



FRALIN LIFE SCIENCES INSTITUTE
VIRGINIA TECH™

Protein Electrophoresis Kit

INFORMATION MANUAL

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We would like to thank the teachers who have made many helpful suggestions for improving the kit and the manual, including Kristin Jenkins, Jill Peters, John McLaughlin, Steven Scheidell, Jim Meadows, Greg Deskins, and Adrienne Warren. We would also like to acknowledge the Biotechnology Explorer Program at Bio-Rad, with whom we traded many ideas during the development of this Biotech-in-a-Box kit and their classroom kit.

Time Required: Electrophoresis of Protein Samples

≥15 min Introduce lab

15–30 min Prepare protein samples

Optional stopping point: Protein samples will keep overnight in the refrigerator. Allow samples to warm to room temperature before loading gel.

15 min Set up gel boxes (can be done before class)

45 min Load samples and run gels

Optional stopping point: If you do not have time to wash and stain the gel immediately, do not open the cassette after running the gel. Wrap in plastic wrap and store flat in refrigerator overnight. (There may be some diffusion of the bands, but it will be minimal.)

15 min Wash gel in water

1 h Stain gel in Bio-Safe Coomassie

Optional stopping point: Gel can be left in Bio-Safe Coomassie for longer periods (for example, overnight). May increase the time needed for destaining.

30 min Rinse (destain) in water

48-72 h Dry gel (optional)

INTRODUCTION TO THE PROTEIN ELECTROPHORESIS KIT

The Protein Electrophoresis Kit from the Fralin Life Sciences Institute contains all the materials needed to prepare samples, run SDS-PAGE gels, visualize the proteins on the gels, and dry the gels to preserve them. The borrower must provide the sample materials (fish, seafood, meat, etc.) and distilled water. The kit is available from Fralin for a two week loan period.

Electrophoresis of proteins using SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) is similar in principle to electrophoresis of DNA in agarose, but there are some significant differences. One of the primary differences is that acrylamide in its liquid form is a neurotoxin, and, as such, is not suitable for classroom use. The gels will be provided ready to use. Another difference is that the apparatus is slightly more complicated to set up and run. Because of this, we ask that the instructor, or perhaps the instructor with a few trusted students, set up the gel boxes for use. The class can prepare the samples and load them on the gels, and then process the gels after the run.

This manual is divided into three sections. The first section includes a page on timing the activity, a list of kit contents, and a section on the theory of protein electrophoresis. The instructor can decide the complexity of the material to be presented to the students. The most important points are highlighted and there are several overheads on the material.

The second section is the experimental section, containing an overview of the experiment, notes on the reagents used, and the student experimental procedure. The experimental procedure is broken into several sections; each section is preceded by information for the instructor, including advanced preparation needed.

The final section is the Appendix, which contains everything else, including gel box assembly, scenarios, pipettor directions, conceptual and technical review material, practice gel analysis, sources for the supplies and equipment, references, and information on muscle proteins and on evolutionary relationships.

If you come up with any other scenarios or study questions, please send us a copy and we'll incorporate them in the manual as well.

Feel free to call or email Dr. Kristi DeCourcy at Fralin with any questions about the material. Corrections and suggestions will be gratefully received. Kristi's phone contact info is decourcy@vt.edu and 540-231-7959.

PROTEIN ELECTROPHORESIS KIT CONTENTS

Item	Quantity
power supply	1
gel boxes	4
buffer dams	4
gel loading guides	4
Fisher micropipettor (5-40 μ l)	1
10- μ l fixed volume pipettor	4
20- μ l fixed volume pipettor	4
yellow tips for micropipettors	1 bag
tube racks	10
scissors	10
forceps	10
spatulas	5
plastic boxes	5

weigh boats	10
microcentrifuge tubes	1 pack
colored microcentrifuge tubes	1 pack
marker pens	2
label tape	2
1-ml graduated transfer pipettes	20
transfer pipettes	20
gel drier rack	1
gel drier frames	5
gel drier clamps	20
gloves	1 box
50-ml graduated cylinder	1
gel cassette opening tool	1

The quantities of the following items vary with the number of gels the instructor plans to run with his or her classes. The maximum number of gels available with the kit from Fralin is five, although additional gels may be purchased by the instructor from Bio-Rad, if desired (see [page 60](#) for ordering information). Fralin will provide, upon request, buffers and materials for up to 10 additional gels.

The sample buffer provided is enough to prepare 50 samples. Additional sample buffer will be available if you plan to prepare more samples. Please make this decision before the kit is shipped, so we can avoid additional shipping charges. Ten samples may be run on each gel (actually, 9 plus a molecular weight standard).

Item	Quantity	storage conditions
pre-cast gels	5	refrigerator
Laemmli sample buffer	25 ml	room temperature
molecular weight markers	20 μ l/gel	freezer
gel running buffer	400 ml/gel	room temperature
Bio-Safe Coomassie stain solution	50 ml/gel	room temperature
gel drying solution	40 ml/gel	room temperature
cellophane sheets	2/gel	room temperature

IMPORTANT NOTES (Please read!)

- **Please** remember that this kit is shipped with **5 gels** and the materials to run them (buffers, cellophane sheets, etc.). If you wish to run more gels while you have the kit, you must purchase them from Bio-Rad directly, and let Fralin know ahead of time the number of extra gels for which you will need materials (maximum: materials for 10 additional gels). If you decide to purchase more gels, please remember that there are 4 gel boxes in the kit, so the most you can run at a time is 4 gels (or 8, if you run 2 gels per box).
- On the checklist, if something is not shaded in the “repacked” column, that means that it is **not** an expendable item, and **all** should be returned, barring a problem, (e.g., if your dog eats a tube rack).
- On the checklist, if something is shaded in the “repacked” column, that means that it is an expendable item. It is not expected that you will return all of these items, but please return any that are unused. **Please do not mix** used items with unused items! Please help us keep our expendable costs down by returning unused items.
- Please rinse the gel boxes and accessories and allow them to air dry (if possible) before repacking them. A number of boxes have been returned with dried buffer residue all over them. Please see clean-up directions later in manual.
- If there is a problem with a piece of equipment, please indicate which piece by putting label tape on it. Please do not just say, “A gel box didn’t work,” or we have to spend time testing every box.
- **Please (!)** keep to the schedule for return shipping! If you are late returning the kit, the next teacher is the one who will suffer, and it’s not fair to any of us!
- Seal the trunks with the cable ties provided. Please make sure that the cable ties are secure. Note that there is a right way and a wrong way to insert the tab in the cable tie. Look at the end you put the tab through. The tab should be put in from the side that is smooth with the tie, not the end that sticks out. If it is done the wrong way, the cable tie will open when you pull on it. Please test the cable tie by pulling to be sure that you’ve done it correctly.
- Be certain that the shipping label is placed on the trunk securely in the plastic sleeve.
- Please be careful with repacking. Use the bubble bags, and place heavy items on the bottom of the trunk. Please make sure that the lids are on the gel boxes for return shipping or the leads can be broken off. Please, **do not use Styrofoam peanuts!!**

INTRODUCTION TO PROTEIN ELECTROPHORESIS

General principles of electrophoresis

Electrophoresis is the migration of charged molecules in an electric field toward the electrode with the opposite charge (Figure 1). This technique is widely used to examine proteins and nucleic acids, answering a variety of questions. For example:

- How pure is this protein?
- How much DNA do I have?
- How big is this protein?
- Has my experiment resulted in any changes in my protein?
- What is the nucleotide sequence of this DNA?



Although charged molecules would migrate to the opposing electrode if the electrophoresis were

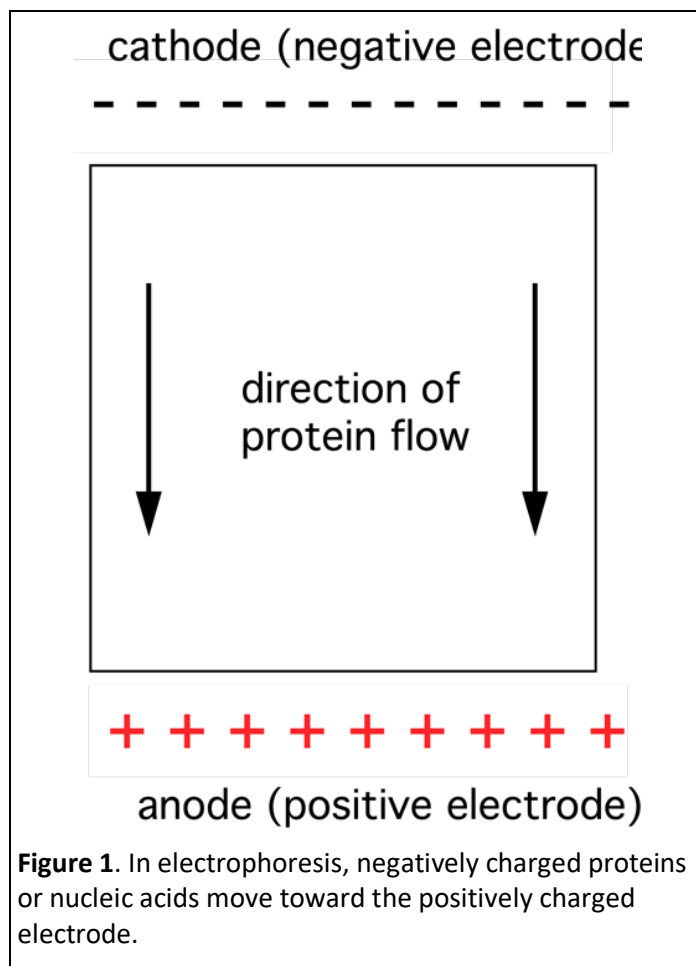
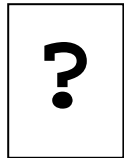


Figure 1. In electrophoresis, negatively charged proteins or nucleic acids move toward the positively charged electrode.

performed in solution alone, electrophoresis is commonly performed by placing the sample in a matrix of either acrylamide or agarose. In gel electrophoresis, molecules separate based on their charge and their mass. The gel matrix acts as a molecular sieve through which smaller molecules can move more quickly than larger molecules. The amount of sieving that takes place during electrophoresis can be increased or decreased by changing the concentration of agarose or acrylamide.

Acrylamide or agarose?

In general, DNA molecules are orders of magnitude larger than proteins. Think about this: DNA codes for proteins. Coding for each amino acid of a protein requires 3 basepairs of DNA, plus all the DNA for the transcription controls: signals for the gene to turn on and turn off, signals that determine where transcription will start and stop, etc.



Proteins range in size from several kilodaltons (kDa) to hundreds or thousands of kilodaltons. The nucleic acids we study are normally larger than a kilobase (kb), and each kilobase is approximately 660 kDa. For example, when cloning DNA, 2 kb pieces of DNA are frequently inserted in plasmids of 3 kb, giving a total plasmid length of 5 kb (~3,300 kDa or 3.3 million daltons).

When the molecules to be separated are large (greater than 200 kDa), then agarose is the matrix of choice. This means that agarose gel electrophoresis is generally used for separating nucleic

acids and some larger proteins. Acrylamide is used for most protein electrophoresis and for separation of small DNA molecules (e.g., for DNA sequencing).

Determining the size of proteins using electrophoresis

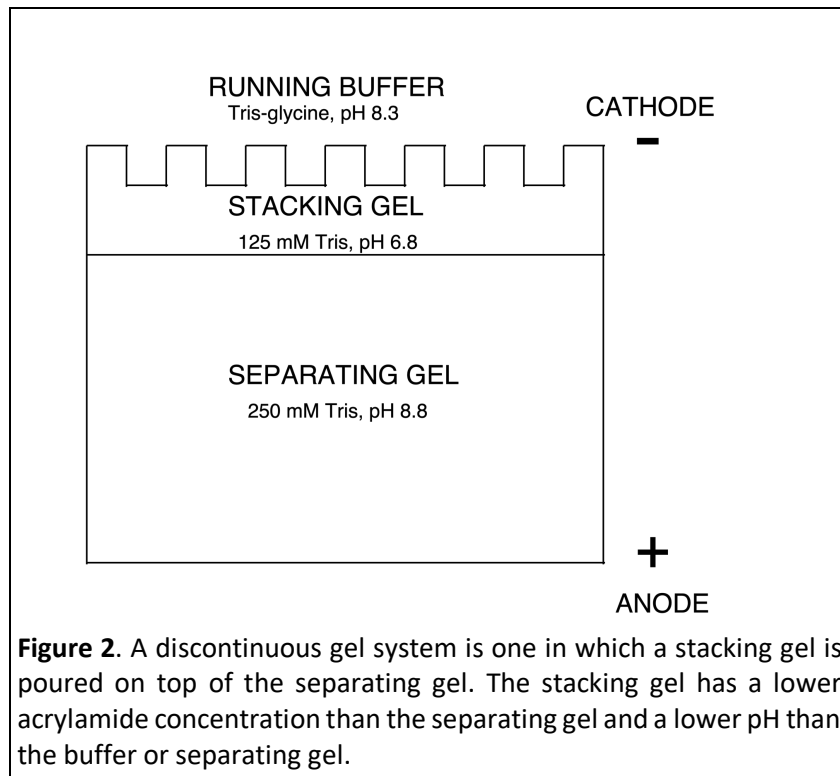
One of the uses of gel electrophoresis is determining the molecular weight of a protein. (Determining the purity of a protein is a second major use.) Proteins will migrate in an acrylamide gel at a rate based both on their electric charge and on their mass. The ratio of charge to mass is called charge density. In order to use this technique for molecular weight determinations, the charge of the protein must be removed as a factor in the migration.

This is accomplished by placing an ionic detergent in both the gel running buffer and the sample buffer. The detergent commonly used is sodium dodecyl sulfate (SDS). Most proteins bind SDS in such a way that they migrate in a gel as if they have nearly identical charge densities (all negative), so mass becomes the only factor in determining the migration rate of the protein. This technique is called SDS-PAGE (SDS-polyacrylamide gel electrophoresis).

Although it will not be discussed here, PAGE is frequently performed without SDS. "Native" PAGE (native, because the proteins are in their native conformations) is used, for example, when it is important that the protein of interest not lose its structure or activity. Also, SDS is not used when sequencing nucleic acids, because, unlike proteins, most nucleic acids have the same charge densities without adding SDS.

SDS-PAGE using a discontinuous buffer system

One final consideration is how one gets all the protein to enter the gel at the same time. Samples usually have microliter (μl) volumes, and not all the protein molecules will enter the gel at the same time.



THE SHORT ANSWER: A "stacking" gel is poured on top of the "separating" gel. The stacking gel has a lower concentration of acrylamide and a different buffer molarity and pH than the separating gel. **As a result of the stacking gel, all the proteins in the sample enter the separating gel simultaneously.** Most, but not all, of the pre-poured gels offered by vendors have stacking gels.

THE LONG ANSWER: The solution is using a discontinuous buffer system which has a "stacking" gel (Figure 2). A gel of lower acrylamide concentration, typically 3-4%, is poured on top of the

"separating" gel, typically 6-15%. The stacking gel (pH 6.8) and the separating gel (pH 8.8) are made with Tris-HCl buffers. The running buffer is a Tris-glycine buffer, pH 8.3. In an electric field at pH 6.8, chloride ions migrate the most rapidly, proteins have intermediate mobility, and glycine migrates the most slowly. As electrophoresis begins, the chloride ions move ahead, causing a zone to form behind them in which there is reduced conductivity. Because of this reduced conductivity, the migration of glycine is accelerated, so that it keeps up with the chloride. Basically, the proteins are "captured" in the zone between the chloride and the glycine in the order of their mobility, so that the protein with the least mobility (the largest) will be closest to the glycine. In other words, the proteins are "stacked" in a very narrow band when they reach the separating gel. When this stack reaches the interface of the stacking and separating gels, the pH increases to 8.8. At pH 8.8, the mobility of glycine increases and it passes the proteins and migrates directly behind the chloride ions. The higher percentage acrylamide in the separating gel results in increased sieving of the proteins, which are no longer stacked, and the proteins separate according to their mass.

A little history

For a historical note, the gel system that we are using is credited to U.K. Laemmli, whose 1970 *Nature* paper has probably the highest number of citations of any scientific paper. Laemmli used the buffer system developed by Ornstein and Davis. This overall system, a SDS-containing discontinuous gel with the Ornstein-Davis buffer system, is the predominant one used in slab gel electrophoresis.

