

TABLE OF CONTENTS

Experimental Design

1. *Design*
2. *Set Up*
3. *Run Experiment*
4. *Select Technique(s)*
5. *Run Technique(s)*
6. *Analyze*
7. *Conclude*

Experimental Techniques

A. *Western Blotting*

General Information

Western Blotting Experimental Technique

1. Sample Preparation
2. Gel Preparation
3. Load Gel
4. Run Gel
5. Transfer
6. Blot
7. Develop

Western Blot Analysis

B. *Flow Cytometry*

C. *Microscopy*

EXPERIMENTAL DESIGN

There are 7 steps to experimental design:

1. **Design**
2. **Set Up**
3. **Run Experiment**
4. **Select Technique(s)**
5. **Run Technique(s)**
6. **Analyze**
7. **Conclude**

1. Design

Experiments in StarCellBio generally involve treating either whole organisms or group of cells with various drugs and/or altering their growth conditions. The goal of a StarCellBio experiment is to determine if a treatment affects the whole organism or cells being treated. There are many ways in which a treatment can affect an organism or a cell. Scientists use various methods to diagnose the effect of a particular treatment on a cell/organism. Some treatments result in a change in morphology while others do not. A lack of change in morphology does not mean a treatment has not affected an organism or a group of cells. Some changes lead to internal changes within an organism or cells without resulting in visible changes.

The first step is to think about the question or objective that you are trying to answer and then design a hypothesis. The user should also start to think about the experimental technique that will be best suited to analyze the samples from the experiment.

[Back to top](#)

2. Set Up

During the experiment set up step, students will think of the different treatments and conditions they would like to test on a strain or set of strains during a particular experiment. StarCellBio contains information regarding the assignment and the necessary background information that is required for a student to design, perform, and analyze experiments within StarCellBio.

Within StarCellBio, these are all the possible variables that a student will be able to specify:

1. strain(s) used during an experiment. The instructor will provide all of the available strains in the software.
2. available treatment(s) and their associated conditions. Possible treatments include drugs and/or media conditions to be used during the experiment. The instructor will provide all of the available treatments in the software. Each treatment has the following conditions associated with it:
 - a. concentration of treatment
 - b. start of treatment
 - c. duration of treatment
 - d. temperature

3. time of sample collection from the start of treatment. The instructor will provide all of the possible options for collection time in the software.

In addition to the variables mentioned above, a user can also treat a particular sample with multiple treatments in StarCellBio. These treatments can either be simultaneous or sequential, depending on the treatment start time and treatment duration variables selected by the user.

At this point in the experimental design, it is critical for a user to set up positive and negative controls. A positive control is a sample in which a specific result is expected. A negative control is a sample in which no result is expected. These serve to demonstrate that the experiment worked as expected and also as comparisons to which a user can compare the results of the experimental samples.

[Back to top](#)

3. Run Experiment

The experiment is performed during this stage and the samples are generated.

[Back to top](#)

4. Select Technique(s)

Now the researcher selects the experimental technique that will be used to analyze the samples. In StarCellBio, there will be three available techniques: western blotting, flow cytometry and microscopy. Currently, only western blotting and flow cytometry are available for spring 2013 and microscopy will be developed starting in summer 2013.

- A.** Western blotting detects overall changes in the amount or chemical modifications of a particular protein.
- B.** Flow cytometry is used to count and analyze the size, shape and properties of individual cells within a heterogeneous population of cells.
- C.** Microscopy is used to study the shape, morphology and properties of cells, tissues or organisms that otherwise cannot be observed with the human eye.

For more information regarding the experimental techniques, see the appropriate section below.

[Back to top](#)

5. Run Technique(s)

The experimental technique is performed at this time and the experimental output is created. For more information regarding the experimental techniques, see the appropriate section below.

- A.** Western blotting detects overall changes in the amount or chemical modifications of a particular protein.
- B.** Flow cytometry is used to count and analyze the size, shape and properties of individual cells within a heterogeneous population of cells.
- C.** Microscopy is used to study the shape, morphology and properties of cells, tissues or organisms that otherwise cannot be observed with the human eye.

