Western Blot Video

Items to highlight during the video:

Lysate Preparation:
How protein lysates are made from whole cells or tissues, including whole cell, nuclear and cytoplasmic lysates
Immunoprecipitation and use of antibodies to create lysates that only contain the protein of choice. We currently don't have IP in StarCellBio, but this will be added in the future.

Gel preparation:
How acrylamide gels are made and why they help separate proteins
Differences in protein mobility according to the % of acrylamide included in the gel. It would be wonderful to include pictures of the same samples (or protein ladders) run on 10%, 12% and 15% gels (the current options in StarCellBio).
Denaturing vs. native gels - but we only have denaturing gels for now.

Gel Loading:
Using SDS and current to separate proteins based on size
Loading dye - the purpose of the loading dye to track the protein front moving through the gel. Even though in real life, you add loading dye (without sample) to otherwise empty wells to prevent smiling/frowning, we do not show this in StarCellBio and so it shouldn't be emphasized in the video.
Protein ladders. Right now we just have one standard protein ladder that can be selected, but you could mention that there are different protein ladders that are intended for use for detection of small or large proteins.

Transferring:
Reasons for transferring proteins from a gel to a blot
How transferring works

Blotting:
Using antibodies to recognize proteins on a blot, including why you need a primary and a secondary antibody, and a secondary antibody that recognizes the primary antibody.
Blocking

Develop:
How chemiluminescence is used to provide a light signal that can be captured on a piece of film
Proteins found in the protein ladder are not recognized by specific antibodies therefore they do not show up in the film (we encountered this as a misconception during our usability tests). [Although if some students are using the new fluorescent antibodies for western blotting, then they will see the bands of the ladder on the blot.]
How we use protein ladders to determine molecular weight of specific proteins
Loading controls

How reprobing works. Make sure to indicate that in StarCellBio we will allow students to reprobe at infinitum but why that would not work in real life
Why do we re-probe instead of loading another gel with the same examples --> detection of another protein in the same gel, for example loading control
Bands are not always perfectly straight. StarCellBio models how the bands are not always perfectly aligned in real life.

Items that do not need to be highlighted in the video:

Lysate Preparation:
StarCellBio does not currently include the quantitation of the proteins in the samples to ensure that you load the same amount of protein. Although we had the functionality to load different amounts of protein in our initial prototype of StarCellBio, it has been removed. We don't need to discuss this right now.

Gel preparation:
We don't need to highlight the purpose of the stacking gel.

Gel loading:
As mentioned above, we do not need to highlight the fact that empty wells would get loading dye alone to prevent smiling.

Troubleshooting Western Blotting "interviews":

Blank blot - didn't select correct secondary antibody, set up transfer the wrong way
Couldn't analyze blot - forgot to re-probe with a loading control, forgot to include protein marker, forgot to include your controls on every gel
Ran gel for too long, and the protein of interest ran off the gel
Oh, thinking of all the troubles is a lot of fun!!