Preparing a polyacrylamide gel

Polyacrylamide gels are formed by the polymerization of acrylamide monomers, and the crosslinking of these polymers by bis-acrylamide (*N,N'*-methylene bisacrylamide). The amount of sieving by the gel is controlled by adjusting the acrylamide concentration, with higher concentrations of acrylamide resulting in increased sieving. For example, to separate proteins of 100 to 200 kDa, the acrylamide concentration should be 5%, but to separate proteins in the range of 20 to 40 kDa, the acrylamide concentration should be 15%. In the experiment in this kit, you will be using a separating gel of 15% acrylamide, with a 4% stacking gel. A 15% gel provides good separation for proteins in the range of 14 to 100 kDa.

Acrylamide, in powdered or liquid monomeric (unpolymerized) form, is a **neurotoxin**, so in most cases it is not feasible to pour acrylamide gels in a high school lab. Fortunately, many companies now sell different types of pre-cast acrylamide gels, making it possible to perform polyacrylamide electrophoresis of proteins in a high school classroom. To cast a polyacrylamide gel, an acrylamide solution containing acrylamide and bis-acrylamide is mixed with a Tris buffer of specific molarity and pH. A catalyst (ammonium persulfate) and a reaction accelerator (TEMED; tetramethylethylene-diamine) are added to the solution to initiate polymerization. The solution is quickly poured or pipetted between glass plates, a well former (comb) is inserted, and the gel is left undisturbed until the acrylamide polymerizes. For a discontinuous gel system, as is provided with this kit, after the "separating" gel has polymerized, the "stacking" gel is poured on top of the separator and the sample comb is inserted in the stacking gel.

Running a polyacrylamide gel

To run an acrylamide gel, the polymerized gel is clamped into a vertical electrophoresis apparatus. The procedure is very similar to performing agarose gel electrophoresis in a horizontal box, except that the two buffer chambers [upper, containing the negative electrode (cathode) and lower, containing the positive electrode (anode)] are connected only by the polyacrylamide gel. (In horizontal agarose gel electrophoresis, the surface of the gel is flooded with buffer.)

After the gel is clamped into position, running buffer, containing Tris, SDS, and glycine, is added to the upper and lower buffer chambers. The comb is removed from the gel, and samples and standard are loaded in the wells. The apparatus lid is placed on the box, and the leads are plugged into the power supply. Gels can be run under either constant voltage or constant current. In this experiment, we will be running the gels under constant voltage. When current is applied, bubbles should be visible rising from the electrodes and the blue dye in the sample buffer should be seen entering the gel.

Sample preparation

Native proteins migrate differently in SDS-PAGE than do reduced, denatured proteins. In determining the molecular weight of a protein, the secondary structure (e.g., β -pleated sheets, α -helices), tertiary structure (e.g., protein domains held in place by disulfide bonds), and quaternary structure (e.g., several polypeptide chains folded

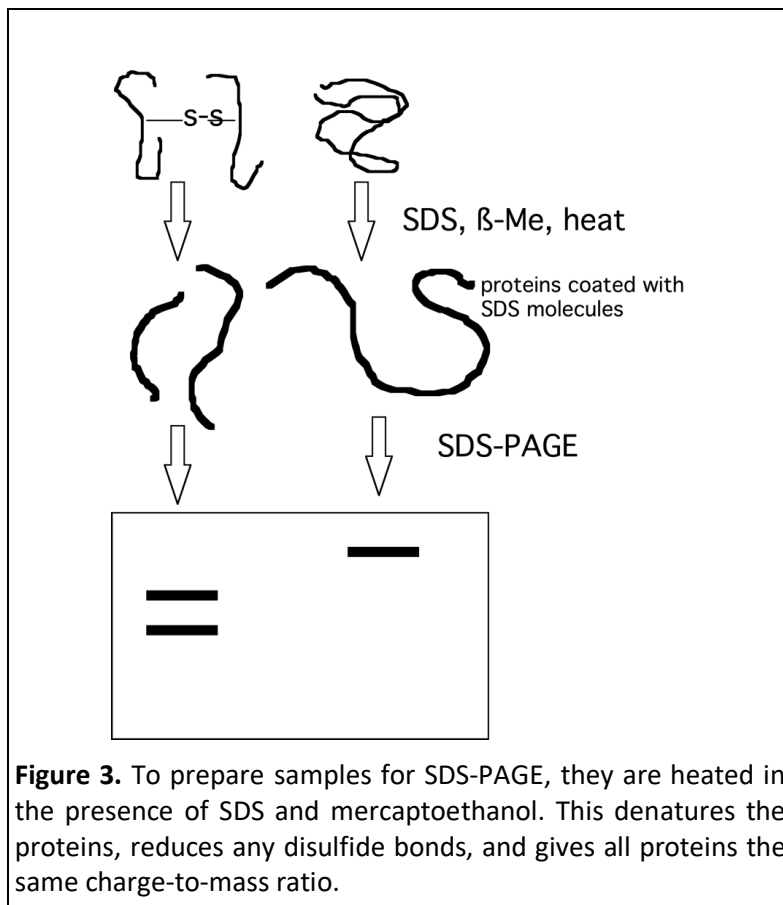


Figure 3. To prepare samples for SDS-PAGE, they are heated in the presence of SDS and mercaptoethanol. This denatures the proteins, reduces any disulfide bonds, and gives all proteins the same charge-to-mass ratio.

together to form a protein; the chains may be held together by disulfide bonds) must be disrupted prior to electrophoresis. This is normally accomplished by boiling the samples for 3–5 minutes in the presence of SDS and β -mercaptoethanol (a reducing agent that breaks disulfide bonds). These three factors (heat, ionic detergent, and reducing agent) disrupt the 2°, 3°, and 4° structures of the proteins (Figure 3). Note: In this experiment we are omitting the

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β -mercaptoethanol from the sample buffer and we do not heat the samples. The results will not be appreciably different. If the samples are not heated, the proteins may not be fully denatured, but the bands are still very similar to those seen in fully denatured samples.

Visualizing the proteins

After electrophoresis is completed, the apparatus is disassembled and the gel cassette is opened. To visualize the proteins, the gel is placed in a solution of protein stain. There are a number of stains available (e.g., Amido black, silver stain, Fastgreen, Ponceau S), but the most commonly used protein stain is Coomassie blue. The gel is soaked in the stain solution and the entire gel absorbs the stain and appears blue. The excess stain, that which is not bound to proteins, is removed by soaking the gel in a solution of methanol and acetic acid, a process called destaining. The stain currently used in this kit is a derivative of Coomassie blue stain called Bio-Safe Coomassie. Unlike standard Coomassie staining, Bio-Safe Coomassie does not have any acetic acid or methanol in the stain or the destain which makes it much safer to use.

For a record of the experiment, the stained gel can be photographed or scanned. Some scanners have a transmitted light source, which should make a nice scanned image of a stained gel. Alternately, the gel can be dried (dehydrated) on a sheet of filter paper or between two sheets of cellophane. Finally, the gel can be sealed in a plastic bag or plastic wrap and kept in the hydrated form.

Molecular weight standards

Standards are needed to make molecular weight determinations of proteins. Molecular weight standards are generally mixtures of proteins of known molecular weight. They are available in a number of protein size ranges. For example, high range standards might contain proteins from 40 to 200 kDa, whereas low range standards might contain proteins from 2 to 30 kDa. There are also wide-range standards available, containing proteins from 4 to 200 kDa. The choice of molecular weight standard should be made based on the size of proteins in which you are interested and on the percentage acrylamide of the gel that is being run. For example, if you are interested in small proteins and running a high percentage acrylamide gel, then choose a low range standard.

Molecular weight standards are available either unlabeled or prestained. The prestained markers are tagged with a dye and are visible as they run on the gel. The unstained markers are not visible until the gel is stained with a protein stain such as Coomassie blue.

Analyzing the results of the gel

Just as with agarose gel electrophoresis of DNA fragments, a standard curve is prepared by plotting migration distances (x-axis) of known protein standards against their molecular weights (y-axis) on semilog graph paper. From the migration distance of an unknown protein, the molecular weight of the protein can be calculated from the standard curve.

The other determination that can be made from a protein gel is the relative abundance of different proteins. A stained band that is broad and dark contains more protein than a lightly stained band. Using this information, one can compare both the abundance of proteins within a sample or the abundance of a particular protein in different samples.

Identifying proteins on an acrylamide gel

It is not possible to identify unknown proteins positively on a SDS-PAGE gel without further experimentation. When doing an experiment like the one below, a complex mixture of proteins

is loaded on each gel. The resulting gel shows a series of blue-stained bands in each gel lane. From this, you can determine the size and relative abundance of the proteins, but nothing more.

However, one can make guesses about the identity of proteins. For example, if a sample is muscle tissue, you might assume that there would be large quantities of muscle proteins such as actin and myosin. And, in fact, in every seafood sample that I have run, there have been very abundant proteins at the correct molecular weights for the subunits of actin and myosin. (See page 55 in the Appendix for more information on muscle proteins and their molecular weights.)

A widely-used method for identifying a protein of interest on a gel is a procedure called Western blotting. In Western blotting, following electrophoresis the proteins in the acrylamide gel are all transferred to a membrane (usually a nitrocellulose membrane). The membrane is then soaked in a solution containing antibodies specific for the protein of interest. The antibodies will bind to the protein on the membrane; they will not bind to any other proteins. The antibody can then be detected on the membrane using another probe that is labeled with an enzyme which will produce a colored product on the membrane. The result should be a colored band of the correct size on the membrane. For more details on Western blotting, refer to the references on page 63.

Nutshell overview of the above material

- Proteins migrate in an electric field because they are charged or can be made charged.
- Generally, acrylamide is used to separate small molecules (e.g., proteins) and agarose is used to separate large molecules (e.g., DNA).
- The percentage of acrylamide to be used in the gel is determined by the size of the proteins of interest. Gels of higher percentage acrylamide are used to separate smaller proteins and gels of lower percentage are used to separate larger proteins.
- In SDS-PAGE, proteins separate based on their sizes. If standard proteins of known molecular weights are also run on the gel, the data generated can be used to calculate the molecular weight of unknown proteins.
- Samples are normally pretreated by heating in the presence of mercaptoethanol and SDS so that the proteins are denatured, have no disulfide bonds, and all have the same charge-to-mass ratio.
- The gels are discontinuous, i.e., have a stacking and a separating gel, so that all of the proteins enter the separating gel at the same time.
- The proteins on the gel will be stained so that they will be blue on the clear gel.

OVERVIEW OF THE EXPERIMENTAL PROCEDURE

1. Samples prepared as described on page 17 will provide enough material for at least 10 gel lanes. Sample preparation can easily be done in a single class period. After the samples are prepared, they can be refrigerated overnight or frozen for longer storage. (Be sure that they are warmed to room temperature before loading them on a gel.)
2. The polyacrylamide gels are prepared for use. This involves opening the gel packet and setting up the gel cassette in the apparatus. Setting up the gel boxes should be completed by the instructor, or perhaps the instructor and a few student assistants.
3. (Optional) The samples and a molecular weight standard are heated in a boiling water.
4. The samples are loaded on the gel.
5. Current is applied to the gel, and the gel is run until the blue dye front reaches or nears the bottom of the gel. This can be done in under an hour, or the gel can be run much more slowly.
6. The apparatus is disassembled and the gel cassette is removed.
7. The gel cassette is opened and the gel washed three times, 5 minutes per wash.
8. The gel is stained in Bio-Safe Coomassie stain for 1 hour. (Longer staining will not cause a problem.)
9. The gel is washed in water for 15–30 minutes.
10. (Optional) The gel is incubated in drying solution, placed between sheets of cellophane, and dried for 12-36 hours.

CHOOSING SAMPLES

There are several kits available that offer prepared protein samples for SDS-PAGE, but it is much cheaper and surprisingly easy to make your own samples using food from your neighborhood grocery store (or local pond!). This kit contains the sample buffer and materials that you will need to prepare your own samples. Your choice of samples will depend on the questions your students chose to pose or on the real world scenario you chose to follow. (The real world scenarios begin on page 43.)

One thing I tried recently with high school students in a workshop worked well. I presented the students with a list of available samples and asked them to consider what questions they could ask using the samples. Each student group chose a question to ask, such as: 1) Are there differences between the proteins in raw vs. processed fish? 2) Are there differences in muscle proteins across species? or 3) What is the difference in protein expression in different organ meats? After the gel is stained, ask the students if they can answer their question?

The samples I have found locally include catfish, Boston blue, salmon, haddock, squid (body & tentacles), monkfish, bay scallop, tilapia, frog legs, flounder, chicken muscle, chicken gizzard, chicken heart, cow kidney, alligator muscle, pig muscle, lamb muscle, cow muscle, fake krab, cow liver, pig stomach, cow heart, shark (thresher), tuna, yellowfin (fresh), crabmeat, claw (fresh), shrimp (fresh), sardine (canned), tuna (canned), oysters (canned), clams (canned), shrimp (canned), and crabmeat (canned). I've shopped at Kroger and other chain grocery stores, plus I have found some different fresh, frozen, and canned samples at my local Asian specialty grocery.

NOTES ON THE REAGENTS IN THE KIT

Safety note: Please follow the guidelines established by your school district for liquid handling, especially in terms of **safety glasses**.

- **Acrylamide** and bisacrylamide monomers, both in solution and in powdered form, are neurotoxins. Once the monomers are polymerized, however, the polyacrylamide gel is relatively non-toxic. A small amount of unpolymerized monomer could remain on the surface of the gel, so it is recommended that gloves be worn while disassembling the gel boxes and transferring the gels to staining solution. It shouldn't be necessary to wear gloves during gel box assembly, as you should not be in direct contact with the gel during this procedure.

Safety notes:

Shellfish! If you prepare samples from real crustaceans (as opposed to imitation crab or lobster), be sure to ask your students beforehand about any shellfish allergies. Anyone with shellfish allergies should have no contact with the shellfish samples, either pre- or post-preparation! Also, it is possible that fake Krab may have some real crab for flavoring, so avoid using it if any of your students have shellfish allergies.

Latex gloves: Nitrile gloves are sent with the kit. Some individuals are sensitive to the latex used in gloves, so if you buy additional gloves, be certain to get non-latex.

PREPARING SAMPLES FOR SDS-PAGE

INSTRUCTOR'S NOTES

Nutshell version of sample preparation:

Part 1:

- Weigh out a 0.15 g piece of sample material. Alternatively, the sample size can be estimated by sight; you want a piece about the size of a Tic-Tac™.
- Place sample in tube containing 0.5 ml sample buffer. Flick the tube 15 times, then let the sample sit for 5 minutes at room temperature.
- Pour the sample solution into a new tube (leaving the muscle tissue in the old tube). Sample can be frozen at this point for storage.

Note: The sample prep procedure demonstrates differential solubilization. Incubation in sample buffer, with only a slight agitation of the sample, is enough to solubilize many of the muscle proteins, but it is not enough to solubilize the fats (lipids), which remain membrane-bound in the cells.

Materials needed for sample preparation (not included in the kit):

- **Samples:** Chose any samples that you want. (For ideas, see Real World Applications, beginning on page 43 in the appendix.) This experiment was developed using seafood, and some of the ones that we have used in workshops are tilapia, flounder, bay scallops, tuna, scrod, ocean perch, sea bass, catfish, salmon, rainbow trout, shark, cod, squid, swordfish, crabmeat, sea trout, shrimp, orange roughy, frog legs, grouper, red snapper, sturgeon, and Crab Delight (imitation crab meat). The meat samples that have been tried include cow (skeletal muscle, liver, kidney, tripe, tongue, and heart), pig, chicken, turkey, buffalo, rabbit, quail, and duck. (If you use meat samples, trim off any fat and do not use ground meats.) Additionally, some processed meat products may work in the experiment, such as hot dogs.

You need only 0.15 grams (g) of seafood or meat to make each sample, and each prepared sample is enough for at least 10 gel lanes.

- (Optional) **Balance(s)** of some sort for weighing samples: The sample weight does not have to be exact. The procedure calls for 0.15 g of sample material, or a piece that has the dimensions of 0.5 x 0.5 x 1.0 cm (about the size of a Tic-Tac™). Ideally you want all the samples to be about the same size, especially if you are planning to compare amounts of proteins from lane to lane.

Instructor's preparation for Part 1:

- Thaw samples, if frozen. Each sample will require 0.5 milliliter (ml) of sample buffer. The instructor can aliquot it if desired, or just let students share a common tube or tubes.
- Unpack other materials listed in the Experimental Procedure.