[Back to top](#)

6. Analyze

A student now analyzes the data that has been generated from the experimental output. For example, in western blotting, the final blot will be analyzed. In flow cytometry, students will analyze either a histogram or a dot plot to determine the number of events of a specific parameter and the size of subpopulations in their sample. With microscopy, students will study images obtained from the microscope.

For more specific information regarding the analysis of each experimental technique, reference the appropriate experimental technique below.

[Back to top](#)

7. Conclude

To construct an overall conclusion for an experiment, a user brings together all of the data that they have generated – possibly from multiple western blotting and/or flow cytometry and/or microscopy techniques – to form a conclusion about the original hypothesis proposed. At this point, the user will need to decide whether the original hypothesis should be revised and/or if additional experiments should be set-up to better address the hypothesis.

[Back to top](#)

EXPERIMENTAL TECHNIQUES

A. Western Blotting

GENERAL INFORMATION

Western blotting is a technique that allows scientists to detect overall changes of a particular protein of interest in an organism or cell. These changes can be:

1. changes in protein concentration.
2. addition or subtraction of small chemical modifications to a protein that can alter its behavior.

Proteins are the workhorses of a cell. Proteins are tightly regulated by changes in the environment so that they are only "expressed" (present) or can only be "activated" at the right time and place. Proteins are regulated by altering their concentration in the cell and/or by adding a chemical modification to the protein that "activates" or "deactivates" it. Either or both of these types of protein regulation can occur for a given protein.

Any particular process in a cell is controlled by the action of many proteins which form complex regulatory networks with some "activating" and others "repressing" the expression or activity of other proteins within the pathway. In addition, most proteins are involved in more than one cellular process, so the whole system looks like an interconnected network of protein pathways. This means that often times a particular treatment will result in a change in the amount of a protein or a change in the activity of a particular protein that is important for a specific cellular process. For example, carcinogens often cause alterations or mutation in DNA sequence, which can have detrimental effects on an organism in the long run. When you treat cells with carcinogens, cells increase the production of proteins that help them deal with the damage incurred by the treatment. In addition, cells will also suppress the production of proteins involved in promoting growth and cell division to allow for the repair of the damage before cell division, and therefore propagation of the damage to the next generation can take place. One can diagnose what type of cellular response the cells are experiencing after treating them with a carcinogen by looking at (1) how the overall concentration of a particular protein involved in repair or cell division, for example, changes after treatment and/or (2) how the particular concentration of the activated or deactivated form of such said proteins changes after treatment.

[Back to top](#)

WESTERN BLOTTING EXPERIMENTAL TECHNIQUE

After the experiment is set up and performed, the protein samples need to be prepared. Proteins in the sample are then separated using a method called “gel electrophoresis”, transferred to a cellulose membrane and then the specific protein(s) of interest are detected and visualized on a piece of photographic film.

Video Resource

An overview of the western blotting procedure can be accessed in the video article entitled: “*Western blotting: Sample preparation to Detection*”¹ on www.jove.com. The link is: <http://www.jove.com/video/2359/western-blotting-sample-preparation-to-detection>.

The 7 steps to perform a western blotting experimental technique are detailed below.

[Back to top](#)

1. SAMPLE PREPARATION

Once an experiment is run and the samples are collected, the samples need to be prepared in a specific way to analyze the proteins within the sample. Once a sample has been prepared for analysis it is called a lysate.

To analyze the proteins within your sample by western blotting, you first need to prepare your lysates. Lysates can be prepared from whole tissue (made of cells as well as connective materials, etc.) or cells. If tissue is being used, then the tissue first needs to be broken down in a mechanical manner by cutting or crushing the sample. The sample can then be processed using different combinations of salts and detergents to isolate the proteins. In addition, the sample preparation is usually performed in the cold to prevent the proteins from being degraded or denatured (un-folded). Enzymes can also be added to the lysate preparation to prevent proteins from being digested or chemically modified by other enzymes contained within cells.

