PRELIMINARY ACTIVITY FOR

# Introduction to Molecular Evolution

## Open Inquiry Version

### Evolution and Classification

An evolutionary tree shows the evolutionary lineages of different species over relative time. Evolutionary trees, (also called cladograms), can be based on many different types of data. The traditional way of constructing evolutionary trees was to look at the physical morphology of organisms, including sizes, shapes and developmental structures of both living organisms and fossils. Today, similarities and differences in molecular data (protein and DNA sequences) are also being used. Both methods are valuable and often complement each other.

You will use the evolutionary tree shown in Figure 1 to make predictions about the relatedness of the species you will examine in this investigation. Following the analysis and interpretation of your electrophoresis results, you will create a cladogram from your own results and compare your cladogram with your predictions.

For this investigation, it is useful to compare both closely related and distantly related fish. In addition to using the tree below to make your predictions, we recommend that you research additional information on the evolutionary histories of fishes, using the Internet, and biology and zoology books.

The data used to construct the evolutionary tree in Figure 1 was obtained from the cladograms on the tree of life web page from the University of Arizona (www.tolweb.org). (Please note that the field of phylogenetics is ever changing and different methods used to construct a phylogenetic tree often result in differences between trees, hence the data on the tree of life web page may not concur exactly with "textbook" evolutionary trees.)

### Classification of Fish

Most fish are contained within the superclass Gnathostoma (jawed vertebrates), which also includes all tetrapods. Only hagfish and lampreys are outside this group. These two fish types are sometimes classed together as Agnatha, but can also be separated into Hyperotreti and Hyperoartia. Hyperotreti (hagfish) are craniates (animals with skulls), but not vertebrates because they have no backbone, while Hyperoartia (lamprey) are very primitive vertebrates, but do not have a jaw. The Gnathostoma fishes are divided into the classes Chondrichthyes (cartilaginous fishes) and Osteichthyes (bony fishes). The Chondrichthyes include the sharks and rays, and the Osteichthyes, include all other modern fishes and all tetrapods (amphibians, birds, and mammals). Below are brief descriptions of some of the major fish groups, in order from most ancient to most recently diverged.

**Hyperotreti (e.g., hagfish)** are eel-like, jawless fishes that have a skull, but no backbone with parasitic and scavenging lifestyles. They are very primitive and may approach the condition of the common ancestor to all craniates.

**Hyperoartia (e.g., lamprey)** are eel-like, jawless fishes that are primitive vertebrates. They are identified by a single nostril and a sucker-like mouth with which they attach to fishes and rocks.

**Chondrichthyes (e.g., shark, ray, skate, and sawfish)** have cartilaginous rather than bony skeletons that reflect a more evolutionarily ancestral state. Their skin is thick and without true scales, and they do not have swim bladders or lungs.

**Osteichthyes (e.g., coelancanth, tuna, and haddock)**, the bony fishes, are the most diverse class of fish. The class is characterized by having bony skeletons, true scales, paired fins, and movable rays in their fins and tail. Osteichthyes are divided into two subclasses:

- The lobe-finned fish, Sarcopterygians, which contains the living fossil, the coelacanth, and the tetrapods (amphibians, reptiles, mammals, birds and dinosaurs).
- The ray-finned fish, Actinopterygians, which contains most other fish.

**Sarcopterygians (e.g., lungfish and coelacanth)** also include modern amphibians, reptiles, birds, and mammals. Coelacanth were thought to have become extinct at about the same time as the dinosaurs, until a live specimen was found in 1938. They form an important evolutionary link between fish and four-legged land animals.

**Actinopterygian (e.g., gar, sturgeon, mackerel and anglerfish)** is the subclass encompassing most modern ray-finned fish including the chondrostei, semionotiformes, and teleosts.

- **Chondrostei (e.g., sturgeon)** are considered relic bony fishes. They lack scales on most of the body, have a cartilaginous skeleton, and have developed a shark-like, heterocercal tail and a rostrum extending past the mouth.
- **Semionotiformes (e.g., gar)** are also ancient fish; they have bony scales and a mainly cartilaginous skeleton.

**Teleosts (e.g., herring, carp and pufferfish)** comprise the remainder of the bony fishes. These include Clupeomorpha (e.g., herring, sardine and anchovy), Ostariophysi (e.g., carp, catfish, minnow, piranha and electric eel), Salmoniformes (e.g., salmon, trout and smelt), Esociformes (e.g., pike), and the diverse group, Acanthomorphia (e.g., tuna, cod and pufferfish).

- **Acanthomorphia (e.g. pollock, bass and sole)** comprises two main superorders, Paracanthopterygians (e.g., cod, pollock and anglerfish) and Acanthopterygians. The Acanthopterygians include the Perciformes (e.g., the scombridae (e.g., swordfish, mackerel and tuna) and the serranidae (e.g., bass, snapper and grouper)), the Pleuronectiformes (e.g., flat fish, flounders and sole) and the Tetradontiformes (e.g., pufferfish).

