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GENERAL INFORMATION

Resources

- Click **Contact** at the top of each page to provide feedback or report software bugs.
- Click **Reference Library** at the top of each page to access the reference material on experimental design and the experimental techniques available in the program. Additionally, the same reference material can be accessed by clicking the **Learn More** buttons on the StarCellBio home page and the **Assignments** page.
- Click **User Guide** at the top of each page to access the StarCellBio user guide. Please note that the **User Guide** does not contain information on biological content and the

experimental techniques available within the program. If you are unclear about any of the content addressed within the program, go to the **Reference Library** instead.

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Navigation Tool Bar

- The Navigation Tool Bar is the green horizontal bar that appears at the top of each page. As you progress through the program, additional buttons will appear within the navigation tool bar. To navigate through previously opened pages within the program, select the appropriate button within the navigation tool bar.

- You can additionally navigate through the program using the blue buttons at the bottom of each page.

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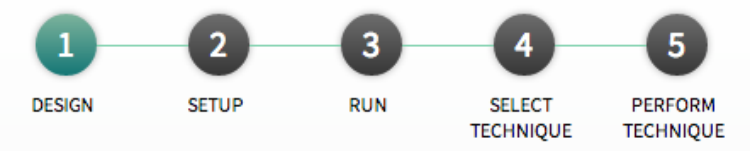
Progress Bar

- A progress bar, which indicates all of the steps of an experiment and your progress within each experiment in StarCellBio, will appear at the top of each page of a particular experiment once you start to design it. The progress bar in StarCellBio has 5 steps, with sub-steps displayed once a particular experimental technique is selected. The 5 steps are: Design, Setup, Run, Select Technique, and Perform Technique.



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Saving

- Your work will be automatically saved for you within the browser in which you completed the work.

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ASSIGNMENTS PAGE

- The **Your Assignments** window contains a list of all of the assignments for a particular course.

- To view the abstract of each assignment, select an assignment in the **Your Assignments** window. The abstract will be shown in the right panel.
- Click **COMPLETE ASSIGNMENT** to view the assignment in its entirety.
- To navigate directly to a previously started experiment(s), select the appropriate experiment underneath the assignment name in the **Your Assignments** window.
- Select **+ New Experiment** underneath the assignment name in the **Your Assignments** window to start a new experiment without viewing the complete assignment.

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COMPLETE ASSIGNMENT PAGE

- The complete assignment is displayed on this page.
- The name of the assignment will be displayed in the Navigation Tool Bar.
- Click **DESIGN NEW EXPERIMENT** at the bottom of the page to start a new experiment.
- Alternatively, select **+ New Experiment** to start a new experiment in the **Your Assignments** window on the left side of the page.
- To navigate directly to a previously started experiment(s), select the appropriate experiment in the **Your Assignments** window.

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DESIGN PAGE

- To rename the experiment, click inside the **Experiment Name** box and type the new name. The new name of the experiment will be displayed above the navigation tool bar once you navigate to the next page of the program.
- The questions on the **DESIGN** page will help you think about and design your experiment. To design your experiment, write the question that your experiment is going to address, your hypothesis for the experiment, and select the experimental technique(s) that is (are) best suited for the analysis of your experiment. Please note that by selecting a technique(s), you will not alter the techniques that are available to you for this experiment. The responses provided to the questions within the **DESIGN** page are currently not required.
- Click **EXPERIMENT SETUP** once you have designed your experiment.

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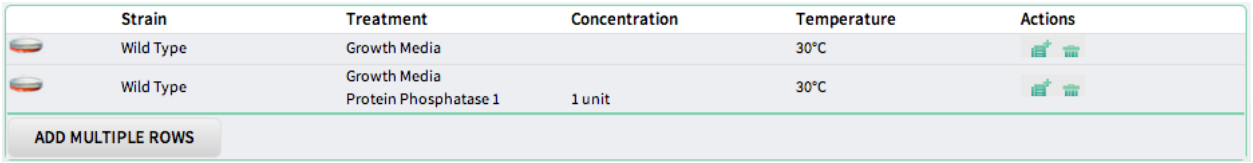
SETUP PAGE

- To get started, select **Create new set-up** or select the appropriate experiment set-up from a previous experiment from the **Select pre-existing set-up as a template** dropdown menu. Some assignments will not have **Select pre-existing set-up as a template** as an option.
- Instructions for setting up your experiment are displayed directly above the experimental setup table.
- In some assignments, the ability to add one or multiple treatment protocols to the setup table will be available by clicking the **Add Multiple Rows** button at the bottom of the setup table. Within the **Add Multiple Rows** pop-up window, select all the strains and treatments you would like to add to the setup table and click **Add Multiple Treatments** at the bottom of the **Add Multiple Rows** pop-up window. If the **Add Multiple Rows** button is not shown at the bottom of the setup table, it means that your current assignment lacks this functionality.