Proteins are found within several cell compartments: the cytoplasm, the nucleus or embedded within a membrane (either surrounding the cell or an internal membrane that surrounds an organelle within the cell). Depending on where a particular protein is located within the cell, different conditions will need to be applied to isolate the protein(s) of interest. All protein preparation protocols involve using detergents and/or salts of various strengths. Detergents cause the membrane that surrounds and protects the cell to fall apart, providing access to the proteins within the cell. Depending on where the proteins that the researcher is studying are localized within the cell, the

¹ Eslami, A., Lujan, J. Western Blotting: Sample Preparation to Detection. *J. Vis. Exp.* (44), e2359, doi:10.3791/2359 (2010).

researcher will use either weak or strong detergent to break apart only the outer membrane or all the membranes within a cell.

There are several different types of protein samples that a researcher can prepare:

1. One type of lysate sample is called a whole cell lysate because it contains all proteins that are located in a cell.
2. Another type of lysate that a researcher may want to prepare is a nuclear & cytoplasmic lysate. In this type of lysate preparation, the researcher uses increasing concentrations of detergent to first isolate proteins that are found in the cytoplasm and then a stronger detergent to isolate proteins that are found in the nucleus. The researcher will end up with two samples: one will contain all of the proteins from the nucleus and the other will contain all the cytoplasmic proteins.
3. A third type of protein sample is called an immunoprecipitate or IP. In some experiments, a researcher will want to determine whether a particular protein is associated with another protein (proteins often time found large complexes – sometimes binding to a complex is the direct result of a particular cellular event and can alter the properties of a particular protein). In this experiment, the researcher would first isolate, or immunoprecipitate (IP) a specific protein, along with anything else it is bound to, from the lysate. To do this, the researcher will use an antibody that will bind and isolate the protein from the lysate sample (see the Blotting section for information about antibodies). Then in later steps of the western blotting procedure, the researcher will probe whether this protein is associated with another protein of interest.

Once the protein sample is prepared – either whole cell, cytoplasmic/nuclear fractionations, or IP – the amount of total protein that is in each sample should be quantified. In particular experiments, it is critical to do a western blot with the same amount of total protein in each sample in order to draw conclusions from the experiment. This is extremely important in experiments that are trying to determine whether a particular treatment has an effect on the amount of a protein in a sample. StarCellBio currently has the functionality to make whole cell as well as cytoplasmic and nuclear fractionations. The capability to make immunoprecipitates will be added in the future.

Now the samples are ready to be prepared to load in the polyacrylamide gel. The appropriate amount of protein is mixed in a specific buffer, and a dye (blue color) is added to the mixture of lysate and buffer. The dye will help the researcher visualize the protein mixture in the gel, prior to and during movement of proteins through the gel. It is important to note that the dye does not actually bind to the proteins; instead, it

provides a visual sense of where the protein mixture front is within the clear polyacrylamide gel. If a denaturing gel is being used, then a chemical to denature the proteins is also added and the samples are heated at 95 °C for 5 minutes prior to being loaded in the gel's well.

[Back to top](#)

2. GEL PREPARATION

Proteins are separated by size and shape by applying an electric field to them in a "gel". The word, gel, refers to a mesh-like matrix made of an acrylamide polymer through which different proteins will travel at different speeds depending on their size and shape (once the electric field is applied).

The gel itself is created by pouring a solution of a chemical, acrylamide, at a specific concentration into a framework that is provided by two vertical plates. Under the appropriate treatment conditions, the acrylamide will polymerize to form a solid, mesh-like matrix that is only 1-2 millimeters thick. The polymerized acrylamide is now called polyacrylamide.