Notes for instructor on sample preparation Part 1:

- The easiest way I've found to get samples of the right size is to trim bits of tissue from the larger piece, letting them drop into the weigh boat on the balance. Try to avoid damaging the tissue; the less tissue damage, the cleaner (sharper bands) the gel. Note: If you are pressed for time, skip weighing the samples.
- The sample tubes need to be labeled so that the samples can be told apart. Marker pens have been included in the kit; label the tubes on the tops, or the writing may rub off during handling (even with "permanent" markers). Finally, tubes can be color-coded.
- The instructor will have to decide how many samples to prepare. Each gel will hold 9 samples (plus molecular weight standard). Perhaps have each lab team prepare 1 or 2 samples. (The procedure below is written for each group to prepare a single sample.) There is enough Laemmli sample buffer included in the kit to prepare 50 samples.

Instructor's preparation for Part 2:

- Aliquot the molecular weight standards. Each student group will load 20 μ l, so prepare 30 μ l aliquots. (Instructions on the use of the Fisher micropipettor can be found on page 28.)

Notes for instructor on sample preparation Part 2:

- At this point, the instructor and/or the students should have decided what samples will run on each gel.
- If more than one student group will be loading the same samples, then the samples need to be aliquoted.

PREPARING THE SAMPLES: Part 1

MATERIALS

- microfuge tubes
- samples
- marker pens
- balance
- scissors
- weigh boats
- forceps
- microfuge tube racks
- graduated transfer pipettes
- Laemmli sample buffer

EXPERIMENTAL PROCEDURE

1. Label a 1.5 milliliter (ml) microcentrifuge tube for the sample. Add 0.5 ml of Laemmli sample buffer to the tube using a 1-ml transfer pipette.
2. Either weigh out 0.15 grams of the sample or prepare a piece that has the dimensions of 0.5 x 0.5 x 1.0 cm (about the size of a Tic-Tac™). Avoid bones, skin, and fat, and try to avoid damaging the tissue.
3. Transfer the weighed sample to the labeled tube using forceps.
4. Close the microcentrifuge tube and flick the tube 15 times with your finger to agitate the tissue in the buffer, then let the tube sit for 5 minutes at room temperature.
5. Label a second microcentrifuge tube and transfer the buffer containing the extracted (or solubilized) proteins by pouring from one tube to the other. Do not transfer any of the tissue to the new tube.

Note: it is not necessary to transfer the entire volume to the new tube. Only a small volume is actually needed for gel loading.

6. Discard the original tube. **You may freeze the samples at this point.**

PREPARING THE SAMPLES: Part 2**Materials:**

- prepared samples
- molecular weight standards

Experimental procedure:

1. Fill in the table with the samples that you will run on the gel. You can use this table as a guide as you load your gel. Each gel has 10 lanes. The first lane will be used for the molecular weight standard. You may use up to all 9 of the other lanes for samples. If you do not use all the lanes, load your samples in adjacent lanes; do not skip lanes between samples.

Lane	Sample
1	Molecular weight marker
2	
3	
4	
5	
6	
7	
8	
9	
10	

RUNNING A SDS-PAGE GEL

Instructor's notes:

- The instructor must decide how much of the samples the students will load on the gel. The procedure calls for 10 μl per well. If you feel that your bands have been too light when they've been loaded with 10 μl , then have the students load 20 μl per well. Fixed volume pipettors of both sizes (10- μl and 20- μl) have been included with the kit. (That excludes the molecular weight standard, of which 20 μl should be loaded regardless of the volume of experimental samples loaded.)
- Directions for use of the fixed volume pipettors can be found on page 28. You may wish to have the students practice using them first, but they're pretty foolproof.
- The next stopping point will be after electrophoresis, when the gels are in the staining solution. However, some users have reported that if the gels are kept sealed between their plates after running, they can be stored overnight in the refrigerator without much diffusion of the bands. If you do store the gels after running overnight in the refrigerator, **do not** open the gel cassette first.

Instructor's preparation for running the gel:

- Dilute the running buffer (see directions on page 29, step 1).
- Set up the gel boxes (see directions on page 29).
- The power supply directions are on page 32–33.

MOST IMPORTANT NOTE: When I have talked to teachers after they have run the gels, the problem that I have heard most frequently is, "One of the gels just stopped running." **In almost every case**, the cause of a gel not running is that the buffer level in the upper buffer chamber has dropped below the level needed for a current to pass through the gel. When you are adding buffer to the upper buffer chamber (i.e., between the buffer dam and the gel plates), the level **must** be above the top of the short plate. You can (and *should*) fill this chamber nearly to the very brim. The photo on page 31 illustrates the correct buffer fill level.

Check the buffer level again right before you start running the gel. If you have to add more buffer at this time, just add it carefully, pouring away from the wells so you don't wash the samples out of the wells. **Check the buffer level again** after the gel has been running for 10 minutes or so. If you have a slow leak, you may note that the buffer level has dropped. (Don't be concerned about the bubbles on the top of the buffer; that's normal.) If it looks like the buffer level has dropped, you may add more buffer to the upper chamber at any time. Just turn off the power, unplug the box and take the lid off, and top off the buffer. Replace the lid and start the run again. (I would normally check the buffer level at least a couple of times during the run.)

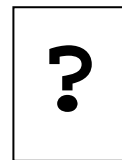
RUNNING AN SDS-PAGE GEL

Materials:

- molecular weight standards
- samples
- prepared gel boxes
- fixed volume pipettors
- yellow pipette tips
- table of sample loading order
- power supply (will run up to 4 gel boxes)

Experimental procedure:

1. Place 20 μl of the molecular weight standards in the first well.
2. Load 10 μl of each sample to the following wells. Follow the loading order that you wrote down on the table.
3. After all the samples are loaded, remove the sample loading guide and place the lid on the gel box, matching red to red and black to black.
4. Plug the leads into the power supply (again, matching red to red and black to black) and start the power supply. Run the gel at 200 volts (unless instructed otherwise by your teacher).
5. Run the gel for 30 minutes or until the dye front reaches the bottom of the gel. Note: The closer to the bottom of the gel the dye front reaches, the better the results will be. Time allowing, run the gel until the dye front reaches the bottom of the gel. It does not hurt if the dye front runs off the bottom of the gel.
6. When the run is finished, turn off the power supply and disconnect the leads from the power supply.



STAINING, DESTAINING, AND DRYING THE GEL

Instructor's notes for staining the gel:

- Removing the stacking gel is optional. When the gel is dried, it is easier to get all the air bubbles out of the gel/cellophane sandwich if the stacking gel has been removed, but it is not necessary to remove it for staining.

Instructor's notes for drying the gels:

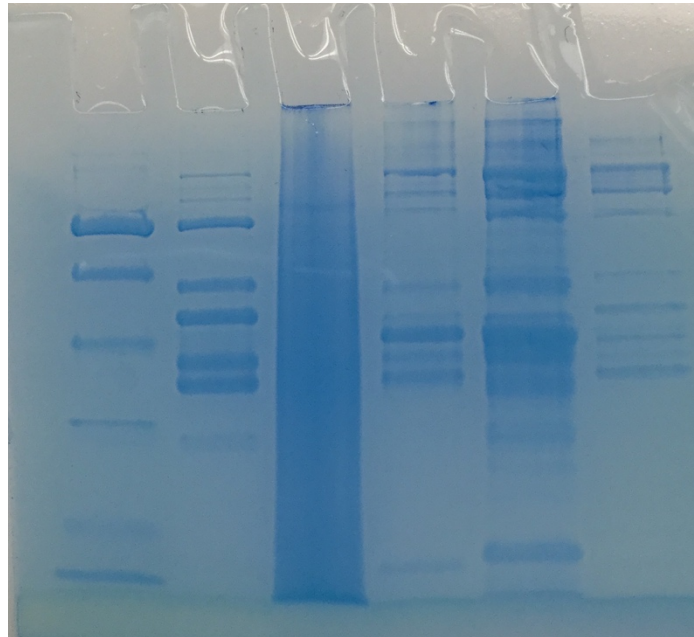
- Five gels can be dried concurrently with the kit materials.
- Before drying, the gel will be impregnated with a drying solution containing glycerol. This will help prevent the gel from cracking as it dries.
- To dry the gel, it will be placed between 2 sheets of cellophane, then allowed to sit in an undisturbed place for 12–36 hours. The water will evaporate, leaving a completely dehydrated gel.
- It is a good idea to pre-wet your gloves before handling a gel, as dry gloves can catch on the gel surface and tear it.
- To prepare the gel for drying, a rack is used to orient the frame, cellophane sheets, and gel. After the frame is clamped shut, it can be lifted from the rack. The frame should be vertical during gel drying.
- The most important part of preparing the gels for drying is to be certain to get rid of **all** bubbles between the gel and the cellophane sheets. If this is not done, the gel will crack during drying.

COMPARING COOMASSIE AND BIO-SAFE COOMASSIE STAINING

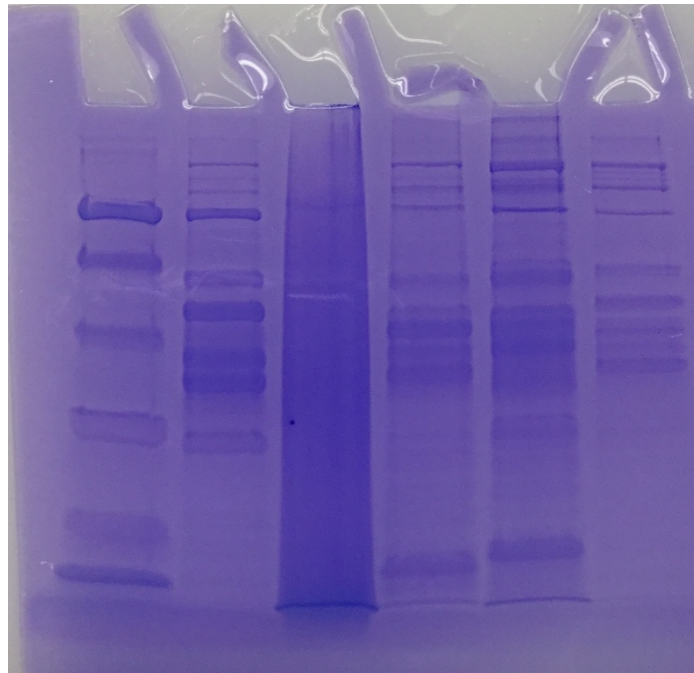
The change to Bio-Safe Coomassie is due to increased safety concerns with Coomassie staining and destaining solutions. Bio-Safe Coomassie does not contain acetic acid or methanol, and the only destain step is in distilled water. The sensitivity of the two stains is similar.

Here is a comparison of the two stains.

Bio-Safe Coomassie stain



Coomassie blue stain



STAINING THE GEL

Materials:

- gel cassette opening tool
- plastic boxes (1/gel)
- distilled water
- Bio-Safe Coomassie stain
- 50-ml graduated cylinder
- label tape

Experimental procedure:

1. Turn off the power supply and disconnect the leads. Remove the lid and lift out the electrode assembly and clamping frame.
2. Dump out the running buffer from the electrode assembly. (Running buffer should not be reused.) Open the cams on the electrode assembly and remove the gel cassette.
3. Lay the gel cassette flat on the bench with the short plate facing up. Pry the plates apart carefully using the gel cassette opening tool. (There are arrows on the cassette indicating where to place the tool.) The gel will adhere to one of the plates. When you see which plate has the gel on it, keep that plate flat with the gel on the top and lift the other plate off the gel.
4. (Optional) Carefully trim off the stacking gel using a razor blade. (Press down with razor blade; do not slice across the gel or it will tear.)
5. Transfer the gel to a plastic box containing 200 ml of distilled water (tap water may work fine here) and place the lid on the box. (An easy way to transfer the gel is to invert the plate with your gel attached into the water. The gel should float off the plate.)
6. Gently agitate the box for 5 minutes. Hold the gel as you pour off the first wash water. Repeat 2 times for a total of 3 washes. The washes are necessary to remove the SDS from the gel. If you do not do this, the staining will not work. Also, this is not a stopping point—if you leave the gel in water before staining, the proteins are not fixed in the gel and will diffuse away.
7. Pour off the third wash and add 50 ml of Bio-Safe Coomassie stain. Put the lid on the plastic box, label the box with your name using label tape, and let the gel sit in stain for 1 hour, rocking the box several times during the staining. (You can stain for a longer time—even overnight—without increasing the background.)
8. While holding the gel in place in the box with your fingers, pour off the stain and rinse the gel in 200 ml distilled water for at least 30 minutes. (Changing the rinse water and longer rinse times will further reduce background.)
9. Can you see differences in the protein bands when you compare different samples?

DRYING THE GEL

Materials:

- white rack
- drying frames (blue plastic)
- plastic clamps (clear plastic)
- drying solution
- distilled water, if available
- plastic box
- 50-ml graduated cylinder
- cellophane sheets (2/gel)

Experimental procedure:

1. While holding the gel in place in the box with your fingers, pour off the water and add 40 ml of gel drying solution to the box. Incubate for 15–20 minutes, rocking the box every few minutes.
2. Place one section of drying frame (blue plastic) on the white rack. Place it with the corner pins facing up.
3. Place 2 sheets of cellophane into a plastic box containing drying solution for 15-20 seconds. This step pre-wets the cellophane. Do not leave the cellophane in the drying solution for over 2 minutes!
4. Place 1 sheet of cellophane centered on the white rack. It will be slightly smaller than the blue frame.
5. Carefully place the gel on top of the cellophane, centering it in the frame. If there are bubbles between the gel and the cellophane, gently push them out with a gloved finger.
6. Place the second sheet of cellophane on top of the gel, trying not to trap any bubbles in the sandwich. If there are any bubbles, gently push them out with a gloved finger. **Bubbles will cause cracks in the gel during drying!** (It helps to pipette some extra drying solution onto the surface of the gel before trying to place the second sheet of cellophane.)
7. Place the second half of the blue frame on the sandwich, with the corner pins facing down. The frame should fit together. Slide the four clamps on the frame, one on each side. Stand the drying frame up on the feet and place in a draft-free area. Let dry for 12-36 hours. Do not leave longer than 48 hours.
8. When the gel is completely dried, it will appear flat. Remove the clamps and take the gel from the frame. There may be a small amount of moisture in the cellophane where it was under the frame. Just trim the edges of the cellophane away with scissors. Place the dried gel under a couple of books for 1-2 days, and it should remain flat. **Note:** Please keep track of the clamps- they are very easy to lose!

DATA ANALYSIS

Data analysis will of course depend on the specific question that your students have asked in their experiment, but some general information follows.

Is the protein there? One of the easiest questions that can be answered from an SDS-PAGE gel is presence or absence of a protein. Compare the protein complements of different samples. Are there bands in some samples that are not in others? If so, is there a detectable pattern? For example, is there a protein band found in shellfish that is not present in bony fish?

Is there more protein? By comparing the size of bands (i.e., thick vs. narrow bands), you can assess whether there is more of a particular protein in one sample than there is in another sample. (It is very hard to load the exact same amount of sample in each well, so the results are not truly quantitative, only suggestive.)

How big are the predominant proteins? By graphing the migration of the protein standards on semilog graph paper, you can generate a line that can be used to determine the molecular weights of the unknown proteins in your samples (just like you find the size of DNA fragments in an agarose gel). See Practice Gel Analysis on page 35 for an example.

Computer analysis! You can scan your gel and analyze your results using gel analysis software (available free on the web; see page 46).

Plan future experiments. Did your results produce more questions than answers? Have your students suggest other experiments that might provide additional information.

Note: At the end of each scenario (see Real World Applications, page 43) there are specific analysis questions.

CLEAN-UP

1. Rinse the gel box, electrode assembly, clamping frame, and buffer dam with water.

Note: Do not dry the electrode assembly! The platinum wires might be damaged.

2. Wash the plastic staining boxes with soap and water. If they are stained blue, a squirt of glass cleaner or alcohol will usually clean them up.
3. Rinse the drying rack, drying frames, and clamps with water. (The drying solution gets very sticky as it dries, so please make sure everything gets well rinsed.)
4. Wash scissors and forceps. Please do not return anything that has touched raw seafood or meat without cleaning it thoroughly.