- Each row in the experimental setup table represents an individual treatment protocol.
- Click the copy icon on the right side of each row to duplicate a particular treatment protocol. If the ability to edit a treatment protocol within the setup table is available to you in your assignment, then edit the duplicated treatment protocol as needed by clicking within the appropriate dropdown menus.
- Click the trash can icon on the right side of each row to delete a particular treatment protocol.
- Click **RUN EXPERIMENT** once you finish setting up your experiment. Carefully review the summary of your experimental set-up and then either click **EDIT SET-UP** to go back to edit your set-up or click **CONFIRM SET-UP & SELECT TECHNIQUE** to run your experiment. After you confirm your experiment's set-up, you will be unable to change your treatment protocols for this particular experiment.

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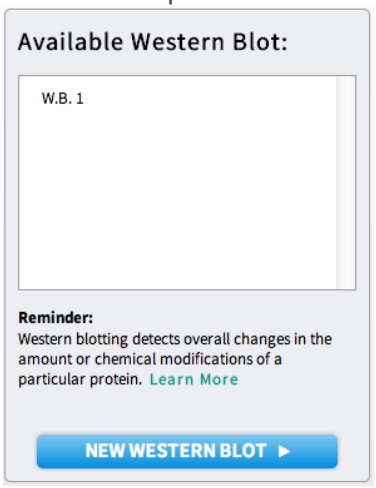
TECHNIQUES PAGE

- Click on **NEW WESTERN BLOT**, **NEW FLOW CYTOMETRY**, or **NEW MICROSCOPY** to start a new western blot, flow cytometry, or microscopy experiment, respectively. Please note that some assignments will only have certain experimental techniques available to use. Experimental techniques not available within an assignment will appear grayed-out.
- To view the results of or to finish a previously started technique, select the particular name of the experimental analysis in the appropriate window on the **TECHNIQUES**

page. The program will navigate to the last edited page for the previously started technique.



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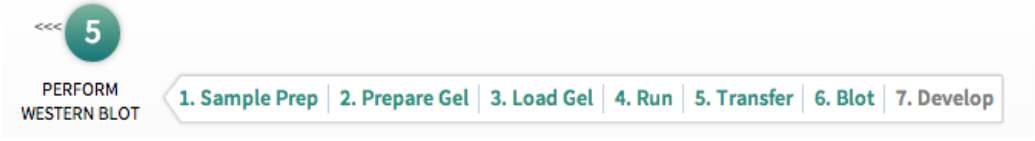
WESTERN BLOT EXPERIMENTAL TECHNIQUE PAGES

General Navigation

- Click **TECHNIQUES** within the Navigation Tool Bar and then click **NEW WESTERN BLOT** to begin a new western blot.
- Each western blot analysis within one particular experiment is displayed in its own tab directly underneath the Navigation Tool Bar. Each western blot analysis tab will be labeled 'W.B. 1', 'W.B. 2', etc. Navigate between various western blot analyses by selecting a particular tab or by selecting a western blot within the **Available Western Blot** window on the **TECHNIQUES** page.
- To delete a western blot, click the trash can icon in the tab for the appropriate western blot on the **WESTERN BLOT** page.
- There are 7 steps to complete a western blot: 1) sample preparation, 2) prepare gel, 3) load gel, 4) run, 5) transfer, 6) blot and 7) develop. Your progress through these steps is illustrated in the horizontal sub-progress bar at the top of each western blot. The instructions for each step of a western blot are detailed below.