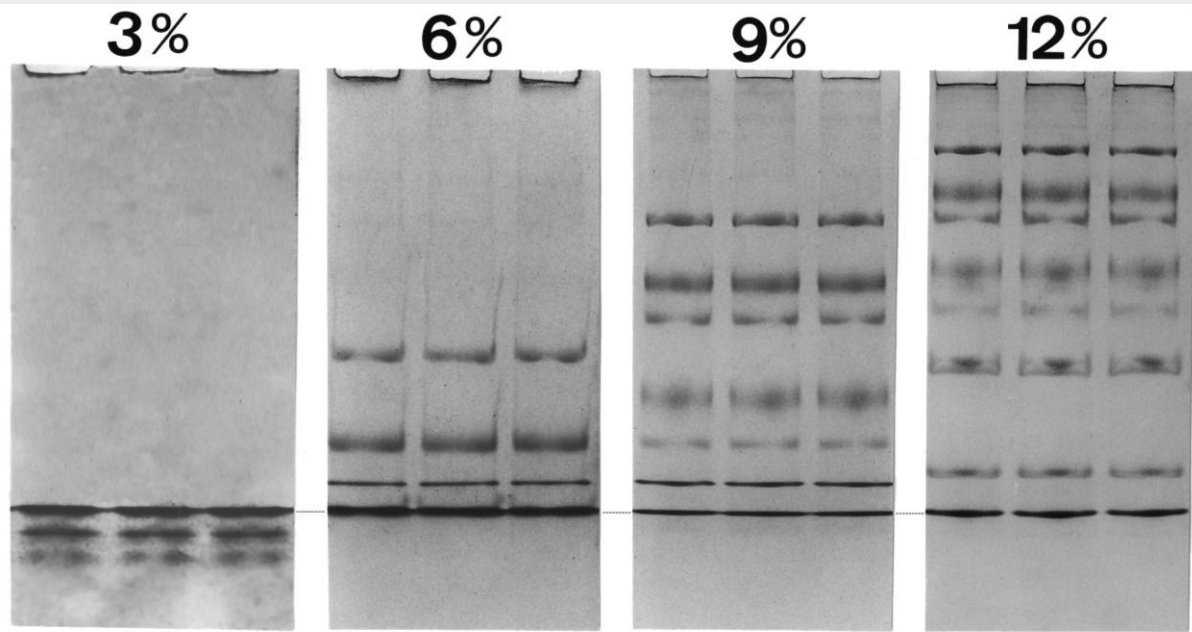


Figure 1. *Separation of proteins by SDS-PAGE at varying concentrations of acrylamide.* The images show the resulting separation of proteins following electrophoresis when separated on 3%, 6%, 9%, and 12% (from left to right) acrylamide gels.

Figure Credit: Ernst Hempelmann (Produced by Ernst Hempelmann) [CC-BY-3.0 (<http://creativecommons.org/licenses/by/3.0/>)], via Wikimedia Commons.

Depending on the size of the protein of interest, the concentration of acrylamide can be adjusted, usually in the range of 6-15%. Lower gel concentrations result in large holes in the matrix and are used to obtain good separation of large proteins. In contrast, high concentrations result in the creation of small holes and crevices through which small proteins can travel and are used to obtain good separation of small proteins (Figure 1). In StarCellBio, users can select 10%, 12% or 15% acrylamide.

Western blotting gels usually contain 10 – 15 wells, or pockets that serve to contain the protein samples prior to the application of an electric field. In StarCellBio, the gels have 15 wells, and one well should be reserved for the protein marker.

There are two types of gels that are used in the research lab. Both of these are polyacrylamide gels (PAGE or polyacrylamide gel electrophoresis).

1. The first kind of gel that is run is a denaturing gel, also called “SDS-PAGE” gel. This gel contains a very strong negatively charged detergent (SDS) that causes the proteins to be denatured (lose their three-dimensional shape) and also coats the proteins with a negative charge. A denaturing gel separates proteins based solely on size.

2. The second kind of gel is a native gel. As the name suggests, this type of gel preserves the native conformation of a protein (no detergent is used) and separates the proteins based on shape and size.

StarCellBio currently only simulates western blotting with denaturing gels. In the future, we may add the capability to run native gels.