APPENDICES

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USE OF THE ADJUSTABLE VOLUME MICROPIPETTOR

The adjustable-brand pipettor measures from 5–40 microliters (or a very similar volume range); it is intended for the instructor's use only. The volume is adjusted by turning the plunger knob. You can feel/hear clicking as the knob is turned. Turn the knob clockwise to decrease the volume setting and counterclockwise to increase the volume setting. The volume setting is displayed digitally on the handle of the pipettor.

NOTE: The biggest problem we've had with the pipettors is their being turned out of their range! *Never* force the adjustment knob and never turn the knob beyond the range of the micropipettor! If the knob does not turn easily, assume that you are making a mistake—check the size of the pipettor and the range!

- 1) Set the pipettor to the desired volume as described above and place a tip on the pipettor.
- 2) The plungers on adjustable-volume pipettors have 2 stops. The first is used to draw liquid into the tip and the first and second stops are used to dispense liquids. To draw liquid into the pipette tip:
 - a) Press the plunger down to the first stop and place the pipette tip in the liquid to be pipetted.
 - b) Slowly release the plunger, drawing liquid into the tip. Do not allow the plunger to pop up, which can draw liquid into the pipettor itself. **NOTE: Be sure that you are only depressing the pipettor plunger to the FIRST stop.** If you are going to the second stop, you are pipetting far more volume than you intend to pipette.
- 3) Withdraw the tip from the sample solution and dispense the sample from the pipettor. To dispense into an empty vessel, touch the end of the pipette tip on the side wall of the vessel and depress the plunger to the first stop. Wait ~1 second and then continue to depress the plunger to the second stop. If you are pipetting a sample into another solution, place the pipette tip into the solution and then dispense.
- 4) Without removing pressure from the plunger, withdraw the tip from the tube or solution, and then allow the plunger to return to the up position. If you release the pressure on the plunger while the tip is in the solution, you will pull solution into the tip again.
- 5) Eject the used tip by pressing down on the tip ejector located on the side of the pipettor handle

STEPS FOR USING FIXED VOLUME PIPETTORS:

1. Place a yellow tip on the pipettor by twisting the tip on while applying pressure. Be sure the tip is on the pipettor tightly.
2. To draw liquid into the pipette tip, press the plunger down until it stops and place the pipette tip in the liquid to be pipetted. Release the plunger, drawing liquid into the tip.
3. Withdraw the tip from the sample solution and dispense the sample from the pipettor. To dispense into an empty vessel, touch the end of the pipette tip on the side wall of the vessel and depress the plunger down until it stops. If you are pipetting a sample into another solution, place the pipette tip into the solution and then dispense.
4. Without removing pressure from the plunger, withdraw the tip from the tube or solution, and then allow the plunger to return to the up position. If you release the pressure on the plunger while the tip is in the solution, you will pull solution into the tip again.

SETTING UP A SDS-PAGE GEL FOR ELECTROPHORESIS

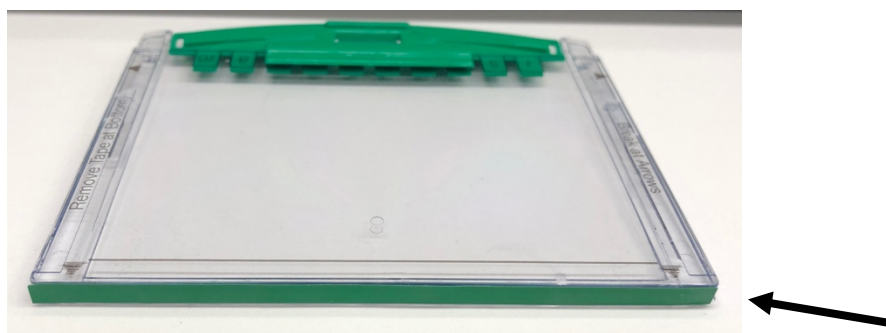
Note: As mentioned above (page 4), this step should be performed by the instructor and perhaps several carefully selected students.

Materials:

- gel box
- pre-cast 12% gel
- buffer dam
- gel loading guide
- gel running buffer

Procedure:

1. Dilute the 10X gel running buffer stock. Approximately 350-400 ml will be needed for each gel. For each liter of buffer, add 100 ml of 10X running buffer to 900 ml of distilled water and mix.
2. Open and prepare the pre-cast gel(s):
 - a. Open the plastic bag and remove the gel.
 - b. Remove the green tape strip from the bottom of the gel.



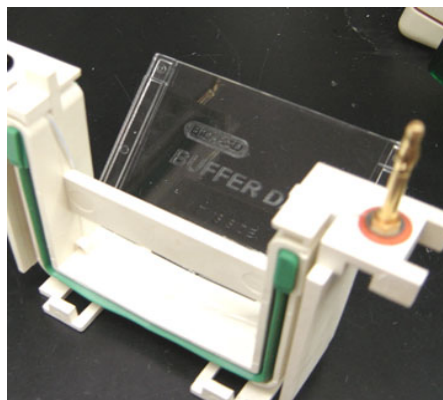
- c. Remove the comb and rinse the wells using 1X gel running buffer and a transfer pipette.

3. Assemble the gel box:

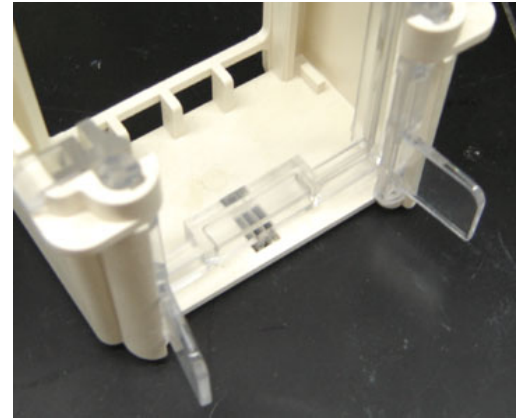
- a. If you are running only a single gel per box (**recommended**), place the buffer dam on the back side of the electrode assembly. Place the side of the buffer dam that says "BUFFER DAM" toward the electrode assembly.

Note: These directions are for running a single gel per gel box. If 2 gels are to be run on a single box, use a second gel instead of the buffer dam.)

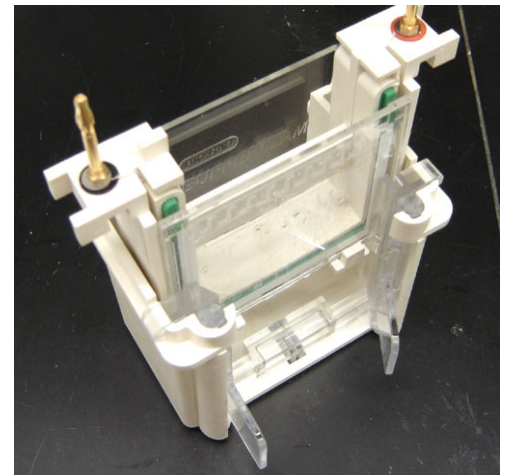
- b. Place a gel cassette on the other side of the assembly, with the **short** plate facing the electrode assembly.



- c. Open the cams on the front of the clamping frame.



- d. Hold the gel and buffer dam against the assembly and place the electrode assembly in the clamping frame.

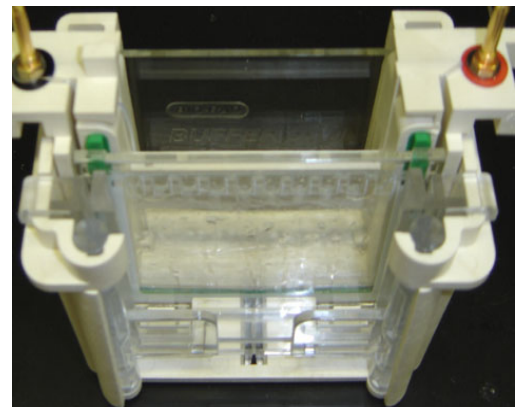


- e. While pressing down on the top of the electrode assembly, close the cams.

- f. Pour a little running buffer (an inch or so) in the upper buffer chamber (between the gel and the buffer dam) to see if the assembly is leaking.

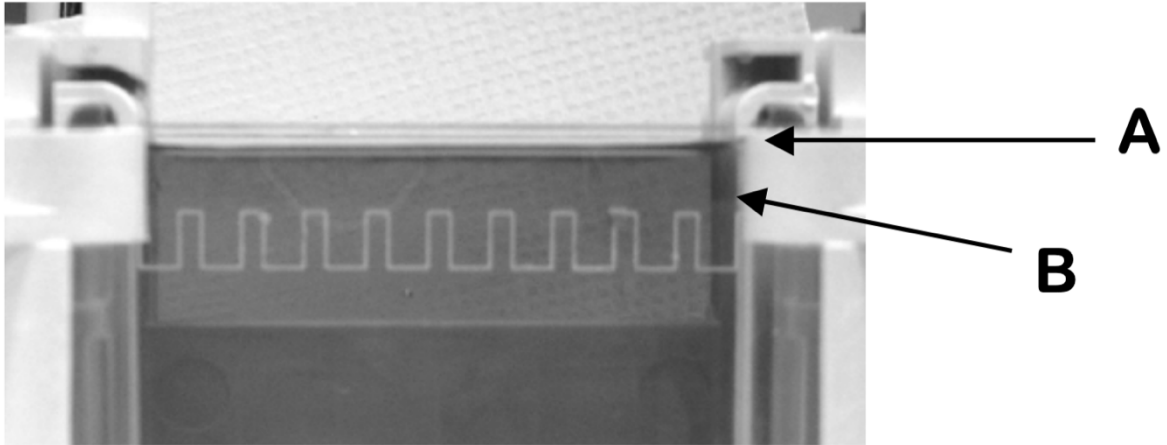
If it is leaking, start the assembly over. Make sure that the buffer dam and gel cassette are facing the right direction, i.e., facing the gasket. Make sure that you are pressing down on the electrode assembly while you close the cams.

- g. If there are no leaks, transfer the electrode assembly and clamping frame into the gel box tank.

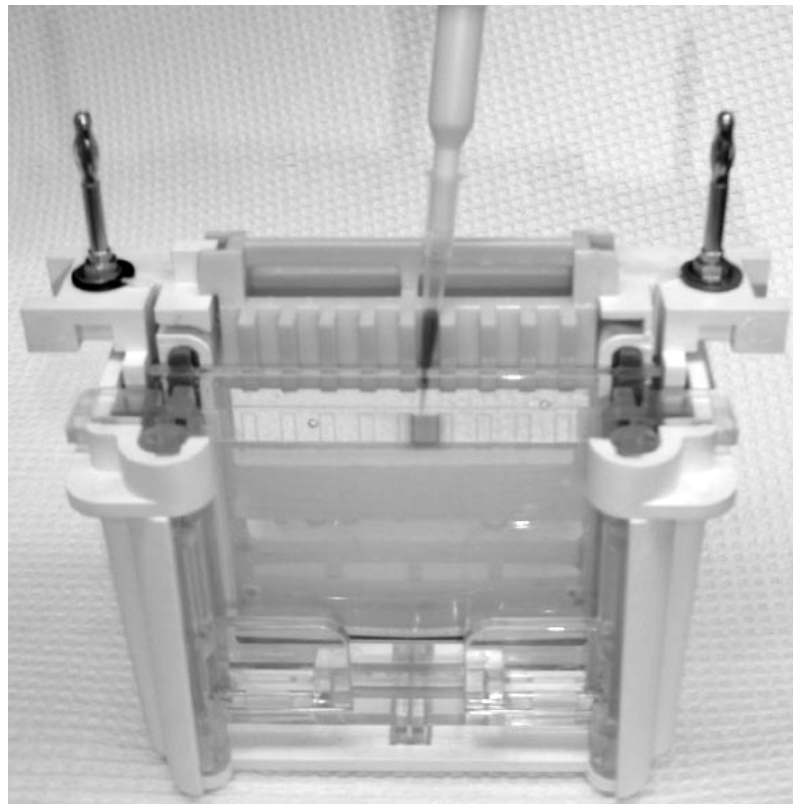


Appendix: Setting up the gel box

- h. Fill the upper buffer chamber with ~125 ml of gel running buffer; the buffer level **(A)** should be **well above the level of the short plate (B)**. (In the photo below, dye has been added to the buffer to make it visible.)

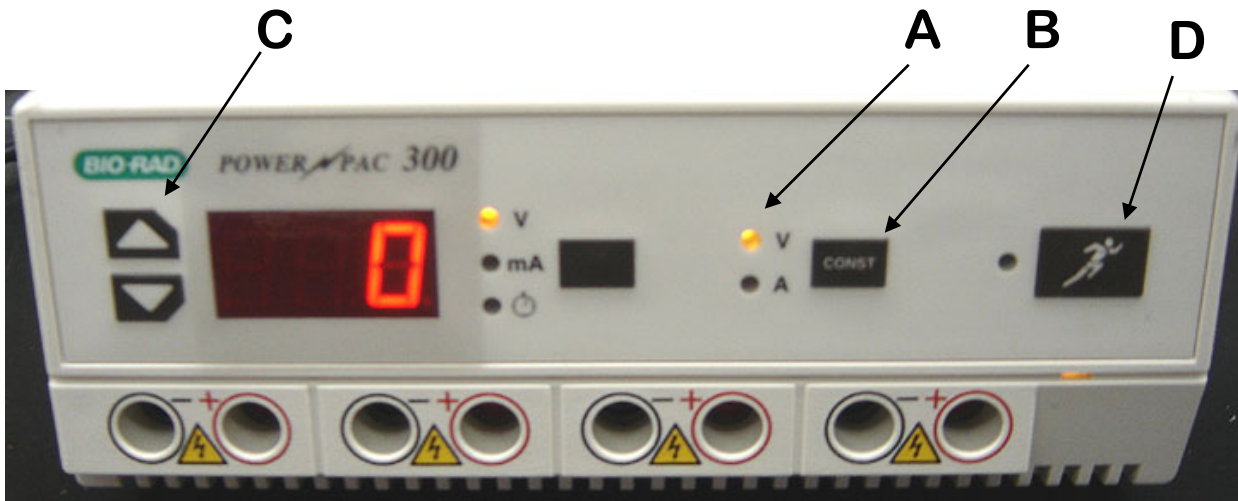


- i. Pour 200 ml of gel running buffer into the lower buffer chamber (the tank).
- j. Place the sample loading guide on the top of the electrode assembly. The guide will direct the pipette tip to the correct position for loading the sample in the well.



POWER SUPPLY DIRECTIONS

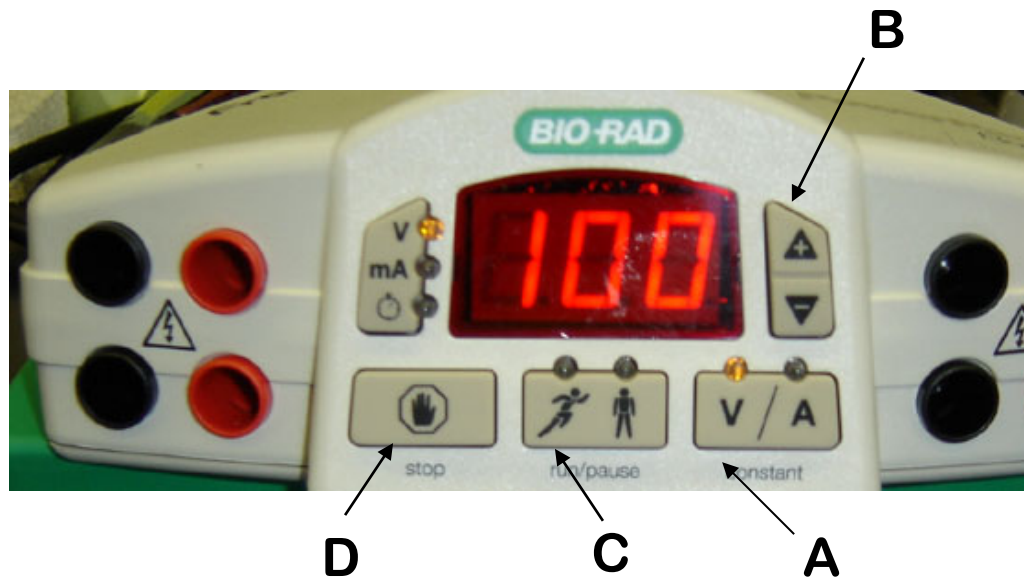
Bio-Rad PowerPac 300 Power Supply



To use:

1. Plug in gel box leads, matching red to red and black to black.
2. Turn power on using the switch on the right side of the unit. Note: If the display flashes an error code such as E-7, turn the power off and back on, and the error message should clear.
3. The V light (A) should be on (the unit defaults to constant voltage). If it is not, push the Const button (B) until the V light comes on.
4. Enter the desired voltage by scrolling up with the arrow keys (C) on the left of the digital readout.
5. Start the power by pushing the Run (running man) button (D).
6. When the run is finished, push the Run button (D) again to stop the run. Stop button (D). The display should then read OFF.