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1. Sample Preparation

- Select the samples that you would like to prepare for western blot analysis by selecting the checkbox to the left of each sample. This will result in the creation of protein lysates that are suitable for western blotting analysis.
- Alternatively, click **Select All** at the bottom of the **Sample Prep** window to select all of the samples at once.
- For each sample selected, select the appropriate lysate type from the **Lysate Type** dropdown menu. Some western blotting experiments will only have one available lysate type.
- If the ability to select more than one lysate type is an available option in your assignment, select the appropriate lysate type(s) in the **Lysate Type** dropdown menu. If you inadvertently add more than one lysate type for one sample, click the **X** icon to the right of the lysate type to remove it.
- Click **PREPARE LYSATES** once you have selected and specified lysate types for all of your samples.

Important considerations:

- Each western blot gel has 15 lanes. When designing your experiments, remember to reserve one lane for loading the protein size marker.
- You can only select samples from your current experiment. To perform a western blot with samples from a different experiment, you will need to navigate to the other experiment in which the samples were created. To navigate to the other experiment, click on the particular assignment name within the Navigation Tool Bar. This will take you to the **Complete Assignment** page for that assignment, where you will be able to select the appropriate experiment in the **Your Assignments** window.

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2. Prepare Gel

- To prepare your gel, select the appropriate percentage of polyacrylamide that you would like to use. If specific percentages of polyacrylamide are not available as options for your assignment, they will appear in grayed-out text.

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3. Load Gel

- Once you have chosen the percentage polyacrylamide gel you would like to use, all of the lysate samples that you previously prepared will be automatically loaded in your gel. Each well in the gel contains one sample to which blue loading dye has been added before loading.

- Click **LOAD MARKER** to load the protein size marker. The marker will be loaded in the well immediately to the right of your last sample.
- Click **RUN GEL & TRANSFER** once you have prepared and loaded your gel.

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4. Run

- Once you click on **RUN GEL & TRANSFER**, your samples will be run through the gel. This step of a western blot is not currently displayed in the program.

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5. Transfer

- Once the samples are finished running through the gel, the proteins will be automatically transferred from the polyacrylamide gel to the blotting membrane. This step of a western blot is not currently displayed in the program.

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6. Blot

- Once the proteins have been transferred on to a membrane, the membrane is ready to be blotted with antibodies to detect the protein(s) of interest.
- From the **Primary Antibody** dropdown menu, select the appropriate primary antibody.
- Then, from the **Secondary Antibody** dropdown menu, select the appropriate secondary antibody. Ensure that you select an appropriate secondary antibody that will recognize your chosen primary antibody.
- Click **BLOT & DEVELOP** once the blotting conditions have been selected.

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7. Develop

- After clicking **BLOT & DEVELOP**, the program generates a simulated western blot film for the chosen blotting conditions. As a reference, the protein sizes (in kiloDaltons or kDa) corresponding to the bands within the protein size marker lane on the blotting membrane are shown to the right of the film.
- To aid in the determination of the size of experimental bands shown on your western blot film, hover your cursor over the film. A horizontal red line will appear and the corresponding molecular weight (in kDa) will be indicated in a text box to the right of the film.

- The **Exposure Time** slider can be adjusted to alter the intensity of the bands on the western blot. To increase the amount of time that the film is exposed to the blotting membrane, move the **Exposure Time** slider to the right. To decrease the amount of time that the film is exposed to the blotting membrane, move the **Exposure Time** slider to the left.
- Click **RE-PROBE** to strip the membrane of the current blotting conditions and probe the same membrane with an antibody that recognizes another protein. An additional tab labeled **BLOT** will be generated. Select the appropriate blotting conditions (primary and secondary antibodies) again, as described in the **Blot** section above.
- Once a membrane has been blotted and developed more than 3 times, a dropdown menu will appear to more easily navigate between the different films.

Important considerations:

- You should control for the amount of protein loaded within the lanes of your gel since differences in protein levels within your experimental conditions could be due to differences in the amount of protein loaded for the various samples. To do this, re-probe each blot with an antibody that detects a protein whose levels are not altered in response to your specific experimental conditions. This protein can serve as a loading control.