Video Resource

Benchfly.com has a great video resource on preparing gels. In particular, the video details how to pour an acrylamide gel and set up the gel for running. The link is: <http://www.benchfly.com/video/106/pouring-acrylamide-gels-for-sds-page/>. There are two additional notes to make about this video:

1. This video will provide you with a visual of what the gel actually looks like. The gel is a polyacrylamide gel. The names of the other chemicals (TEMED, etc.) mentioned are used to solidify the gel and the names of each of the chemicals are not relevant for our purposes.
2. In the description above, we have not described the two layers (upper and lower) that are contained within the gel. Because the goal of running the gel is to separate proteins based on size (and sometimes also shape), you need to have the proteins enter the gel at the same time. However, the well that holds the protein prior to the application of the electric field is relatively tall. As a result, we use gels with two layers. The sole purpose of this is to ensure that all of the proteins contained in the well enter the lower portion of the gel at the same time.

[Back to top](#)

3. LOAD GEL

Once the gel has solidified, it is placed in a transparent box surrounded by a liquid buffer, which will help conduct the electric field once it is applied. Each of the samples can now be pipetted into a well in the gel. Once all of the samples of interest are added to their own individual wells, the gel is ready to run.

In StarCellBio, all of the samples are loaded into the gel for the user. Users can organize their samples into by dragging and dropping the samples in the Samples Window into the particular order in which they should appear in the gel.

In order to determine the actual size of a protein, we always run a sample that is composed of proteins of known sizes in one of the gel's wells. We refer to this sample as a protein marker or ladder and it usually consists of a wide range of protein sizes, from

approximately 10-250 kDa. Kilodaltons (kDa) is the term of measurement for the molecular weight of proteins.

Video Resource

Benchfly.com has a great video resource on loading a gel. In particular, the video provides a nice visual of the wells and how they hold the protein sample prior to applying the electric field. The link is: <http://www.benchfly.com/video/120/how-to-load-and-run-sds-page-gels/>

[Back to top](#)

4. RUN GEL

To separate the proteins in the gel, a uniform electric field (usually around 120-200V for about an hour) is applied. The electric field is applied in such a way that proteins will be drawn into the gel and will travel through the gel at various speeds depending on their size and shape. For the first iteration of StarCellBio, the protein samples are denatured, which means that the proteins are no longer found in their natural shape in which they are found inside a cell. Instead, they are in a linear form and they will be separated in the gel only by size, and not by shape or charge.

If multiple samples have been loaded into separate wells in the gel, then each sample will run in a parallel fashion in their individual lanes. A "lane" refers to the physical vertical space that is located beneath the well. Larger proteins will take longer to run through the gel and as a result will remain closer to the starting point (the wells) of the gel. Smaller proteins will be able to squeeze and move through the gel matrix much more easily and will travel farther through the gel matrix. As a result, smaller proteins will move closer to the bottom of the gel (farther away from the wells). Proteins in different lanes that travel the same distance will be approximately the same size.

Depending on the proteins of interest that are being examined, the electric field will need to be shut off after different lengths of time for different experiments. There are two visual indicators to alert the researcher to when the electric field should be turned off.

1. The protein samples all contain a blue dye (see sample preparation). This blue dye travels through the gel at the same rate as the smallest proteins. When the dye front gets to the bottom of the gel, this indicates to the researcher that the smallest proteins in each sample will be running off of the gel and into the buffer (lost forever!).

2. The protein ladder creates samples of known sizes that are all marked in various patterns and/or colors. Unlike the proteins in a researcher's samples that are not dyed, the proteins in the protein ladder are visually apparent. The protein ladder can be used as a visual guide to tell the researcher where proteins of specific sizes are present in the gel as the gel is running.

If the researcher would like to visualize ALL the proteins in the gel, and not a specific protein of interest, then the gel can be stained at this point with a dye that will bind all proteins in a non-specific manner. The dye that is most commonly used is Coomassie blue, which dyes all of the proteins in the gel blue (Figure 2). It is important to note that the stain binds all proteins in a non-specific manner and as a result, a researcher cannot tell at which proteins he/she is looking. This step is not always necessary and it is often skipped during a western blotting experiment. Up to this point, this procedure can be called just "gel electrophoresis". It is the detection of specific proteins of interest that is at the heart of a western blot. StarCellBio does not currently have the capability of performing Coomassie blue staining, but it will be added in the future.