Bio-Rad PowerPac Basic Power Supply



To use:

1. Plug in gel box leads, matching red to red and black to black.
2. Turn power on using the switch on the right side of the unit. Note: If the display flashes an error code such as E-7, turn the power off and back on, and the error message should clear.
3. The V light (A) should be on (the unit defaults to constant voltage). If it is not, push the Constant button until the V light comes on.
4. Enter the desired voltage by scrolling up with the arrow keys (B) on the right of the digital readout.
5. Start the power by pushing the Run/Pause button (C).
6. When the run is finished, push the Stop button (D).

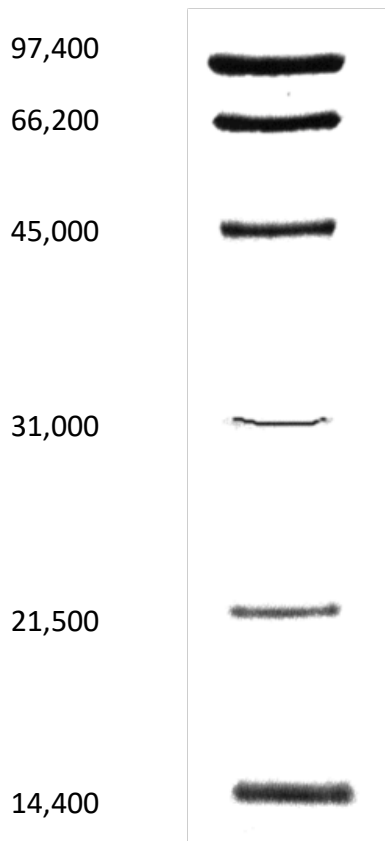
MOLECULAR WEIGHT STANDARDS

The molecular weight standards provided with the kit were purchased from Bio-Rad, and are low range standards. They were chosen for two reasons, the range of proteins in the standards and the fact that they are stable at room temperature for shipment. However, they should be stored in the freezer after you receive the kit.

The proteins in the standard and their sizes are:

protein	size (kDa)
phosphorylase B	97.4
albumin, bovine	66.2
ovalbumin, hen egg white	45
carbonic anhydrase	31
trypsin inhibitor, soybean	21.5
lysozyme	14.4

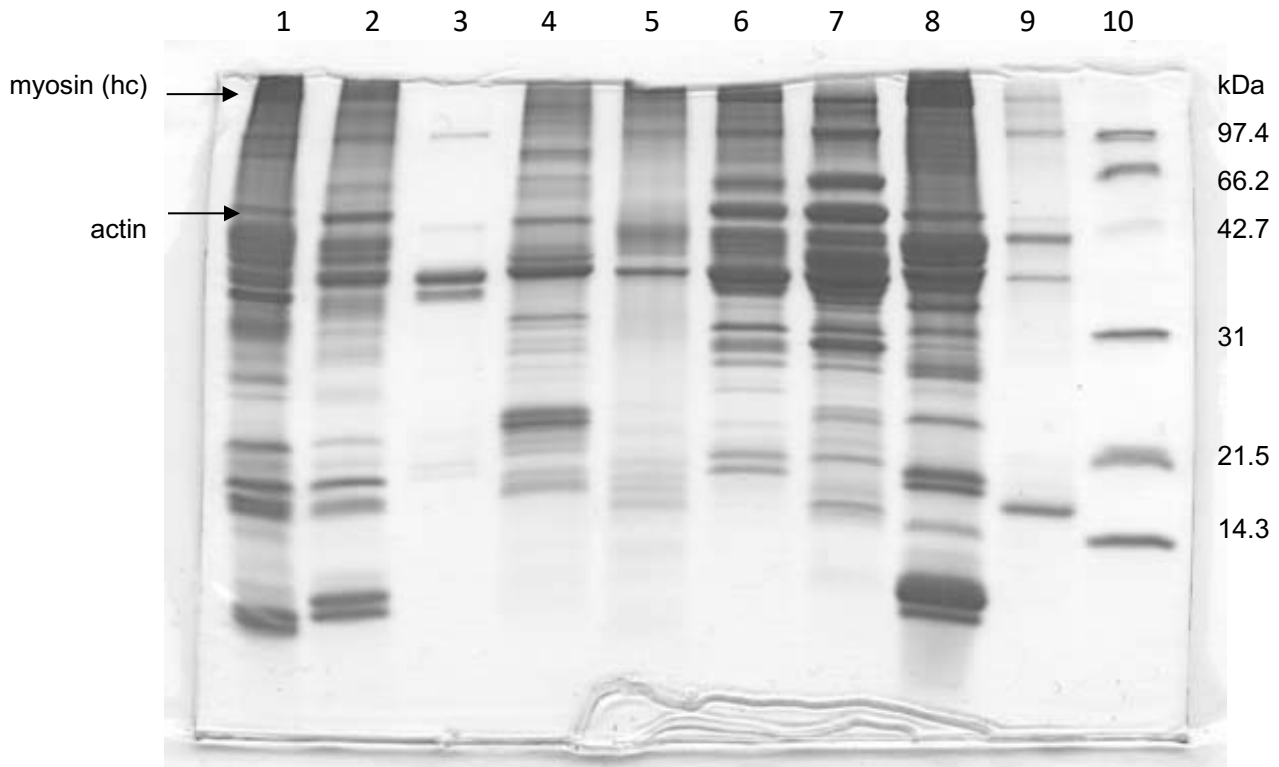
Here is a scan of the markers as they run on a 12% acrylamide gel. The sizes are in daltons.



PRACTICE GEL ANALYSIS

Analysis of an SDS-PAGE gel is very similar to analyzing the results of agarose gel electrophoresis of DNA. The migration of the standards is measured, then graphed against the known molecular weights of the standards.

Here is a sample gel, with the approximate locations of actin and myosin heavy chain marked with arrows:



Lane 1: scallop
2: catfish
3: tuna
4: swordfish

5: shark
6: shrimp
7: Lobster Delite
8: orange roughy

9: flounder
10: molecular weight standard

To calculate the migration of the markers, draw a line across the top of the gel to use as a baseline. Then measure the migration from that point. It does not matter to which part of the band you measure (e.g., center, leading edge, etc.), as long as you are consistent for all of your measurements. Enter the measurements in the following table.

standard protein	size (kDa)	migration (mm)
phosphorylase B	97.4	
albumin, bovine	66.2	
ovalbumin	42.7	
carbonic anhydrase	31	
trypsin inhibitor, soybean	21.5	
lysozyme	14.3	

Graph migration (in mm) on the x-axis and size (in kDa) on the y-axis, using single cycle semi-log graph paper. Fit a line to the points. You will notice that the biggest marker (97 kDa) is off the line. This is because the gel is not linear in the upper region (like an agarose gel of DNA fragments). If you wanted the gel to be linear in that size range, you would reduce the percentage of acrylamide.

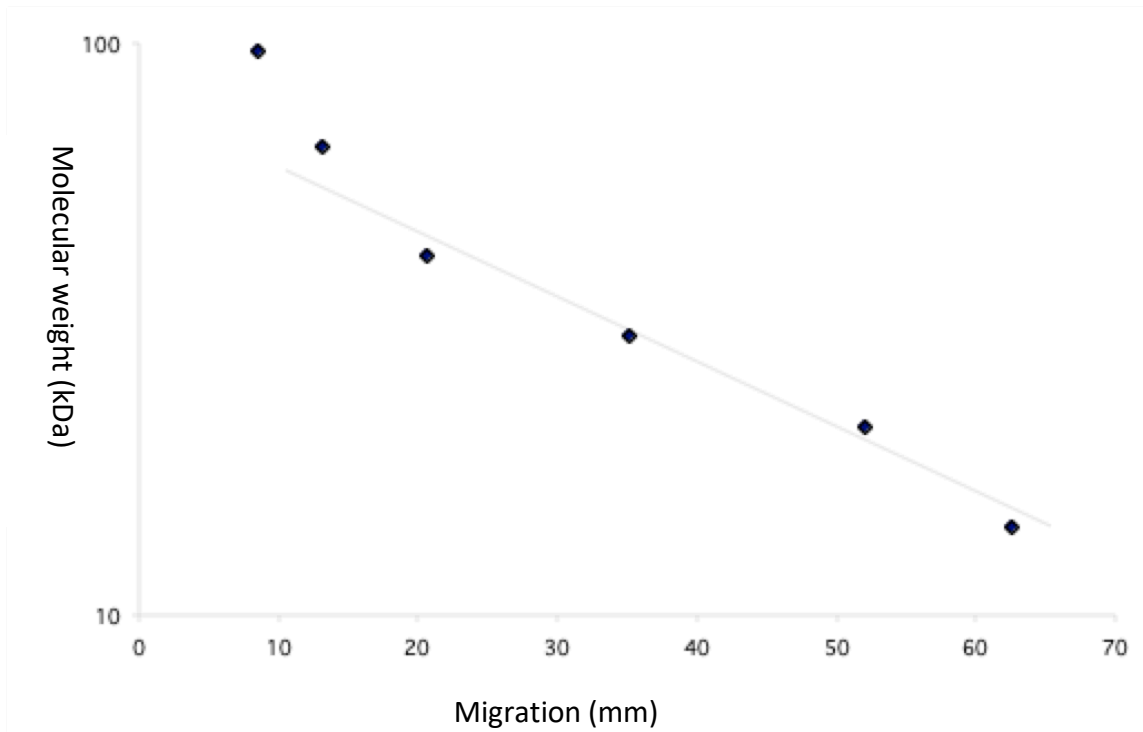
To calculate the size of an unknown protein, measure the migration of the band from the baseline. Place a ruler vertically at that point on the x-axis and find the intersection of the ruler with the line that you generated. Then determine the y-axis coordinate for that point on the line. That will be the molecular weight of the unknown protein.

If you want to compare your measurements and graph to mine, see the table and graph below.

SAMPLE DATA FROM GEL

Here are the measurements that I got from the gel photo on page 35. The graph is computer-generated, but yours should look something like this.

standard protein	size (kDa)	migration (mm)
phosphorylase B	97.4	8.5
albumin, bovine	66.2	13
ovalbumin	42.7	20.5
carbonic anhydrase	31	35
trypsin inhibitor, soybean	21.5	52
lysozyme	14.3	62.5



CONCEPTUAL REVIEW MATERIALS

Two different icons have been placed throughout the manual to note points at which students can be asked Thinking Questions (signified by a question mark) and Real-World Applications (signified by a globe).

Thinking Questions

These questions are designed to prompt deeper thinking about the concepts covered.



1. What is a protein fingerprint?

A protein fingerprint is the pattern of proteins made by a specific type of cell or tissue, it's like a barcode for identifying that cell or tissue.

2. Which is larger, a strand of DNA or a single protein?

Obviously, it depends on the strand of DNA and the protein in question. Yet, a DNA strand typically contains more than one gene, and, therefore, codes for multiple proteins. Thus, DNA is generally larger than most proteins. The fact that proteins are very small molecules means that acrylamide rather than agarose must be used as the gel substrate for electrophoresis. Alternatively, very high percentages of agarose (2.5–4%) can be used but the gels are difficult to manipulate.

3. Does each type of cell make the same type of proteins? Why or why not?

Each type of cell does not make the same type of proteins because each cell has a different job to do. Each cell only makes the proteins it needs.

4. Why do the proteins flow downward through the gel during electrophoresis?

The proteins are negatively charged, so they will move toward the positive electrode (at the bottom). The proteins in this experiment are negatively charged because SDS, which is negatively charged, is in the gel buffer and sticks to proteins, making them negative as well.

5. Why do bubbles form on the electrodes in the electrophoresis set-up? What are the bubbles?

Electrophoresis involves an oxidation-reduction (redox) reaction - H^+ from the water is being reduced to form hydrogen gas (H_2), and O^{2-} from the water is being oxidized to form oxygen gas (O_2). Because H^+ is positively charged, it will migrate to and be reduced at the negative electrode (black). The O^{2-} will migrate and be oxidized at the positive electrode (red). The electrodes are more easily observable in a horizontal gel electrophoresis apparatus than a vertical set-up.

PROTEIN ELECTROPHORESIS QUESTIONS

courtesy of Adrienne Warren

1. What is electrophoresis?
2. What are some uses for electrophoresis?
3. What is a cathode? ...an anode?
4. How is the amount of sieving done by a gel controlled?
5. What would determine whether agarose or acrylamide matrix should be used?
6. What are the major functions of acrylamide gel electrophoresis?
7. What is charge density?
8. How does charge density relate to electrophoresis?
9. What step is taken to do molecular weight determinations using PAGE?
10. What does SDS-PAGE stand for?
11. What is the function of SDS?
12. What is native PAGE?
13. When is native PAGE used?
14. What is done to get the proteins to enter the gel at the same time?
15. What is a stacking gel? ...a separating gel?
16. Why should precautions be taken when making up a polyacrylamide gel?
17. When is acrylamide safe to handle?
18. Why does this lab use a separating gel of 15% acrylamide?
19. What are the differences in the way an agarose and an acrylamide gel are run?
20. What function does heating serve in preparing a protein for electrophoresis?
21. What stain is commonly used for proteins?
22. How is a protein gel destained?
23. What are molecular weight standards and what function do they serve?
24. What does the size of the band indicate?
25. What two proteins are likely to be the most abundant in muscle samples?
26. What is the primary method for identifying proteins?
27. Explain the three steps involved in Western blotting.

PROTEIN ELECTROPHORESIS ANSWERS

1. Electrophoresis is the migration of charged molecules in an electric field toward the electrode of opposite charge.
2. Uses of electrophoresis include determining the purity of a protein sample, the amount of DNA in a sample, the size of a protein, and the nucleotide sequence of a DNA sample.
3. cathode – negative electrode
anode – positive electrode
4. Sieving is controlled by changing the percentage (%) of agarose or acrylamide in a gel.
5. The size of the molecules being separated determines the matrix to be used (e.g., >200 kDa use agarose).
6. The major functions of acrylamide gel electrophoresis are separation of most proteins and electrophoresis of small DNA molecules.
7. Charge density is the ratio of charge to mass.
8. Charge density relates to electrophoresis because proteins can be separated according to their charge density since both their charge and mass will affect their migration. Often, however, charge is removed as a factor and only mass will determine rate of migration.
9. In order to determine molecular weights using PAGE, charge must be removed as a factor. SDS is put in the gel buffer and in the sample buffer to give the proteins an overall negative charge. SDS also gives all proteins identical charge to mass ratios, thus removing charge as a factor in migration.
10. SDS-PAGE stands for sodium dodecyl sulfate polyacrylamide gel electrophoresis.
11. The function of SDS is to remove charge as a factor, so the molecular weight of a protein can be determined.
12. Native PAGE refers to running a protein in its natural state without breaking bonds or removing charge as a factor.
13. Native PAGE is used when the structure and activity of a protein need to be preserved.
14. To get proteins to enter the gel at the same time, a stacking gel is poured on top of the separating gel.
15. A stacking gel is a gel that is poured on top of the separating gel and is of a much lower acrylamide concentration and a lower pH than the separating gel. Its function is to allow the

proteins to enter the separating gel at the same time. The separating gel is used to separate the proteins according to molecular weight.

16. Acrylamide is a neurotoxin in its unpolymerized state (before the gel solidifies).
17. Acrylamide is safe to handle when it is polymerized (in gel form).
18. A 15% acrylamide gel provides good separation for proteins in the range of 14 to 100 kDa.
19. An agarose gel is run in a horizontal chamber and the gel is completely surrounded by buffer. An acrylamide gel is run in a vertical chamber and has two separate buffer chambers. One chamber allows buffer to be in contact with the upper portion of the gel and the other chamber with the lower portion of the gel.
20. Heating in the presence of SDS denatures the proteins, allowing them to become linear for running in the gel.
21. Coomassie blue is the stain that is commonly used for proteins. In this exercise, you are using a safe form of Coomassie called Bio-Safe Coomassie stain.
22. A protein gel stained with Bio-Safe Coomassie stain is destained by soaking the gel in distilled water.
23. Molecular weight standards are a mixture of proteins with known molecular weights. They are used to plot a standard curve for the gel so that the molecular weights of the other unknown proteins in the gel may be determined.
24. The size of the protein band indicates the amount of the protein present in the sample. A dark wide band indicates a lot of protein, whereas a narrow light band indicates a smaller amount of protein.
25. Actin and myosin are the two muscle protein likely to be found in large quantities in muscle samples.
26. Western blotting is the primary method used in identifying proteins.
27. The three steps involved in Western blotting are: (1) transfer the proteins to a membrane; (2) soak the membrane in a solution containing antibodies specific for the protein of interest; and (3) soak the membrane in another solution containing an enzyme which will make the antibody visible, thereby making the location of the protein known.

