Western blot technique movie

https://www.dropbox.com/s/k5ua9bfynmsx0nc/OctoberCutSmall.mov

General comments:

- Add captions (maybe with http://www.3playmedia.com/ - I just looked at their pricing and it does not seem too unreasonable for a 7 min video. You can check it out.

	Per Minute	Per Hour
Pay as you go *No file minimums – prorated to the exact duration of each file* *Minimum commitment: $150*	$2.50	$150
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- All captions and transcript output formats
- Indefinite storage and access to your captions and transcripts
- Free use of interactive transcripts and video plugins (limited)
- Integrated workflows with lecture capture and video platforms
- Ability to edit your captions post processing
- Unlimited use of search and clipping tools
- API access
- No minimum file size
- Exact billing – each file is prorated to the nearest second

- Credits, acknowledgments slide at the end
- Create Chapters

Specific Comments:

- **0.18 min** - "Mammalian cells that have been engineering to express a different protein" - Can we clarify what that means?
  - Are they expressing a protein that they generally do not express (from a different organism or mammalian tissue?) or are they overexpressing a protein that they already express?
  - Are all the plates expressing the same protein? Or different proteins?
- **1.31 min** - "The first sample…" --> arrow to indicate the first sample.
- **1.35 min** - "DNA ladder" --> change to protein ladder
- **2.07 min** - "monitor the dye front" --> indicate with an arrow the dye front & label the "-" and "+" sides of the box.
- **2.53 min** - At this point, Larry indicates that it is always from "black" to "red", but the students won't know what this means. Can we add something here that the black and red colors are there to help you to orient the cassette in the right direction (negative to positive)?
- **3.47 min** - Label here the - and + charge parts of the box and if there is space, we can even point out that if they put the membrane in the wrong orientation (positive to negative) the proteins will migrate off the cassette and into the transfer buffer (the opposite direction) - not towards the membrane.

- **4.08 min** - arrow for the ladder
- **4.15 min** - label the "gel" and the "membrane" so that students do not get confused seeing a little still of the ladder on the actual gel.
- **7.08 min** - I know you mention that Larry is actually comparing the ladder with the film, but making a more explicit statement that about the reason for why you need to line up the film with the blots and why you can't see the ladder in the film will be good --> **add "on the blot" at the end of this sentence.**

Feedback on first short draft

Notes

- In the comments below, I mention at times that voice overs should be added, but this can actually be a voice over or text on the screen depending on what you think is best. If it's a definition, then it could just be text.
- When you are doing a voice over, please convert my notes into your own voice! :)
- The reference library is a good resource if you need to look things up. :)
- Also I have tried to change the chapter titles to match the western blotting steps in the program itself. I'm wondering if we should remove the numbers for each chapter because they don't correspond to the steps of a western blot experiment in StarCellBio (we're missing prepare gel because L. is using a pre-cast gel).

Introduction

LOVE!

Chapter 1. Lysate Preparation

Change chapter title to be: Preparing Samples (or Sample Preparation)

- When discussing the samples, voice over with types of cells (mammalian), cut Larry off after expressing different proteins (don't include the part about amino acids). you can say that he has 6 different samples, he has engineered each plate of cells to express a different protein.
- Voice over with Larry's action of removing the media (need to remove the media and wash the cells for the lysis buffer to work). Cells are washed to remove all media present. Mention that this step is not currently shown in the video.
- Mention types of lysate preparations - whole cell vs fractionations and say that L. is preparing whole cell lysates based on the lysis buffer he is using.
- After Larry confirms that he is adding lysis buffer, voice over (quickly) with what the lysis buffer does. - and mention that need to scrape cells/proteins/lysis buffer mixture off the plates to work with them in eppendorf tubes
- At the end of chapter 1, mention that all of the protein samples are now transferred to eppendorf tubes.

Chapter 2. Gel Loading

Change chapter title to be: Loading Gel (Load Gel)

- As Larry is setting up the western blotting box, mention that companies now produce precasted gels that are available for purchase. Delete Larry saying Tris and Glycine buffer, and either keep Larry saying or voice over with "polyacrylamide gel". Point to the actual gel so that students know what the "gel" is.
- After L. says that there is an SDS buffer in the gel, voice over with what the SDS buffer does. SDS buffer is added to the reservoir where the gel is until it covers the back of the gel where the wells are located.
- When asking Larry about native gels, cut him off before he says "stuff like that"
- When L. is loading the gel, voice over with the fact that he is loading 2 gels - actually I'm not sure this is important. You can delete Larry talking about two gels and ignore this information.
- Voice over at some point that the samples are all blue because they have had loading dye added to them. Importance of loading dye...
- After L describes difference between 12 and 16% gels, voice over with a short clear description that 16% helps to differentiate smaller proteins, 12% can better differentiate larger proteins (I think that's right off the top of my head but I always have to think about it carefully!) and why.
- Mention need for protein ladder - Do you have zoomed-in video footage of him loading the protein ladder so you can voice over saying what it is and why you need it? If not, just mention it anyway at some point.

Chapter 3: Run Gel

Should this be Running Gel to match your other titles?

- Delete part about mass/charge ratio
- Larry saying that everything (meaning the proteins/samples) is negatively charged due to the SDS is great. Delete the part about "exact same principle" And voice over with information about proteins running toward the positive charge at the bottom of the gel box. As a result, the proteins will run through the gel to the positive charge.
- It seem to me that the running part of the video was incredibly short. It would have been nice to see the migrating dye front a couple of times so that students can see it progressing through the gel.
- Mention that once the dye front has reached almost the bottom of the gel, you should stop running your gel so that your proteins of interest do not run off the gel.

