUE

NOTES FOR PAGE 5

Note: When the instructor first gets to this page, all of the sliders for each sample will be automatically at a “normal” default level of relative intensity and the size of the band(s) will be automatically filled in (from the prior question). If a particular sample does not have a band, then the user moves the slider all the way to the left and no band will appear. The instructor can manually adjust the intensity of the band here by adjusting the slider.

The slider will have about 10 preset points on it (like the exposure slider we have in western blotting). The slider needs to be labeled with the left side being “no band” and the right side being “big band”. It may also be helpful to have the slider labeled in some way - dashes or numbers - that will help the user as they move the slider along the horizontal line.

The size column is read-only since the instructor has already set the size of the band(s) in a prior question.

Since this list of samples can be long and there may be multiple primary/secondary antibody combinations, we may need to think of an easier way to navigate between antibody combinations rather than having a really long list. But we can go with a really long list for simplicity and speed at the beginning.

For right now, the preview button is for each antibody combination. Having the preview button close to the antibody combination will be useful for the instructor to easily navigate back and forth between preview and the editing features. An example preview window appears on the next page.

There is also a “copy to other sample(s)?” column where the instructor can select which sample conditions he/she would like to copy to the rest of the samples. This will copy all of the slider values for a sample to the rest of the samples.

This table will be formatted with light gray lines between bands for a sample and dark gray/black lines between samples.

This is what the preview blot function will look like:

Notes: This page will provide a preview of how the blots will appear in StarCellBio. Because the instructor may have many more samples than can appear on one blot (one blot is limited to 15 samples), representative samples for each protein size and intensity will be shown on this previewed blot.

If the instructor has selected more than one percentage of acrylamide for the gel, then we provide them with the first question which asks which gel percentage they would like to view. If the instructor only selected one gel percentage, then the previewed blot will show up.

PREVIEW FILM

Notes: If there are more samples than will fit on a blot (>15 samples) then multiple blots will be generated. These can be accessed using different tabs in this window. They will be labeled as Samples 1-15, Samples 16-30, etc.

X

Which percentage of acrylamide would you like to use for your gel?

(•) 10% ( ) 12% ( ) 15%

Samples

1. Strain A, Treatment A, 100 ng/mL, 30 C
2. Strain A, Treatment A, 100 ng/mL, 37 C
3. Strain A, Treatment B, 200 ng/mL, 30 C
4. Strain A, Treatment B, 200 ng/mL, 37 C
5. Strain B, Treatment A, 100 ng/mL, 30 C
6. Strain B, Treatment A, 100 ng/mL, 37 C

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Insert protein ladder sizes here.

Blotting Conditions
Primary antibody: Primary antibody 1

Secondary antibody: Secondary antibody 1

Exposure Slider
[insert exposure slider here so the instructor can adjust the exposure.]

7. Would you like background bands to appear?

- (•) Yes
- ( ) No

a) If yes, which antibody combination(s) should yield background bands?

- [X] Primary Antibody 1, Secondary Antibody 1
- [ ] Primary Antibody 2, Secondary Antibody 2
- [ ] Primary Antibody 3, Secondary Antibody 2

b) Define the size and intensity of each desired background band:

Primary Antibody 1, Secondary Antibody 1:

	Size (kDa)	Intensity	
1	80	1	
2	100	1	x

Add

We may not need this page because the instructor can in put background bands in question 5 and define the intensity in question 6. But this is a little easier than going through each row to adjust the intensity for each sample.

The instructor would then select the antibody combination that should yield background bands and define the sizes/intensities of those background bands.

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Finish western blotting