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
- [ ] Nuclear fractionation
- [ ] Cytoplasmic fractionation

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Prepare Gel

2. What percentages of acrylamide are available?

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

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Blotting

3. What are the names of the available primary antibodies?

1 Primary Antibody 1

2 Primary Antibody 2 x

3 Primary Antibody 3 x

Add

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

4. What are the names of the available secondary antibodies?

1 Secondary Antibody 1

2 Secondary Antibody 2 x

Add

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

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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, separate the sizes of the bands by a comma. For example: 13,42,60. Do not include the units in the textbox.

Blank film?	Primary Antibody	Secondary Antibody	Size(s) (kDa)
☐	Primary Antibody 1	Secondary Antibody 1	13, 45
☒	Primary Antibody 1	Secondary Antibody 2	*Sizes*
☒	Primary Antibody 2	Secondary Antibody 1	*Sizes*
☐	Primary Antibody 2	Secondary Antibody 2	25
☒	Primary Antibody 3	Secondary Antibody 1	*Sizes*
☐	Primary Antibody 3	Secondary Antibody 2	60

Notes: The instructor will fill in the table 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.

The size text box only allows numbers that are comma separated. The instructor does not need to type in the units.

The checkboxes on the left are for the instructor to select whether a particular antibody combination will yield no protein bands. Instead of checkboxes, we could have yes/no radio buttons (like on the next pages).

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

A. Primary Antibody 1, Secondary Antibody 1

Sample	Band?	Size (kDa)	Relative intensity
1. Strain A, Treatment A, 100 ng/mL, 30 C	Yes / No	13	3
	Yes / No	45	3
2. Strain A, Treatment A, 100 ng/mL, 37 C	Yes / No	13	10
	Yes / No	45	10
3. Strain A, Treatment B, 200 ng/mL, 30 C	Yes / No	13	3
	Yes / No	45	3
4. Strain A, Treatment B, 200 ng/mL, 37 C	No / Yes		
	Yes / No	45	3
5. Strain B, Treatment A, 100 ng/mL, 30 C	Yes / No	13	3
	Yes / No	45	3
6. Strain B, Treatment A, 100 ng/mL, 37 C	No / Yes		
	No / Yes		

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

See page 7 for the next antibody combination - The next antibody combination will appear directly below this one.

Preview Film

Note: When the instructor first gets to this page, all of the samples will have the "yes" radio button automatically selected, the size of the band(s) automatically filled in (from the prior question) and a default intensity value. If a particular sample does not have a band, just select "no" and the band size and relative intensity text box will disappear. The instructor can also manually adjust the intensity of the band here.

If the instructor selects "no" then the size and relative intensity values disappear. Another option is that they become opaque and uneditable, but we don't want the instructor to think that the band will appear at the given intensity.

The size column is currently 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.

I'm not sure whether there should be a preview button for each antibody combination or have the instructor navigate to the next page to preview the bands on the blot. For right now, I have set the preview button to be for each page. An example preview window appears on the next page.

Note: There needs to be some sort of copy to all rows for the band yes/no and intensity value. We're not sure what this should look like or where it should go. Maybe a yes/no button with a drop down menu to select the sample to be used as a template would work.

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

X

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

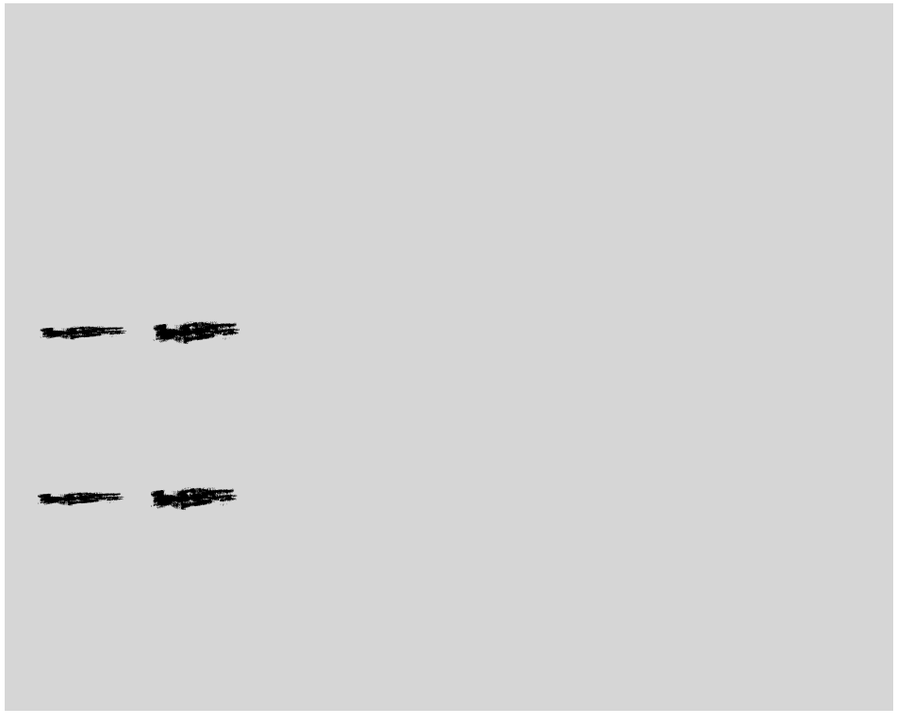
(•) 10% ( ) 12% ( ) 15%

Samples

1. 13 kDa, I = 3; 45 kDa, I = 3

2. 13 kDa, I = 10; 45 kDa, I = 10

1 2



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.]

Insert protein ladder sizes here.

B. Primary Antibody 2, Secondary Antibody 2

Sample	Band?	Size (kDa)	Relative intensity
1. Strain A, Treatment A, 100 ng/mL, 30 C	(•) Yes ( ) No	25	1
2. Strain A, Treatment A, 100 ng/mL, 37 C	(•) Yes ( ) No	25	10
3. Strain A, Treatment B, 200 ng/mL, 30 C	(•) Yes ( ) No	25	1
4. Strain A, Treatment B, 200 ng/mL, 37 C	( ) Yes (•) No		
5. Strain B, Treatment A, 100 ng/mL, 30 C	(•) Yes ( ) No	25	1
6. Strain B, Treatment A, 100 ng/mL, 37 C	( ) Yes (•) No		

Same notes as page 5…

This antibody combination will be directly below the preceding antibody combination.

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

Preview Film

See next page for the next antibody combination - The next antibody combination will appear directly below this one.

C. Primary Antibody 3, Secondary Antibody 2

Sample	Band?	Size (kDa)	Relative intensity
1. Strain A, Treatment A, 100 ng/mL, 30 C	Yes / No	60	3
2. Strain A, Treatment A, 100 ng/mL, 37 C	Yes / No	60	3
3. Strain A, Treatment B, 200 ng/mL, 30 C	Yes / No	60	3
4. Strain A, Treatment B, 200 ng/mL, 37 C	Yes / No	60	3
5. Strain B, Treatment A, 100 ng/mL, 30 C	Yes / No	60	3
6. Strain B, Treatment A, 100 ng/mL, 37 C	Yes / No	60	3

Same notes as pages 5 & 7…

This antibody combination will be directly below the preceding antibody combinations.

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

Preview Film

Next

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