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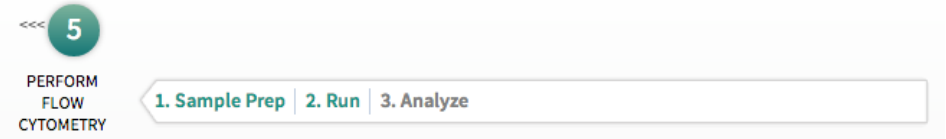
FLOW CYTOMETRY EXPERIMENTAL TECHNIQUE PAGES

General Navigation

- Click **TECHNIQUES** within the Navigation Tool Bar and then click **NEW FLOW CYTOMETRY** to add a new flow cytometry experimental analysis to your experiment.
- Each flow cytometry analysis within one particular experiment is displayed in its own tab directly underneath the Navigation Tool Bar. Each flow cytometry analysis tab will be labeled 'F.C. 1', 'F.C. 2', etc. Navigate between different flow cytometry analyses by selecting a particular tab or by selecting a particular flow cytometry analysis within the **Available Flow Cytometry** window on the **TECHNIQUES** page.
- To delete a flow cytometry analysis, click the trash can icon in the tab for the appropriate flow cytometry analysis.
- There are 3 steps to complete each flow cytometry analysis: 1) sample preparation, 2) run and 3) analyze. Your progress through these steps is illustrated in the horizontal bar at the top of each flow cytometry experimental technique window. The instructions for each step of a flow cytometry experimental technique are detailed below.



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1. Sample Preparation

- Select the samples that you would like to prepare for flow cytometry by selecting the checkbox to the left of each sample.
- Alternatively, click **Select All** at the bottom of the **Sample Prep** window to select all of the samples at once.
- For each sample selected, select the appropriate treatment of cells in the **Cell Treatment** column. If only one cell treatment is available in your assignment, the available treatment will be automatically selected for you.
- For each sample selected, select the appropriate DNA content treatment in the **DNA content treatment** dropdown menu. Please note that in some assignments only one type of treatment will be available.
- Click **PREPARE SAMPLES** once you finish selecting and preparing your samples.

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2. Run

- Click **RUN SAMPLES** at the bottom of the **Samples** window to run your samples through the flow cytometer.

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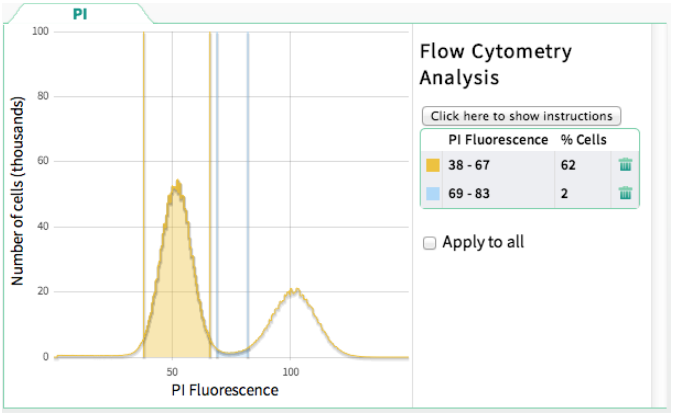
3. Analyze

- Select each sample name in the **Samples** window to view a graphical representation of the flow cytometry data for each sample. The displayed graph corresponds to the currently highlighted sample.
- The graphical representation of the flow cytometry data displayed is a histogram of the results. The X-axis corresponds to the amount of fluorescence emitted by each cell and detected by the flow cytometer due to the selected DNA content treatment. The Y-axis corresponds to the number of cells with a particular level of fluorescence.
- Click **Analyze Data** at the bottom right to analyze the data represented by each histogram.
- The flow cytometry analysis tool will allow you to 1) divide the flow cytometry histogram into color-coded segments that represent populations of cells with varying levels of fluorescence and 2) determine the percentage of cells within each segment. To create a segment within your graph, click and drag within the graph to create your desired segment. A segment will be created and the vertical lines will indicate the left and right

boundaries of the segment. To alter the position of the segment, drag the left and right vertical lines to the desired position. Repeat to create a different segment.

- The range of fluorescence levels represented by each segment and the corresponding percentage of cells within each fluorescence range will be displayed in the **Flow Cytometry Analysis** table.



- Select **Apply to All** to apply the designated segments in one histogram to all the other histograms within your active flow cytometry analysis. The same flow cytometry analysis will be applied to all of the flow cytometry histograms. To make changes to an individual graph, uncheck **Apply to All** and analyze the specific histogram as desired.
- Click the trash can icon in the **Flow Cytometry Analysis** table to delete a particular segment.

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