Figure 2. *Coomassie Blue staining of two gels.* Coomassie blue staining is used to visualize all of the proteins in the gel, but does not allow for detection of specific proteins in a sample.

Figure Credit: Stephen Helms from Dallas, TX, United States (My Pretty Gels) [CC-BY-SA-2.0 (<http://creativecommons.org/licenses/by-sa/2.0>)], via Wikimedia Commons.

[Back to top](#)

5. TRANSFER

In this step, the proteins are transferred from the gel to a membrane. After the gel has run for an appropriate length of time to allow for proteins to separate sufficiently on the gel, the electric field is turned off. At this point, although you can't see all the proteins, all of the proteins in a given sample are separated based on their size and are present at different places in the gel. We then immobilize the proteins by transferring them from the gel to a membrane. We do this for three reasons:

1. The gel is very thin and fragile and will not hold up well to further manipulations.
2. The proteins are contained within the gel, and are not accessible to detection by antibodies, which is the next step of the experiment. Once the proteins are transferred to a membrane, the proteins will be localized on the surface of the membrane and they will be accessible to antibody detection.
3. The proteins are not immobilized in the gel. Although the electric field is no longer applied, proteins will continue to slowly diffuse through the gel, and the diffusion will no longer be in a specific direction. As a result, we cannot use proteins in the gel to differentiate between proteins of different sizes.

To transfer the proteins from the gel to the membrane, we set up a “sandwich” in which the gel is placed next to a membrane and protected by filter paper and sponges. The membrane is a type of cellulose (looks like a thick, flexible and impermeable piece of paper) and is usually made up of nitrocellulose or PVDF (polyvinylidene difluoride). The sandwich is placed in a box and again surrounded by a liquid buffer that will help to conduct the electric field.

Once the transfer apparatus or “sandwich” is assembled, we again utilize an electric field to pull the proteins from the gel and onto the membrane. The proteins are still negatively charged from the use of the strong detergent in the sample preparation and gel running steps. As a result, the proteins will migrate out of the gel and move toward the positive charge and, due to the membrane’s properties, the proteins will “stick” to the surface of the membrane.

This step can either occur at room temperature for approximately an hour at around 100 V or can be done overnight at 4 °C at 20-30 V.

[Back to top](#)

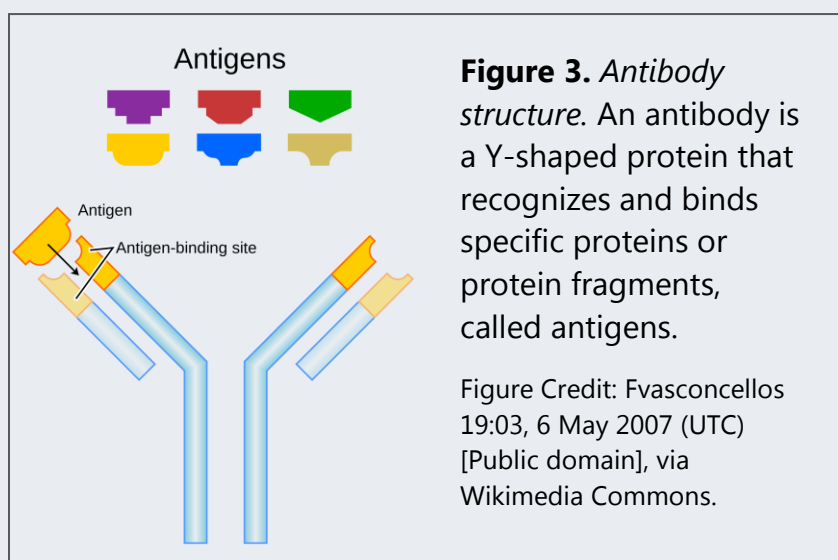
6. BLOT

Following the transfer of the proteins from the gel to the membrane, we are now ready to detect a specific protein(s) of interest that is bound to the membrane. In this step, we probe, or “blot”, the membrane for our protein of interest using antibodies. Antibodies are naturally made by our immune systems in response to infection to specifically bind proteins directly. Foreign invaders contain proteins that are not recognized by our bodies as our own. The immune system then uses the foreign proteins to produce antibodies that recognize them and therefore will recognize the same foreign agent in other cells within our bodies in a current and future infection. Researchers utilize this natural process to isolate antibodies that specifically recognize proteins of interest.

