Meeting w/ Janet Iwasa - November 20th, 2014

Animation Feedback - Western Blotting Part III

General comment: I think that the pattern of the bands shown (when appropriate) should be more realistic. Most of the bands should be placed at the same level. In some wells, there should be less and in some wells there should be more. We can include on or two wells in which the band is slightly higher in molecular weight, but not so substantially as shown in the examples.

0:00:

- The narrative should say “In this animation we will introduce you to the third part of the experimental technique called Western Blotting.” or “In this animation we will introduce to the third part of the Western Blotting procedure."
- Do you think we need an additional transition sentence that connects to the prior video? It only occurred to me as I am playing it.

0:07:

- Change the location of the ladder to the left
- Bands should not be visible, only those in the actual ladder

0:15:

- Change the location of the ladder to the most left well
- Band should not be visible in the membrane either

0:25: Add arrows to the labels.

0:40-0:52:

- In this section the narrative is a little out of sink with the animation. It would be really good for students to see more carefully the gel and how it comes into contact with the membrane. For this, I was actually thinking that a different angle would be more effective but we can discuss this - maybe not be important.
- Label the gel and the membrane.
- I am not sure that we need to show all the pieces that the sandwich is made up being individually placed on top of each other - particularly because they won’t know what they are and we won’t be taking the time to name/explain them. Just showing the gel, then the membrane and then the rest of the sandwich all together (sponge + blotting paper) should be sufficient.
- Is the sponge the black looking paper? If so it does not look like one.

0:53: For the students to see where the negative and positive electrodes might be with respect to the cassette, it would be more effective to see a top shot of the cassette and then, with both the gel and membrane labeled within he cassette, show the respective location of the negative and positive fields.

1:16: Could we start with the membrane and then zoom in to see the proteins bound? I worried that the students here might not realized where the membrane actually is. By the way are the proteins on top of the membrane or within it?

1:28: I think we need to start with the membrane and them zoom in otherwise, students will have a hard time seeing what the actual green band is.

1:46: Label “Proteins within the blocking agent” with arrows pointing to the proteins already bound to the membrane

1:50: I think it would be good to zoom out a little bit here so that students can see more than one band and the areas surrounded by it to get a better sense of this.

1:57:

- Label the primary antibody
- From this angle it is hard to see which antibodies are binding and which are actually floating in the solution. Adding more bound antibodies to the band will help but I think we might also need to look at the band/membrane from a different angle as well.

2:06: Move the ladder to the most left well

2:24: Label the secondary antibody

2:37: Introduce the animation of washing the membrane again for a few seconds followed by the animation of antibodies leaving (might need a pause in the narration here to accommodate for this) to provide a break between the two processes (binding and washing).

2:41: Should this reporter be present when the secondary antibody is first introduced in 2:24?I worry that they won’t make the connection that the reporter was already represent.

2:50:

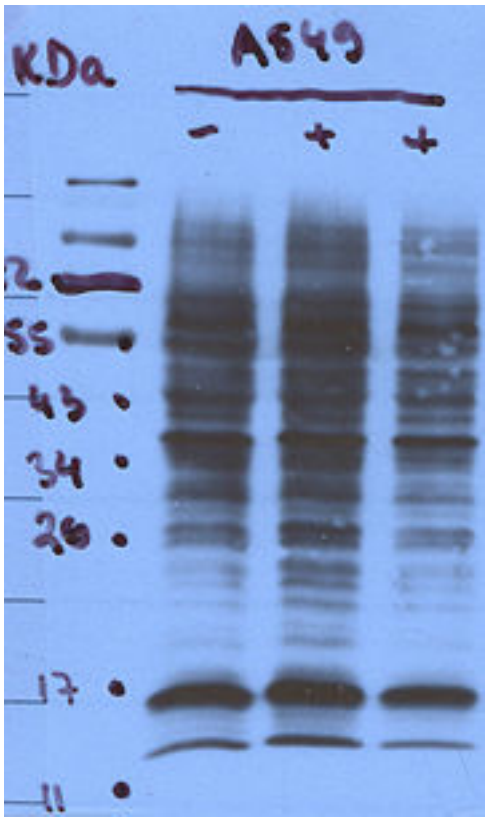
- Can we show more than two secondary antibodies bound?
- Let’s delete the word “enzyme” and just leave “reporter” since not all reporters are enzymatic

3:11: “The amount of light produced is directly related to the amount of horse radish peroxidase antibody bound” - I think we should illustrate this somehow - maybe with a comparison of a small amount of antibody complex bound with a lot.