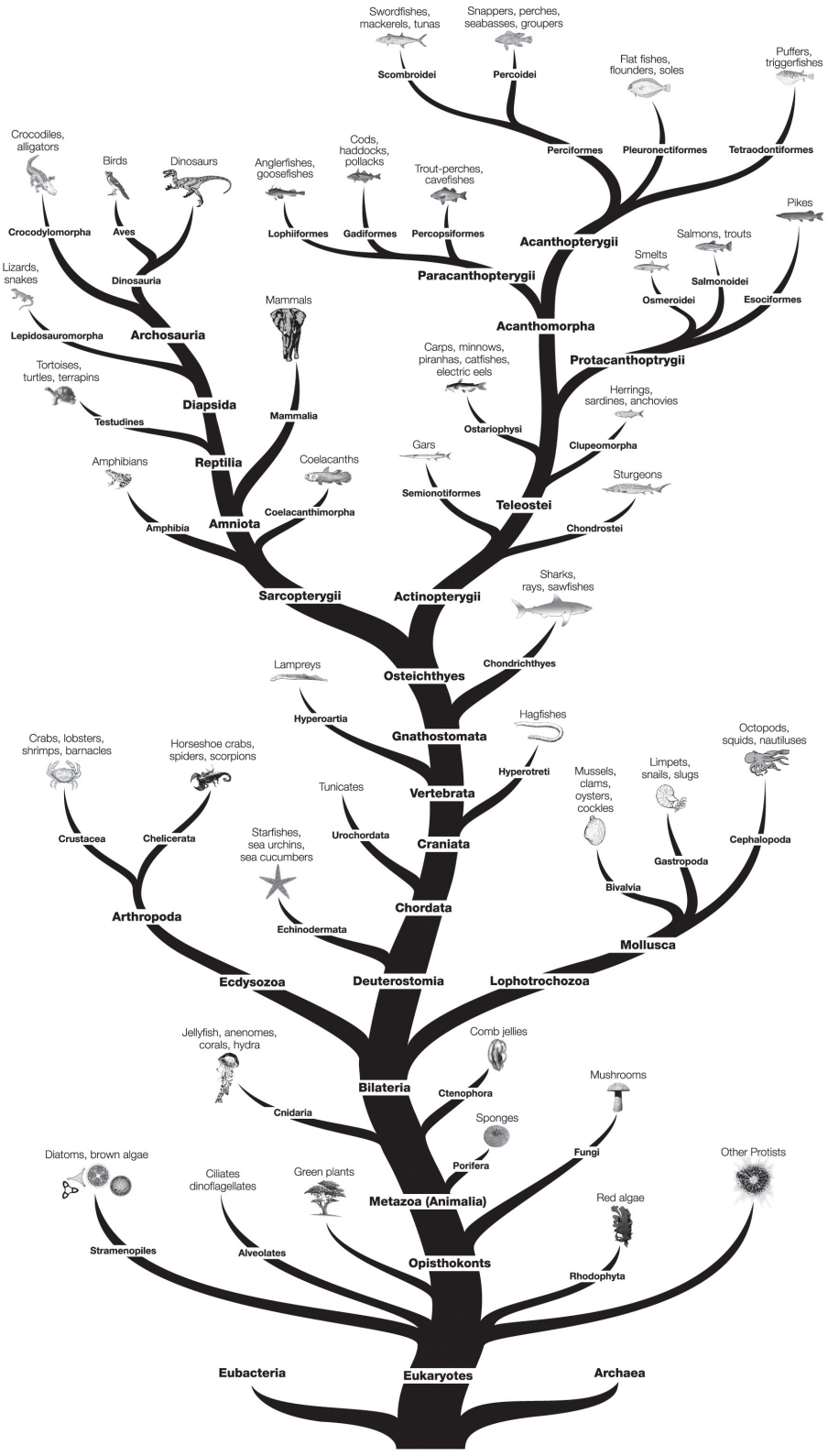



**Figure 1** *Evolutionary tree showing the relationships of eukaryotes. (used with permission)*

## Introduction to Proteins, Electrophoresis, and SDS-PAGE

### How Can We Study Proteins Found in Muscle?

Polyacrylamide gel electrophoresis (PAGE) can be used to separate small molecules such as proteins. Understanding protein structure is important to understanding how we can use PAGE for protein analysis.

Proteins are composed of monomers called amino acids. All amino acids have a carboxyl group, an amino group, and a central or alpha carbon. The central carbon of each amino acid contains a side chain that is often referred to as an R group. Amino acids form polymers when the carboxyl group and amino group of two amino acids form a peptide bond as shown in Figure 2. Water and a dipeptide are formed in the reaction. More amino acids can be added to the carboxyl group of this dipeptide until a polypeptide is formed.