Companies now make antibodies commercially available and can isolate antibodies that specifically bind a protein of interest.

Antibodies are a Y-shaped protein (Figure 3). The tips of the two “arms” of the Y structure vary between antibodies and specifically recognize and bind different proteins. The “stem” portion of the Y structure is the same between antibodies of the same organism. All antibodies made in a particular organism, for example, will have a similar stem structure.

To detect proteins on the membrane, we use multiple steps designed to specifically recognize and bind a protein of interest and then amplify this detection through a second detection step and a chemical reaction, which allows the visualization of protein



of interest.

We call an antibody that recognizes a specific protein of interest a primary antibody². A secondary antibody³ is an antibody that recognizes the primary antibody. More specifically, it recognizes the stem portion of the antibody. If you use a secondary antibody that does not bind to the primary antibody, then no signal will appear on the western blot at the end of the experiment.

The secondary antibody that is used in a western blot experiment is bound to an enzyme that will then catalyze a chemical reaction to create a light signal upon the addition of a chemical substrate. This light signal can be detected using photographic

² If a primary antibody is made in a mouse to detect protein X, then it will be called “mouse anti-protein X”.

³ If a secondary antibody is made in a rabbit to detect a mouse primary antibody, then it will be called “rabbit anti-mouse”.

film. Alternatively, the secondary antibody can be bound to a fluorescent molecule, which eliminates the need for the chemical reaction to detect the protein. StarCellBio currently simulates detection of proteins through a chemiluminescent reaction.

The multiple steps of blotting the membrane to detect proteins using antibodies are as follows:

1. The membrane is washed with a "blocking" solution that is designed to block non-specific binding of an antibody to the membrane or proteins. Although antibodies will bind specific proteins tightly, then can also bind nonspecific proteins weakly. The blocking solution will prevent weak nonspecific binding.
2. The membrane is incubated in a dilute solution of the primary antibody. The first (primary) antibody will specifically bind to target proteins that are bound on the membrane.
3. The membrane is then washed in a very low concentration of detergent to remove any weakly bound or non-specifically bound antibody to the membrane or the proteins bound to it that were not successfully blocked by the blocking solution. Any primary antibody that is tightly bound to the protein of interest will remain.
4. A second antibody incubation step is performed in which the membrane is incubated in a dilute solution of the secondary antibody. The secondary antibody will specifically bind the primary antibody. The secondary antibody is also bound to an enzyme that will be used to detect the protein. Many secondary antibodies can bind to each primary antibody, which serves to amplify the light signal that will be created in a later step.
5. The membrane is then washed in a very low concentration of detergent to remove any weakly bound or non-specifically bound antibody to the membrane or proteins bound to it. The antibodies that are tightly bound will remain.

The blot is now ready to be exposed to film to detect the protein of interest.

[Back to top](#)