NAME _____

DATE _____

Protein Electrophoresis Quiz

1. What does SDS-PAGE stand for?
2. Why is it important to wear gloves and eye protection when making gels for SDS-PAGE?
3. What is the purpose of a stacking gel?
4. What is the purpose of a separating gel?
5. When is it best to use agarose and when is it best to use acrylamide?
6. What are the differences in the way agarose and acrylamide gels are run?
7. What is the function of SDS?
8. When is native PAGE used?

9. Why are molecular weight standards run in a gel with unknown proteins?

10. What does the size of the band indicate?

11. What two muscle proteins are likely to be found in samples?

12. What is Western blotting used for?

Bonus: What are the three steps in Western blotting?

REAL-WORLD APPLICATIONS

Because students can easily access a variety of protein samples, it is straightforward to conduct protein fingerprinting experiments in a way that is quite similar to real-world applications. Below are several scenarios that outline real-world applications. The subsequent pages include additional information that may be helpful for each of the approaches.



SCENARIO 1. Comparing Proteins Among Different Organisms (Evolutionary Relationships)

Protein fingerprinting, or electrophoresis of protein samples, can be used to characterize the evolutionary relationships among organisms of different phyla. Students can collect data by conducting a protein electrophoresis experiment with muscle tissue from different organisms (fish, fowl, mammals, invertebrates, etc.).

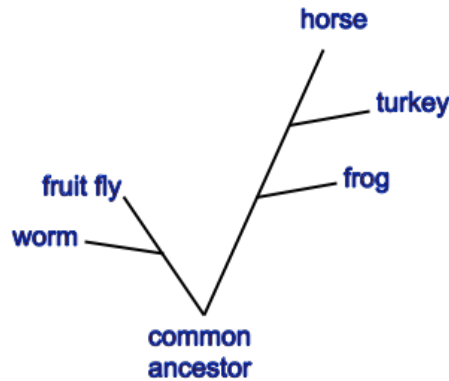
Mutations in an organism's DNA can change its characteristics, and these characteristics can help the organism to survive and reproduce. Sometimes, organisms can change so much over many generations that their offspring become a new species. For example, the ancestors of cats and dogs were directly related (i.e., brother and sister). As individual organisms, each with its own DNA, proteins, and characteristics, they went their separate ways and had their own offspring, and the offspring had their own offspring, and so on—one set of great-great-great grandchildren resembled cats and the other set resembled dogs. Even though their ancestors were related, they have evolved into two different species that can no longer reproduce with each other.

Although some of their characteristics became different, other cat and dog characteristics stayed the same as the characteristics of their ancestors. Therefore, some of their DNA and proteins will be very different, and some will be the same. Ask students to picture a cat and a dog. What characteristics do cats and dogs have in common? What characteristics are unique to cats or to dogs? Ask students to list as many as they can.

Students can create protein fingerprints of several species to determine how their ancestors were related. First, challenge students to think about the animals' characteristics and how the animals might be related. It may be helpful for students to display their thinking as a **cladogram**.

How to design a cladogram: Here is an example cladogram of five organisms: fruit flies, worms, horses, turkeys, and frogs. These organisms have some characteristics in common (they all are made of many cells, they all have mouths, etc.), and some characteristics that are different (some have hair, some have feathers, some have eyes and some don't, etc.). The simpler the organism, the closer it is placed to the common ancestor in the diagram (like the worm). The more complex the organism, the farther it is away from the common ancestor (like the horse). Finally, the more closely related two animals are, the closer they are on the cladogram (fruit flies are more closely related to worms than to frogs, turkeys, or horses).

Sample cladogram:



Challenge students to design a cladogram of the organisms whose protein fingerprints they will examine.

Possible samples

- All fish
- All vertebrates
- All animals
- Samples from living organisms (plants, fungi, invertebrate animals, vertebrate animals)

Analysis

The following questions may help students analyze their data. Answers are not provided because they will depend on the samples used. As long as students examine their data carefully and can justify their answers based on their data, that's science! Data are always right and there isn't necessarily a 'right answer.' Students can answer these questions individually or as a class.

1. Which organisms' protein fingerprints look the most similar? Why?
2. Based on the fingerprint similarities, which organisms do you think are most closely related?
3. Which organisms' protein fingerprints look the most different? Why?
4. Based on the fingerprint differences, which organisms do you think are least related?
5. Each band (line) in the fingerprint represents a single protein. Are there any bands in the fingerprints that look the same for all or almost all of the animals? Why might some proteins be the same in many different animals?
6. Are there any bands observable in one or two organisms' fingerprints? Why might some proteins not be made by all of the organisms?
7. Keeping the observations from the protein fingerprints in mind, draw a new cladogram of the organisms.
8. Is this cladogram different from the initial tree? Why or why not?

Biological Classification

Until we could use DNA and proteins as evidence, scientists used observable characteristics (like hair, feathers, legs, etc.) to 'classify' relationships between organisms and their ancestors. Organisms classified in the same kingdom are more closely related than those in different kingdoms. Organisms in the same phylum are more closely related than those in different phyla (plural of phylum), and so on. Challenge students to conduct textbook or online research to determine the kingdoms, phyla, and classes of the organisms.

Organism	Kingdom	Phylum	Class

9. According to the biological classification, which organisms are most closely related? Why?
10. Which are least closely related? Why?
11. Does the protein fingerprinting evidence support or contradict the classifications? In other words, are the organisms more closely related according to the fingerprints (or genotype) also the organisms more closely related according to classification (or phenotype)? Why?
12. Which is a more reasonable way to examine relationships between different organisms, their genotypes (evidence = protein fingerprints), their phenotypes (evidence = observable characteristics), or both? Why?

SCENARIO 2. An Experiment About Trout

Jim Meadows, a teacher at Greenbrier West High School in Crawley, West Virginia, has developed a really nice experiment using trout found in his state. The five trout samples that he was able to find are:

- brown trout: in the Atlantic salmon family
- rainbow trout: in the Pacific salmon family
- golden trout: a rainbow trout selected for color (i.e., should be same as rainbow in the experiment)
- brook trout: a char
- tiger trout: a hybrid between brown and brook trout

After his students ran and stained their gels, they scanned them and analyzed the results using a gel analysis program that is part of NIH Image and Scion Image. The program compares the locations and densities of bands from lane to lane within a gel. (Actually, this sounds like a great idea for any protein gel, no matter what the samples!)

Update (2019): The only free software package I can find right now is [ImageJ](#).

I don't know if all these trout varieties are widely available. Some are available at the Virginia fish hatcheries, and Jim Meadows says that they are all available at West Virginia hatcheries. If someone finds sources in Virginia, please let me know so I can share the information.

If various trout species are available, it should be interesting to see the results, especially since the students shouldn't know ahead of time the relationships between the species. Jim reported that there were no statistical differences between rainbow and golden rainbow, and I think he said that the hybrid also got the expected results. See the following page for more information on trout.

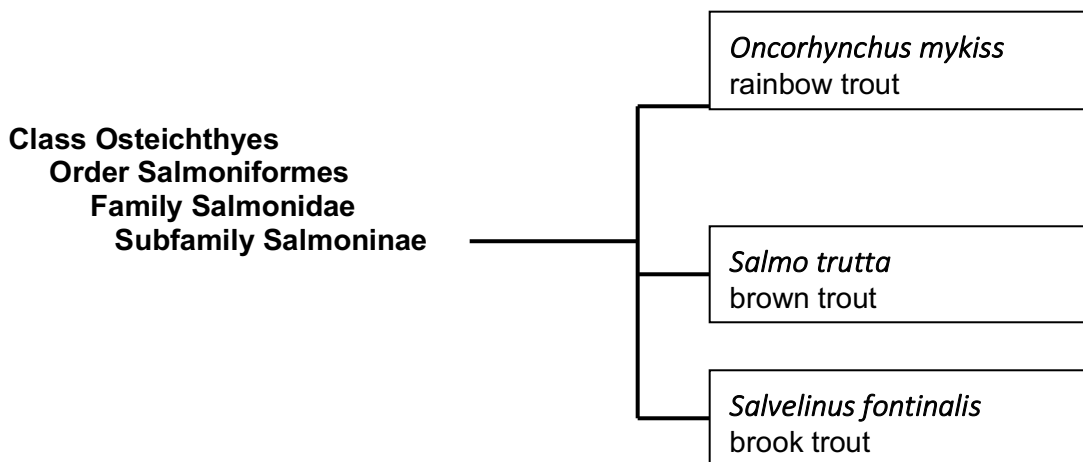
A PAGE ON TROUT

There is a surprising amount of information out there about trout. (The Virginia Tech library has 6-7 books just about trout!) Here are the trout species found in our general area:

- **Brook trout** (*Salvelinus fontinalis*) are actually chars. They are found in the northeastern US, and their range extends into western Virginia and North Carolina.
- **Brown trout** (*Salmo trutta*) are Atlantic trout (closely related to Atlantic salmon). They are native to Europe, but they have been widely introduced in North America.
- **Rainbow trout** (*Oncorhynchus mykiss*) are native to the west coast of the US, but have been introduced into every state. They are Pacific trout (closely related to Pacific salmon).
- **Golden rainbow trout** (*Oncorhynchus mykiss*) is a color variant of the rainbow trout. (The golden rainbow trout is not the same as the Golden Trout (*O. mykiss aguabonita* or *Oncorhynchus aguabonita*), a subspecies of rainbow trout found in California.)
- **Tiger trout** are sterile hybrids from a cross between a female brown trout (an Atlantic salmon) and a male brook trout (a char). They have been grown in hatcheries for release programs around the country.

I checked the State of Virginia webpage and there is a list of all hatcheries in the state, most of which raise trout: [Virginia fish hatcheries](http://www.dgif.virginia.gov/wildlife/fish/). Virginia raises only brook, brown, and rainbow trout (plus a wide variety of other freshwater fish). There is a description of the three trout species and their biology at <https://www.dgif.virginia.gov/wildlife/fish/>. The page includes information on all Virginia fish.

Here is a limited family tree for the species listed above. There are many more trout genera and species.



Other members of the Salmonidae family include salmon, char, whitefish and grayling.

SCENARIO 3. Comparing Proteins from the Same Organism (Gene Expression)

Every cell in an organism has the recipe for every single protein in that organism's body, but which proteins does each cell actually make? Using this approach, students can separate the proteins from different tissues (e.g., cow skeletal muscle, heart muscle, liver, etc.) of an organism using protein gel electrophoresis. This will create a 'protein fingerprint' of different types of cells or tissues.

Almost every cell in a cow's body contains the DNA to make every protein the cow needs. The cells that don't have a complete genome (entire DNA sequence for the cow) are red blood cells, which have no nuclei, and eggs and sperm, which are haploid and contain different DNA because of recombination during meiosis. Each type of cell or tissue makes only the proteins it needs to accomplish its functions. For example, muscle cells make an abundance of actin and myosin, the proteins responsible for muscle contraction. Liver cells make LDL receptor protein in order to take up cholesterol from the bloodstream. Students will be able to observe protein fingerprints for each type of cell or tissue to determine which proteins are expressed by all cell types and which are expressed by specific cells or tissues.

Suggested samples

- Pig or cow heart, stomach (tripe or smooth muscle), liver, kidney, skeletal muscle (stew meat works best, ground beef is ok but the fat smears the proteins bands a bit) – these can be acquired from local meat labs at agriculture schools or slaughterhouses
- Chicken heart, skeletal muscle (breast, leg, wing), liver
- If students or their families hunt, samples can be acquired from game.
- Different plant tissues can also be used, including leaves, stems, roots, flower components, etc.

NOTE: The raw meat samples should be handled carefully, as when preparing food to eat, to avoid any risk of contracting disease. Students should use aseptic techniques, including washing their hands after handling the samples prior to touching anything else.

Analysis

The following questions may help students analyze their data. Sample answers are provided, but most of the questions are open-ended, so students may have correct answers that aren't included. As long as students examine their data carefully and justify their answers based on their data, that's science! Data are always right and there isn't necessarily a 'right answer.' Students can answer these questions individually or as a class.

1. Compare the protein fingerprints from the different tissues. What can be determined about what proteins each type of cell makes?

The protein fingerprints for each type of tissue should look different. Each type of cell makes different types of proteins, although some proteins each cell makes might be the same because some cells have to do the same jobs (e.g. cellular respiration).

2. Which tissues have the most similar proteins? Which tissues have dissimilar proteins? Why do you think so?

The more similar the types of cells (e.g., skeletal muscle and cardiac muscle), the more similar their proteins will be. The more different the types of cells (e.g., skeletal muscle and liver), the more different the proteins in those cells will be.

3. Why would proteins from different types of cells look different? Why would they look the same?

Since each cell has specific functions to perform, the more similar the cells' functions are with other cells, the more similar their proteins will be.

SCENARIO 4. Identify the Mystery Meat!

Students can conduct an experiment to determine the components of a 'mystery meat,' for example, hot dogs or imitation crab. Students can choose comparison samples on the mystery meat ingredients list.

Suggested samples

- Mystery meat is 'krab.' Comparison samples are different types of fish and real crab (look at ingredients list for possibilities).
- Mystery meat is beef hot dog. Comparison samples are cow tissues.
- Mystery meat is hot dog (unknown origin). Comparison samples are muscle tissues from cow, pig, chicken, turkey (look at ingredients list for possibilities).

Analysis

The following questions may help students analyze their data. Only some answers are provided because the answers depend on the samples used. As long as students examine their data carefully and can justify their answers based on their data, that's science! Data are always right and there isn't necessarily a 'right answer.' Students can answer these questions individually or as a class.

1. Compare the protein fingerprints from the different samples. Which comparison samples most closely match the mystery meat? Which comparison samples are most different?
2. Can all of the proteins found in the mystery meat be found in the comparison samples? If not, what are possible explanations for why this is the case?

If extra proteins are observable in the mystery meat, it is possible that some of the proteins are from tissues not used for comparison. For example, smooth muscle may be used in hot dogs but may not have been used as a comparison sample.

Alternatively, the processing and cooking of the mystery meat may have destroyed some of its proteins. For example, imitation crab may most closely match catfish, but some of the catfish proteins aren't found in the mystery meat because they have been destroyed by cooking or other steps in preparation.

3. Which comparison samples are most likely used to make the mystery meat? Why?

SCENARIO 5. The Case Of The Crummy Crab!

Contributed by Steven Scheidell, Murray High School, Murray, Utah

Background

Last week, Jeff Johnson, staff reporter and food critic from the Murray High School Newspaper, was invited to eat at Chez Spartan, a new restaurant in town. The restaurant was featuring a Crab Louie appetizer. Mr. Johnson went to Chez Spartan and ordered the Crab Louie. He was quite surprised when he tasted the appetizer. It seemed to him that the crab was an imitation. He confronted the owner about the questionable crab. She became very angry. She said that they only used the finest ingredients and that Johnson was a fool. The owner asked Mr. Johnson to leave. Johnson told the owner that he would write an article that would close Chez Spartan. Mr. Johnson hid a sample of the crab as he left the restaurant.

Mr. Johnson remembered that the Murray High Biotech Lab was able to conduct sophisticated biochemical analysis of tissues. He took the sample to Dr. Rick Cox, the director of the laboratory. Johnson explained the problem and indicated that he thought the questionable crab was some sort of fish. Dr. Cox said that the lab could determine whether or not the sample was really crab by means of a muscle protein analysis. He also said that if the sample was imitation, they could determine what type of fish it really was.

Assignment

1. Isolate muscle proteins from the Chez Spartan sample, the real crab sample and four other types of fish according to the attached protocol.
2. Conduct SDS-PAGE analysis of muscle proteins from the samples.
3. Write a detailed lab report comparing 5 significant proteins for each sample (use proteins other than actin and myosin). Include a cover letter to Mr. Johnson that explains what the sample most likely was and why you came to that conclusion.