Chapter 4: Transferring

Should this be Transfer to match your other titles?

- Before L starts describing the components of the sandwich, describe what happens in this step. "Proteins move from the gel to a membrane" Also mention why this is important (gel is really fragile and thin, proteins are not accessible to antibody detection in the gel, etc.)
- Describe the fact that Larry removed the wells of the gel before transferring --> they do not contain any proteins. It would have been nice not to have to mention this but it will not be good to show Larry something and then omit what he is doing.
- Describe "black to red" (I can't tell if he is actually saying "blot to red" or "black to

red" - but there is a pause after he says this which would be a good time to insert information about what this means) and reiterate why you have the blot on the positive side of the gel (proteins are still negatively charged and will move from the gel to the membrane). Basically mention how the charge applied in this step works. I think this whole section about how the proteins actually are transferred from the gel to the blot is not very well described and needs more details. Since he mentioned the sandwich, explain that the reason we have a sandwich is to be able to provide weight or pressure (not sure what the right word would be here) so that transfer from gel to blot is maximized --> analogy of putting a bunch of books on top of things you glue together or something like it.

- Mention why the blot, gel and sandwich are kept wet.
- Before you ask Larry "what happens if you get a bubble", mention that what Larry is doing is removing bubbles between the gel and the membrane.
- Once Larry says how many western blotting experiments he has done, highlight this in some way with text or a voiceover to emphasize his expertise! Would also be neat if we could add the number of hours and days he has spent doing western blot.
- Remove the part about L. saying that they're "exciting" because he's being sarcastic here. I like the part about him saying it's the "day to day" because that shows that he does this a lot!
- Once he puts the sandwiches in the transfer box, voice over with information about the elector charge - can even include voltage, time, temperature, etc. Or put this after he says that it will be done in 2 hours (and before he goes into the blocking step) to elaborate on what he has said. Delay any talk about blocking until we get to blotting.
- Mention (either here or in chapter 3) about the bubbles showing the researcher that the electric field has been applied and that things are working correctly.
- Delete you saying "Ok" as you watch the transfer run.

Chapter 5: Blocking

Change this chapter to "Blotting" to match the program

- When looking at the membrane to see if the proteins transferred, I think you should just include your conversation about the ladder transferring, but there's no need to mention the proteins in the other lanes transferring since that will be confusing to the students. Mention here that this is how you know your gel has transferred correctly, by looking at the ladder and seeing if it also transferred. Since he did not show the ladder before, mention that each band in the ladder represents a different protein whose size we know and that has been colored with a dye for easy identification.
- I think the rest of this section can use some more information regarding what is happening for the incubations. Mention why Larry cut the membrane.
- Mention what he is doing when he transfers the small portion of membrane into buffer - to rinse it. You don't have to include the part about "going to the sink" but definitely show the blots on the shaker in the milk near the sink.
- Mention what the block is doing- Blocking non-specific binding of the primary/secondary antibody to the protein of interest. Mention that we use milk

because it is incredibly rich in lots of proteins and that therefore these proteins will bind nonspecifically to the membrane to block antibodies from binding nonspecifically to the membrane in the blotting step. Mention that it is powder milk dissolve in buffer.

- Describe what he is doing when he is creating the little "baggy" for his antibody incubations - Antibodies are really expensive and by creating these small bags (is there another word?), L can use very small amounts of liquid (and save money) and still ensure that his blot will remain moist and not dry out during incubations.
- Voice over with something about adding the primary antibody when he is pipetting it into the bag. Small amount of antibody is added to milk solution.
- Then say, now the blot incubating with the primary antibody is put on a shaker to make sure that the antibody has a chance to bind with all of its protein binding partners on the whole membrane.
- So he says that the incubation goes overnight at 4 C. May need to repeat that since I had a hard time hearing him say the incubation information. Also, was this primary or secondary? It's great to only show one, but definitely mention that he repeats this process with the other antibody. Remove the "goes overnight at 4C" since this changes based on preference. I did all of my antibody incubations for 1 hour at room temperature and blocking overnight so let's not say anything about overnight.

Chapter 6: Developing

- Importance of washing after primary and after secondary. Washing happens multiple times, etc in buffer solution with a small amount of detergent to remove non-specific binding.
- Voice over and describe what he is doing when he has the membranes in a dish with the developing solution. An enzyme in the solution will catalyze a reaction converting the HRP (horseradish peroxidase) which is bound to the secondary antibody to a light molecule, which will be detected on the film. The reaction occurs quickly and only 1 minute or so is needed for catalysis of all the HRP. At this point, we are racing against time to catch the light given off during the reaction.
- Then voice over with information about placing the blots that are ready to be developed in a film cassette to take them to the dark room - where he will place a piece of film on them. Anywhere
- Voice over with something about saying "moment of truth" or something like this as he is walking down the hall to the dark room.
- When he says he is going to do a 30 second one just for you and then a 10 minute one, can you cut out the "just for you" part? (love that you went into the darkroom).
- Describe what is happening when his film goes into the developer.
- Voice over: The final product of a western blot is a piece of film where the dark bands represent where the antibody has detected and bound to a protein of interest. This binding reaction is amplified through by the secondary antibody binding to the primary antibody and then converted into a light signal in the last step of developing a blot. The relative amount of the protein in a sample corresponds to the intensity of the band on the film.

- By laying it over the blots, L. can determine the size of the protein bands on his film by comparing them to the known sizes of the proteins in the ladder. This is great that L says "50 kD, 100kD, etc"