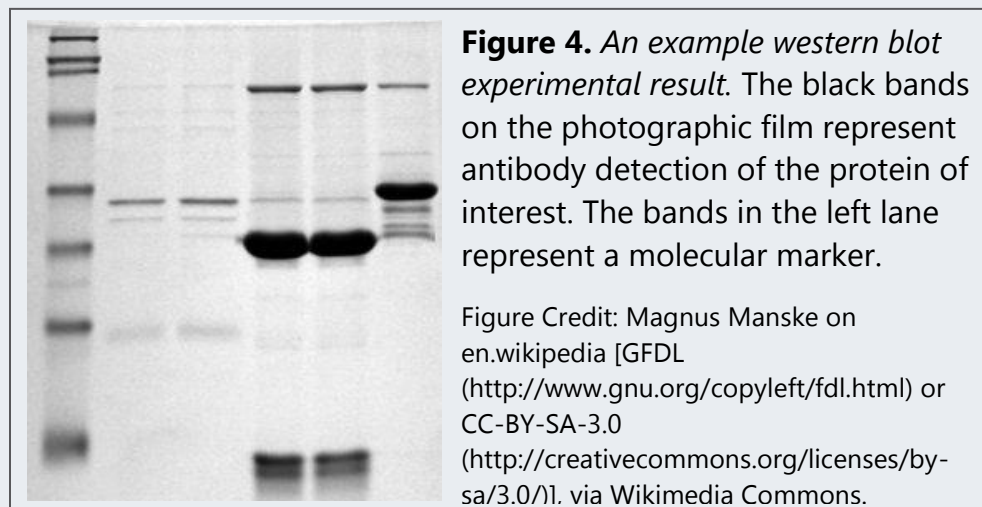
7. DEVELOP

After blotting the membrane with primary and secondary antibodies, the blot is now ready to be exposed to film to detect the protein of interest. The secondary antibody is bound to an enzyme that will catalyze a chemical reaction to create a light signal that will be detected on film. There are two steps to develop a western blot:

1. To catalyze the chemical reaction to create a light signal, the necessary substrate of the reaction is added on top of the membrane. This will create a light signal wherever there is a secondary antibody bound to the primary antibody, which is bound to the protein of interest.
2. A piece of photographic film is applied to the membrane in a dark room to visualize the light signal because the light signal is not visible to the eye. The location of the protein will be marked by a light signal, which manifests itself as a dark band on the photographic film. The membrane will typically be exposed to film for a length of time that varies anywhere from seconds to hours. The amount of luminescent signal is proportional to the amount of protein that is present on the membrane. Additionally, the luminescent signal appears in the physical location of the protein on the membrane and can be used to determine the size of a protein by comparing its location with that of the protein ladder.

At this point, the researcher will have a piece of photographic film that (hopefully!) has black bands on it (Figure 4). The black bands represent the protein of interest.

If a researcher would like to look at another protein(s) of interest, then the blot (the membrane) can be erased or “stripped” of the antibodies (first and secondary) that are bound to the first protein of interest.



Following the stripping of the blot, the new blotting conditions can be selected to detect the second protein of interest utilizing a different set of antibodies. This process can potentially be performed several times, although usually the clarity of protein detection decreases with each stripping procedure. In StarCellBio, select **RE-PROBE** to detect another protein.

[Back to top](#)

WESTERN BLOT ANALYSIS

The user will analyze the presence, position and intensity of the bands in all the lanes in order to determine the effect of a specific treatment on the protein of interest. The location of the protein bands on the membrane relative to the protein ladder will enable the researcher to determine the size of the protein. The comparison of the presence, position and intensity of the bands in the experimental samples to those for the positive and negative controls is critical to the analysis of the western blot. While the film depicts the location of the protein of interest, it does not depict the proteins that were present in the protein ladder. This is because the antibody used to detect the protein of interest does not also detect the proteins in the protein ladder (although there are cases when an antibody non-specifically binds to proteins in the protein ladder). As a result, the researcher will need to align the membrane with the film to determine the size of the proteins relative to the known sizes of the proteins in the ladder.

The analysis of the western blot is a critical educational experience and as a result, students are required to interpret their particular western blot on their own.

[Back to top](#)

B. Flow Cytometry

Flow cytometry is used to count and analyze the size, shape and properties of individual cells within a heterogeneous population of cells.

[Back to top](#)

C. Microscopy

Microscopic analysis is used to study the shape and morphology of the objects (cells, tissue slices after fixation and staining) and in counting the number of objects per unit volume (e.g. counting the number of cells or bacteria). Microscopy is also used in determining the presence and absence of certain type of cells or organelles in a sample or specimen of tissue.

[Back to top](#)