SCENARIO 6. Comparing Protein Expression During Development

How does an undifferentiated cell develop into a mature organism? For example, how does a seed become a plant with many differentiated tissues like leaves, stems, roots, and flowers? Students can examine the role of protein expression during development and differentiation by germinating plant seeds (soak seeds in water, place on wet paper towels under light source to allow to germinate) and examining changes in protein expression as the plant develops.

Suggested samples

- Alfalfa sprouts: seeds, one-day sprouts, two-day sprouts, seven-day sprouts
- Bean plants: seeds (may need to be hydrated for a few hours), one-day sprouts, two-day sprouts, seven-day sprouts, 14-day sprouts

To prepare samples, grind up the plant tissue in a mortar and pestle with dry ice. Combine 2 small spatula scoops of the ground material with the Laemmli buffer. Leave the material in the buffer overnight in the refrigerator for better protein extraction.

Analysis

The following questions may help students analyze their data. Sample answers are provided, but the questions are open-ended, so students may have correct answers that aren't included. As long as students examine their data carefully and justify their answers based on their data, that's science! Data are always right and there isn't necessarily a 'right answer.' Students can answer these questions individually or as a class.

1. Compare the protein fingerprints from the different plant stages. Are there similar proteins among all of the stages? If so, why might this be the case?

There are likely to be similar proteins in all of the samples because some proteins are needed for all stages of development, for example, proteins involved in cellular respiration.

2. Are any of the proteins unique to one or a few stages of development? If so, why might this be the case?

There are likely to be proteins expressed only in seeds, only in young plants, or only in mature plants because some proteins are only needed at certain stages of development.

3. The location of each protein band indicates the protein's size, and the thickness or intensity of the band indicates the protein's abundance. Are there any proteins that are more abundant in one stage vs. another? If so, why might this be the case?

There are likely to be some proteins that are more abundant at specific stages because that is when they are most needed. For example, proteins needed for mitosis will be highly expressed in rapidly developing tissues and not so highly expressed in mature tissues.

One caveat: The amount of protein loaded into each well will affect the abundance of the proteins in the gel. Challenge students to determine whether proteins are abundant because more were loaded on the gel (i.e., all of the proteins in a sample are more abundant) or because one protein in particular is more abundant (i.e., only one protein in particular looks more abundant).

**PROTEIN ELECTROPHORESIS SAMPLES AND
KITS AVAILABLE COMMERCIALY**

Company	Catalog #	Cost	Kit title	Comments
Bio-Rad (800) 424-6723 BR Life Science Education Prices include EDU discount	166-2700EDU	\$178.00	Comparative Proteomics Kit I: Protein Profiler	Enough for 8 student workstations; gels sold separately.
	166-2850EDU	\$370.00	Comparative Proteomics Kits I & II: Protein Profiler & Western Blot	Includes both Protein Profiler and Western Blot kits; gels sold separately
Carolina Biological (800) 334-5551 www.carolina.com	211515	\$64.00	Fish Protein Sample Set	Contains extracts from 7 species of fish; enough for 13-14 gels
Edvotek (800) EDVOTEK www.edvotek.com (gels from Edvotek kits do not fit Bio-Rad gel boxes.)	Cat.# 110	\$99.00	MW Determination of Proteins	Uses horizontal gel box and <i>agarose</i> ; materials for 6 gels.
	Cat.# 111	\$99.00	Electrophoretic Properties of Native Proteins	Uses horizontal gel box and <i>agarose</i> ; materials for 6 gels.
	Cat.# 150	\$60.00	Survey of Protein Diversity	For 6 groups sharing 3 gels; kit does not include gels.
	Cat.# 153	\$60.00	Determination of Protein MW	For 6 groups sharing 3 gels; kit does not include gels.
	Cat.# 252	\$60.00	Bacterial Protein Fingerprinting	For 6 groups sharing 3 gels; kit does not include gels.
	Cat.# 253	\$69.00	Diversity of Fish Proteins	For 6 groups sharing 3 gels; kit does not include gels.
	Cat# 1110	\$79.00	Cell Types in the Brain	For 6 groups sharing 3 gels; kit does not include gels.
	Cat.# 1115	\$239.00	Detecting Risk Factors for Alzheimer's Disease Using Western Blot	For 6 groups sharing 3 gels; kit does not include gels.
	Cat.# 151	\$79.00	Simulation of HIV Detection by Protein Electrophoresis	For 6 groups sharing 3 gels; kit does not include gels.
Modern Biology (765) 446-4220 www.modernbio.com	EXP-106	\$64.83	Protein Fingerprinting	Uses horizontal gel box and <i>agarose</i>
	EXP-201P	\$54.86	Molecular Weight Determination	Materials for four 12-well gels; gels not included.
	IND-11P	\$77.97	Contractile Proteins from Cow Heart	
	IND-25P	\$60.18	Isolation of Chromosomal Proteins	

SOME INFORMATION ON MUSCLE PROTEINS

Much of the following material is paraphrased from *Molecular Cell Biology* by Darnell *et al.* and Alberts *et al.* (see References on page 62).

Actin is the most abundant cytoplasmic protein in most eukaryotic cells. The protein myosin is intimately associated with actin in all muscle cells. In rabbit muscle, actin comprises 19% of total cellular protein, and myosin another 35%, so these 2 proteins together make up 54% of the proteins in rabbit muscle. These quantities will be similar in the muscle cells of other species. Striated muscle is made of a regular array of actin and myosin filaments. Smooth muscle also contains both proteins, but not arranged in the regular array seen in voluntary muscles. (Smooth muscle myosin is different than myosin in striated muscle, however.)

Below are some details on the most abundant muscle proteins, and on the following page is a table of muscle proteins and their molecular weights. There is also a scan showing several of these proteins. (This scan was provided by Bio-Rad.) Please note that the molecular weight standards on the scanned gel are not the same as the ones provided in this kit.

Actin: If you have studied cell biology, you will have heard about microfilaments, which are actually polymers (long chains) of actin. Actin is a globular protein with a molecular weight of 42 kDa (= 42,000 daltons). This means that if you denature the actin, as we have done in this experiment, and run it on an SDS-PAGE gel, it will appear as a single, strong (i.e., abundant) band at 42 kDa. For example, if you look at my sample gel on page 35, you will see a dark band at 42 kDa.

If you place actin monomers (single protein units of 42 kDa) in a solution that contains Mg^{2+} , K^+ , or Na^+ at concentrations similar to that found in the cell cytoplasm, the actin monomers will polymerize into long chains. Actins from organisms as diverse as mammals and slime molds will polymerize together, so there is not much difference between actins from different species, i.e., actin has been highly conserved during evolution.

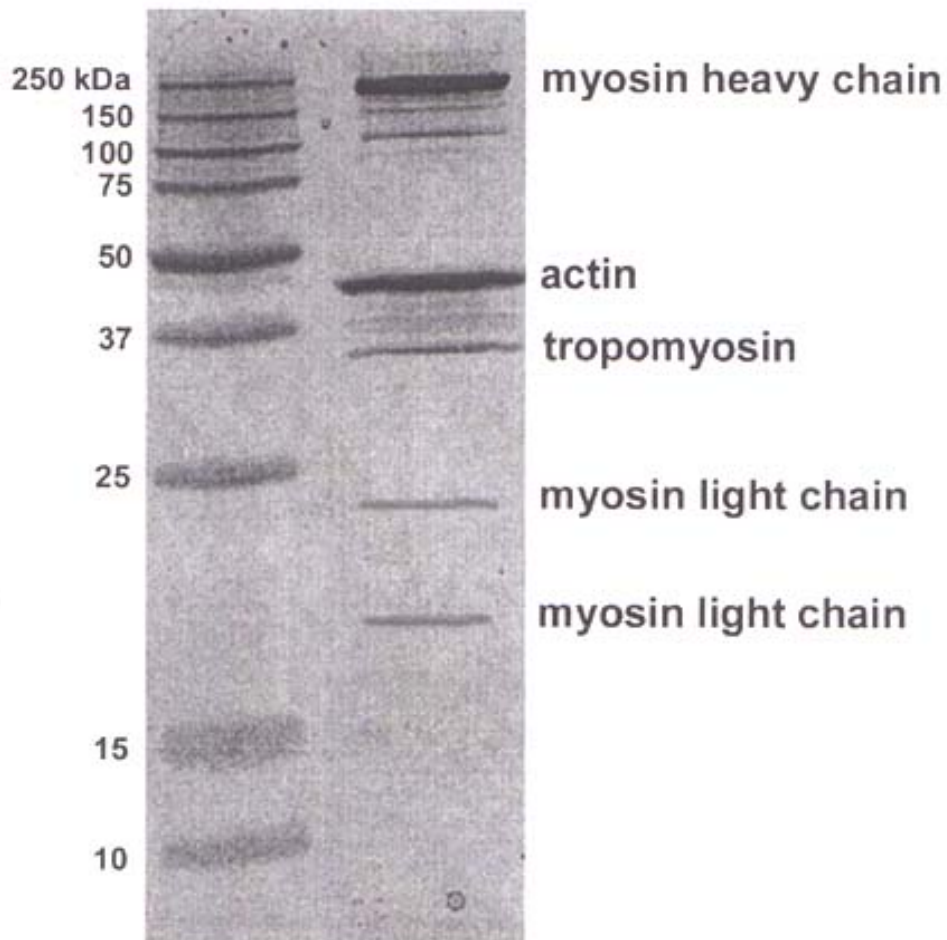
Myosin: Myosin forms filaments also. The structure of myosin, however, is a bit more complicated than that of actin. A myosin molecule has four subunits, two of which are identical "heavy chains" or large polypeptides of 230 kDa. In the 15% acrylamide gels that are part of this kit, proteins of that size (>150 kDa) will just barely enter the top of the separating gel. A strong band should be visible at the top of most lanes in the sample gel on page 35.

The other two subunits of myosin are different, but both have a molecular weight of about 20 kDa. There is a band of that size in the sample gel, but it is not as strong as the bands that are likely actin and the heavy chains of myosin.

Other muscle proteins: There are other proteins in muscle, of course. Muscle cells are eukaryotic cells, so they will contain all the proteins found in the normal cytosol and in subcellular organelles, such as nuclei, endoplasmic reticulum, and mitochondria. The quantities of the other proteins will be much smaller than the amounts of actin and myosin, and the other proteins cannot be identified on the gel. There will also be other proteins associated primarily with muscle, such as tropomyosin (MW 35 kDa) and three types of troponin (two of which have molecular weight of 37 and 22 kDa).

Muscle Protein	MW (kDa)**
titin	3000
myosin heavy chain	230
α -actinin	95
actin	42
troponin-T	37
tropomyosin	33
troponin-I	23
myosin light chains	~20
troponin-C	18

** These are the molecular weights of the subunits, i.e., the sizes that would appear on an SDS-PAGE gel.



SOME INFORMATION ON EVOLUTIONARY RELATIONSHIPS

You should be able to find lots of information on the evolutionary trees in many biology and zoology books, but here is some information that I dug up. The evolutionary trees are not complete, just excerpts from several references (page 62). There is also a good website called Tree of Life that has excellent information and resources on evolutionary relationships.

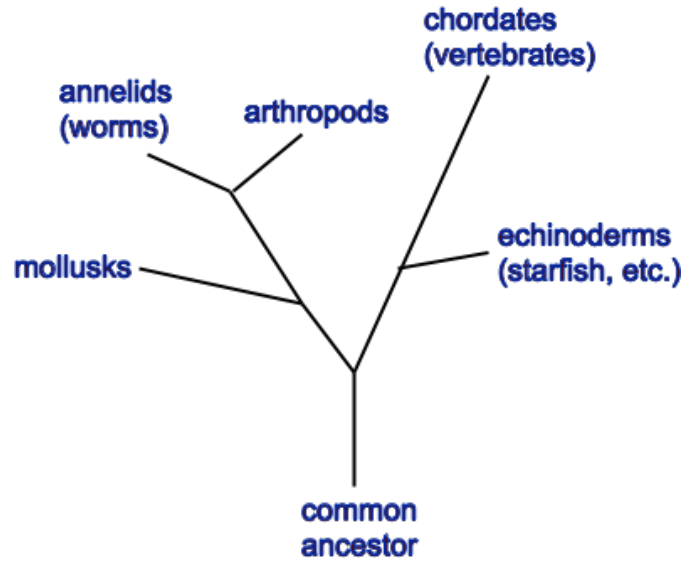


Figure 4. Rough evolutionary tree showing the relationships between vertebrates (including all fish), mollusks, and arthropods

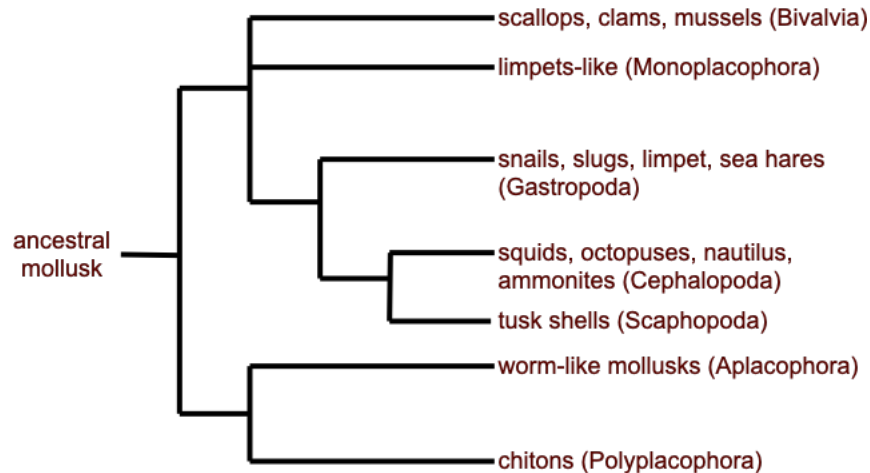


Figure 5. Cladogram showing the relationships between some mollusks.

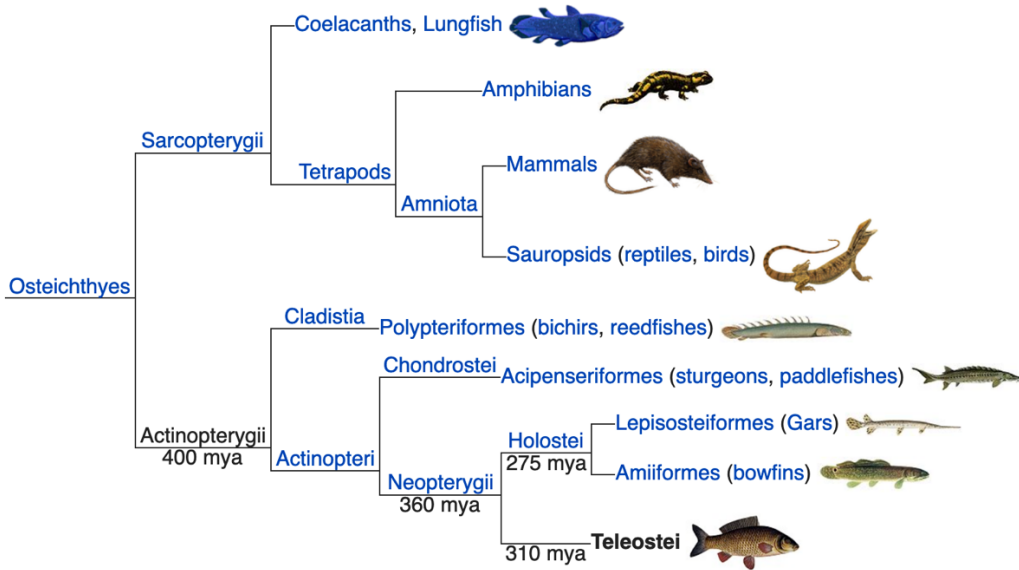


Figure 6. Evolutionary relationships between some of the fishes and other vertebrates; mya: million years ago (source: <https://en.wikipedia.org/wiki/Teleost>)

NON-PISCINE SAMPLES

Non-piscine samples that could be used in this experiment include mollusks (e.g., scallops, octopus, clams, squid), and arthropods (e.g., crab, shrimp, lobster), reptiles and amphibians (e.g., frog, alligator), birds (e.g., turkey, chicken, quail, duck), and mammals (e.g., cow, pig, rabbit, buffalo).