3:20:

- Move the ladder to the most left well.
- The membrane should still be white as to not introduce misconceptions.

3:26: I think that we should illustrate how after you place the x-ray film on top of the membrane, you would record to the left of right on the actual film the size tick marks to indicate the bands in the ladder which is only shown in the membrane. Maybe using a sharpie and actually illustrating markings being made and the size of the band written next to it with a handwriting font will be good. You can later use these markings and handwriting in all subsequent films. See examples below:


KDa
A549
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+
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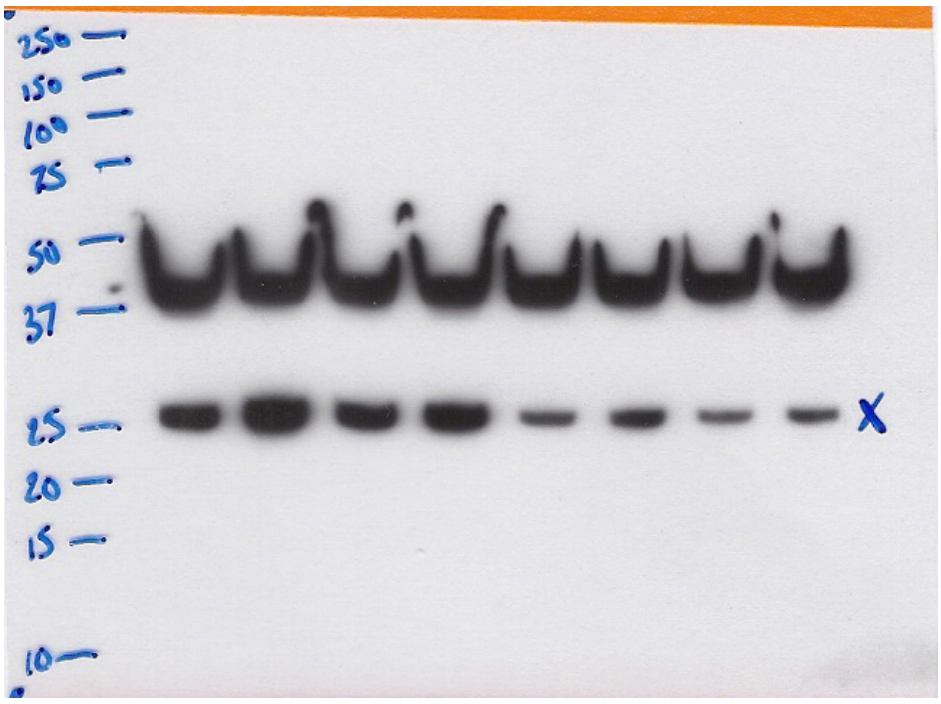
72
55
43
34
26
17
11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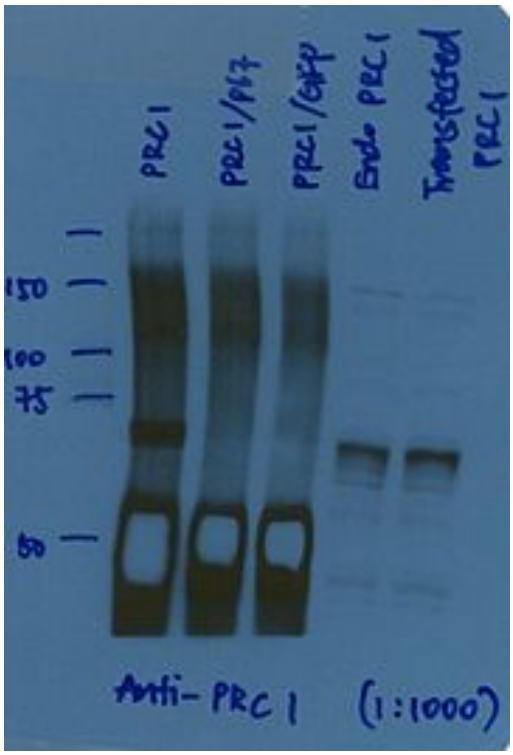
150
100
75
50
37
25
20
15
10
X



3:43: Labels should say “protein of interest” on the left and “control protein” on the right.

3:58: Move the ladder to the most left well.

3:56: I think we need to actually illustrate stripping molecularly. Also we should illustrate stripping differently. Maybe adding an immersion circulator that has a digital thermometer (I think it is 60C is the temperature under which stripping takes place but I will check).