EVOLUTION OF FISHES

The fish are divided into the chondrichthyes (cartilaginous) and the osteichthyes (bony) fishes, with the lampreys and hagfishes forming a separate evolutionary path from the ancestral fishes. The chondrichthyes include the sharks and rays, and the osteichthyes, a very diverse group, include all other (evolutionarily) modern fishes. Below you will find brief descriptions of some of the major fish groups.

Lampreys and hagfishes are eel-like, jawless fish. They do not have scales or paired fins. Although lampreys and hagfishes both possess many of the characteristics of the ancestral Agnatha, they are very different from each other.

Sharks, rays, skates, and chimaeras are members of the class Chondrichthyes. They have cartilaginous rather than bony skeletons, and do not have swim bladders or lungs. Their skin is thick and does not have true scales. There are about 900 species.

OSTEICHTHYES (Bony fishes)

The most diverse class of fish is the osteichthyes. The class is characterized by having true scales, paired fins, bony skeletons, and movable rays in their fins and tail. Osteichthyes are divided into two subclasses, the ray-finned fish (the Actinopterygians) and the flesh-finned or lobe-finned fish (the Sarcopterygians). The Sarcopterygians are the fish most closely related to modern tetrapods (amphibians, reptiles, birds, and mammals), and the subclass includes the lungfish and the coelacanth. Most modern fish are members of the ray-finned subclass, the Actinopterygians.

Sturgeons, bowfins, and gars are considered relic bony fishes. Each is in a separate order in the subclass Actinopterygii. There are about 50 species of these relic fish extant around the world today, although there were many more in the past.

The remainder of the bony fishes are in the subdivision **Teleostei**. Teleostei includes about 40 orders and over 26,000 species.

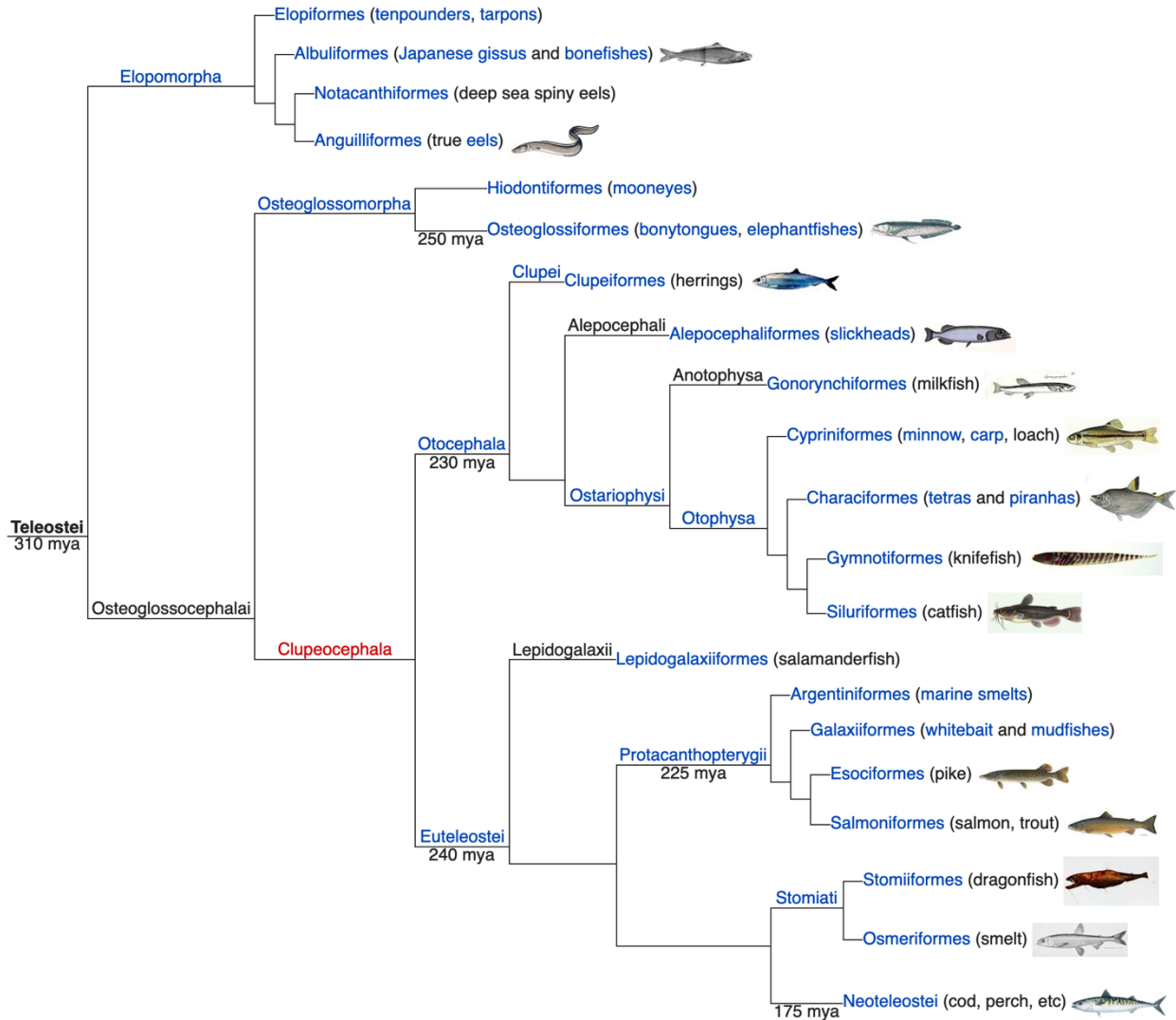


Figure 7. Evolutionary relationships of the Teleosts; mya: million years ago (source: <https://en.wikipedia.org/wiki/Teleost>)

EVOLUTION OF MAMMALS

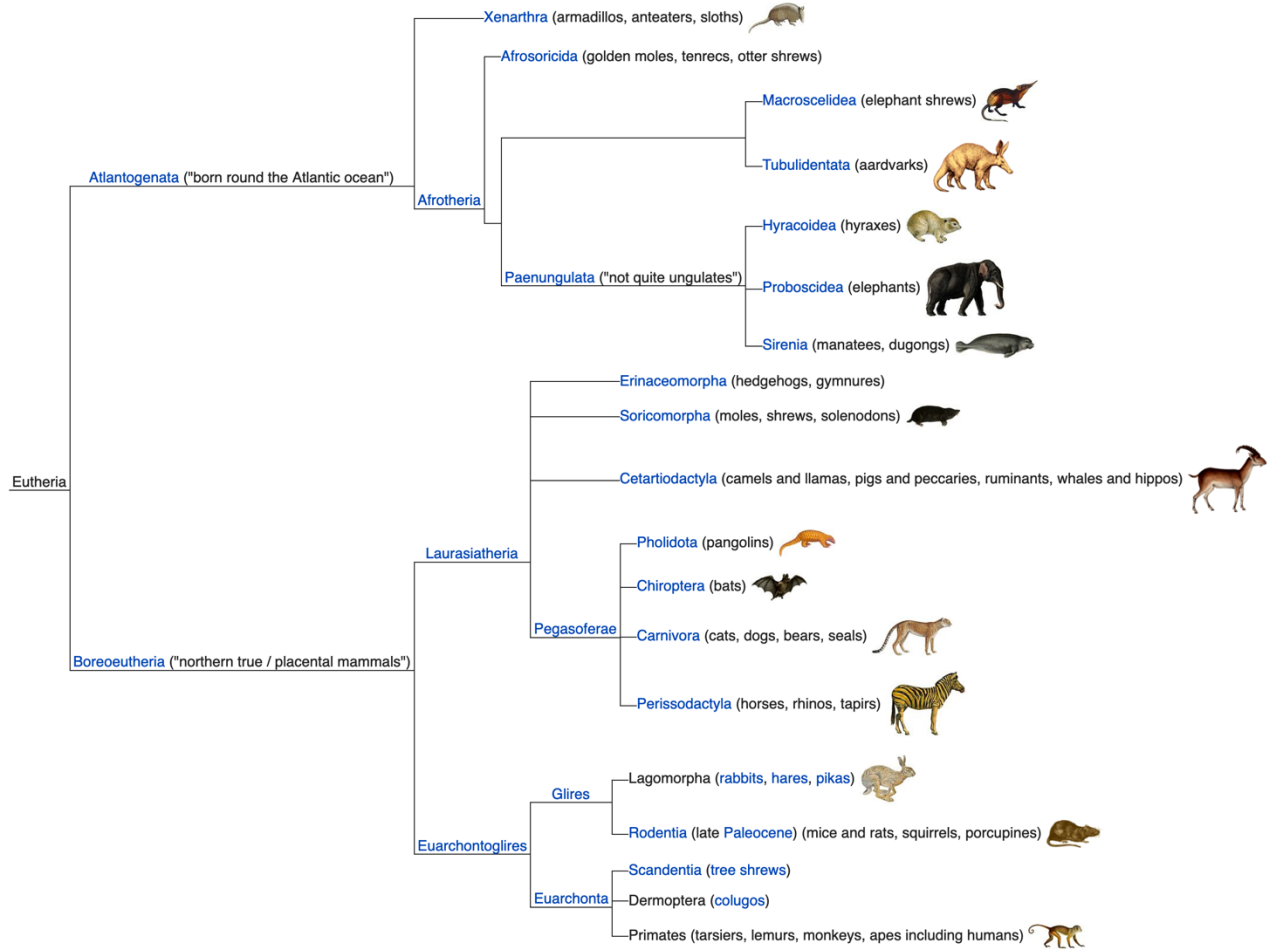


Figure 7. Evolutionary relationships of placental mammals (source https://en.wikipedia.org/wiki/Evolution_of_mammals)

SOURCES FOR EQUIPMENT AND MATERIAL USED IN THIS KIT

Gel boxes and power supplies were purchased from [Bio-Rad](#) (800-424-6723). The gel boxes (Mini-Protean 3) are no longer available. The current Mini-PROTEAN Tetra cell will run 4 gels. (catalog number 165-8005EDU; price \$412). The PowerPac Basic Power supply (catalog number 164-5050EDU; price \$286) will run 4 gels. Note: these are EDU prices.

Precast acrylamide gels were purchased from Bio-Rad. They are 12% Tris-glycine gels (catalog number 456-1043EDU; price \$92.80/10 gels, plus shipping costs).

Gel drying system was purchased from [Fisher Scientific](#) (800-766-7000). The system (catalog number NI2387) includes 2 frames, 8 clamps, 1 drying base, 200 sheets of cellophane, and 500 ml of Gel-Dry™ Solution, and costs \$220. Individual refill item prices:

- Cellophane sheets (pack of 200): \$95.25 (catalog number NC2380)
- Gel-Dry Drying Solution (500 ml): \$49.13 (catalog number LC4025)

Molecular weight markers were purchased from Bio-Rad, however the item is no longer available. Once our current supply runs out, a new marker will be provided and this information updated.

Fixed volume pipettors: The fixed volume micropipettes were purchased from Bio-Rad. The educational price is \$26 each. The 10- μ l pipettor is catalog number 166-0512EDU, and the 20- μ l is 166-0513EDU. The fixed volume pipettors are also available from Edvotek and Carolina Biological Supply Company.

Micropipettors: Micropipettors similar to those in the kit are available from most or all scientific supply houses for between \$150–\$200.

Yellow pipette tips: The yellow tips are from [VWR](#) (800-932-5000) purchased in bulk bags of 10,000 tips for \$219 (catalog #53508-812). Smaller quantities (bagged or racked) are available from most or all scientific supply houses.

Solutions: 10x Tris/Glycine/SDS buffer (161-0772EDU) is \$81.60 for 5 liters. Bio-Safe Coomassie Stain (161-0787EDU) is \$272 for 5 liters. Both are from Bio-Rad (EDU pricing). Smaller volumes are available.

Chemicals were purchased from various supply companies. The following table gives some catalogs and prices from Sigma. The chemicals should be available from most supply houses. Most of the supply houses also carry the pre-made buffers.

Item	Catalog number	Quantity	Cost
SDS	L-3771	25 g	44.80
glycine	G-8898	500 g	55.20
Tris	T-6066	100 g	33.10
bromophenol blue	B-8026	5 g	52.10
glycerol	G-5516	100 ml	44.00

SOLUTIONS

All of these solutions can be prepared in the lab or purchased pre-made from the vendors listed below.

1X gel running buffer (25 mM Tris, 192 mM glycine, 0.1% SDS)

Add 3.03 g of Tris base, 14.4 g of glycine, and 1 g of SDS (sodium dodecyl sulfate) to 900 ml distilled water. Mix, then bring to final volume of 1 liter by adding distilled water. Do not adjust the pH. Store at room temperature. (To prepare one liter of 10X running buffer, add 30.3 g of Tris base, 144 g of glycine, and 10 g of SDS to 800 ml of distilled water. Mix, then bring to final volume of 1 liter by adding distilled water. Dilute 1:10 in distilled water before using.)

We have been buying this from Bio-Rad as a 10x solution (see above for ordering information). Dilute 1:10 in distilled water before use.

Laemmli Sample Buffer

Final concentration	To make 100 ml
62.5 mM Tris, pH 6.8	12.5 ml of 0.5 M Tris-HCl, pH 6.8
2% SDS	2 g SDS powder or 10 ml of 20% SDS
25% glycerol	25 ml glycerol
0.01% bromophenol blue	1 ml of 1% bromophenol blue solution

Add the above to 40 ml distilled water and mix. Bring to 100 ml final volume with distilled water. Aliquot and store at room temperature.

Gel Drying Solution

The gel drying solution is 30% methanol and 5% glycerol in water. To prepare 1 liter, mix 50 ml of glycerol, 300 ml of methanol, and 650 ml of water. Store at room temperature.

Other resources

Bio-Rad has a kit based on this experiment (using seafood samples), and they have a [PowerPoint presentation](#) linked to their website. You can download the presentation (free!). There are some slides that are very useful and provide information that is not included in this manual.

We have a YouTube [video](#) on setting up the gel boxes and running a protein gel (developed for our Cell & Molecular Biology Lab here at VT) that might be useful.

COMPANIES

Bio-Rad
(800) 424-6723
<http://www.bio-rad.com/>

Carolina Biological Supply Company
(800) 334-5551
www.carolina.com

Edvotek
(800) 338-6835
www.edvotek.com

Fisher Scientific
(800) 766-7000
www.fishersci.com

Modern Biology Inc
(765) 446-4220
<http://www.modernbio.com/>

Sigma Chemical Company
(800) 325-3010
<https://www.sigmaaldrich.com/>

VWR
(800) 932-5000
https://us.vwr.com/cms/life_science

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- Stolz, J., and Schnell, J. (eds.) 1991. *Trout*. Stack pole Books, Harrisburg.

DIRECTIONS FOR RETURN SHIPPING

Please return the checklist with the kit.

- There is no need to return the used gel plates and combs. I have bunches of them.
- DO return the buffer dams. (It's easy to get the buffer dams mixed in with the gel plates, so please make sure you count the buffer dams when you're finished running the gels.)
- Remove the cord from the power supply and place the power supply in bubble wrap. (Don't forget to put the cord in the kit.)
- Place the gel boxes in bubble bags. (Be sure that the lids are on the gel boxes or the electrical leads will be damaged.)
- Repackage the materials in the shipping container. Please be sure that heavier items are on the bottom of the box, and that the gel boxes do not have anything heavy on top of them.
- **Please** check off items as they are repacked. This kit has a lot of small parts that are easy to lose track off if you are not careful. **Any items that are not shaded on the form are expected to be returned!** (See Important Notes on page 6.)
- Please return *unused* expendables, such as microcentrifuge tubes, pipette tips, etc. Do not return used items. Particularly, do **not** mix used items with unused items.
- If you need additional packing material, please use wadded paper. Please **do not** use Styrofoam peanuts!
- Shipping:
 - Remove the original FedEx shipping label from the shipping container.
 - Seal the box with cable ties.
Please make sure that the cable ties are secure. Note that there is a right way and a wrong way to insert the tab in the cable tie. Look at the end you put the tab through. The tab should be put in from the side that is smooth with the tie, not the end that sticks out. If it is done the wrong way, the cable tie will open when you pull on it. Please test the cable tie by pulling to be sure that you've done it correctly.
 - Remove the backing paper from the plastic airbill sleeve and place the pre-printed airbill on top of the box.
 - Call FedEx for pickup. They require at least a 3-hour notice on pickups.