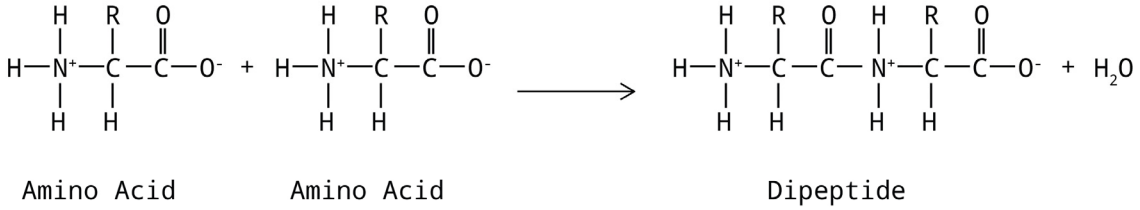



**Figure 2**

There are 20 different amino acids that are found in proteins, and each one has a different R group. These side chains are very important because they impart each amino acid with different characteristics. The sequence of amino acids that make up a polypeptide are referred to as the primary structure of the protein. The primary structure determines how the protein will fold, which will determine its function. The shapes within a polypeptide are referred to as secondary structure.

The three dimensional structure of an entire polypeptide is referred to as its tertiary structure. The tertiary structure of the protein is determined by the interaction of the hydrophilic and hydrophobic side chains with the aqueous environment. The hydrophobic regions aggregate to the center of the molecule. The hydrophilic regions orient themselves toward the exterior. These ordered bends and folds make the protein compact. Examples of tertiary protein structure are structural and globular proteins.

The quaternary structure of proteins is achieved from the interaction of polypeptide chains with others. Multiple polypeptides can combine to form complex structures such as the muscle protein myosin, or the blood protein hemoglobin, which are both composed of four polypeptide chains. These complex proteins are often held together by disulfide bonds between cysteines, a type of amino acid. In fact, PAGE analysis was first carried out in 1956 to show the genetic disease sickle cell anemia is caused by a change to a single amino acid of the hemoglobin protein.

Prior to electrophoresis, the proteins are treated with the detergent sodium dodecyl sulfate (SDS) and heated. SDS and heat denatures (destroys) the protein tertiary and quaternary structures, so that the proteins become less three dimensional and more linear. SDS also gives the protein an overall negative charge with a strength that is relative to the length of its polypeptide chain, allowing the mixture of proteins to be separated according to size.

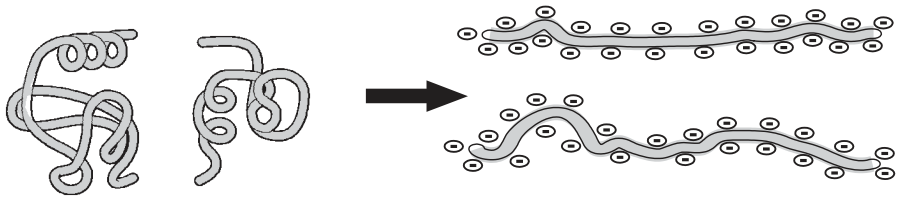


**Figure 3** *The combination of heat and the detergent SDS denatures proteins for SDS-PAGE analysis.*

The proteins, in their SDS-containing Laemmli sample buffer, are separated on a gel with a matrix that acts to sieve the proteins by size upon addition of an electric current. A polyacrylamide gel is positioned in a buffer-filled chamber between two electrodes, protein samples are placed in wells at the top of the gel, and the electrodes are connected to a power supply that generates a voltage gradient across the gel. The SDS-coated, negatively charged proteins migrate through the gel away from the negatively charged anode toward the cathode, with the larger proteins moving more slowly than the smaller proteins. This technique was developed by U.K. Laemmli.

As soon as the electric current is applied, the SDS-coated proteins move toward the positive electrode. The smaller proteins can move through the gel more quickly than the larger ones, so over time, the proteins will separate according to their size.

Protein size is measured in *daltons*, a measure of molecular mass. One dalton is defined as the mass of a hydrogen atom, which is $1.66 \times 10^{-24}$ g. Most proteins have masses on the order of thousands of daltons, so the term *kilodalton* (kD) is used to describe protein molecular weight. Given that the average weight of an amino acid is 110 daltons, the number of amino acids in a protein can be approximated from its molecular weight.

- Average amino acid = 110 daltons
- Approximate molecular weight of protein = number of amino acids x 110 daltons

In this investigation, you will use SDS-PAGE to separate and analyze the protein profiles of the muscle tissue of different fish. By comparing the protein profiles of different fish species you can test the hypothesis that protein profiles are indicators of genetic and evolutionary relatedness.

#### Visualizing your Proteins

Proteins in your samples are not visible while the gel is running. The only visible proteins will be those in the Precision Plus Protein Kaleidoscope standard that have been prestained. You should see these proteins resolve into multicolored bands that move down the gel as power is run through the gel. If the electric current is left on for too long, the proteins will run off the bottom of the gel. To guard against this a blue tracking dye is mixed with the sample buffer used to prepare your protein samples. This blue dye is negatively charged and is also drawn toward the positive electrode. Since the dye molecules are smaller than the proteins expected in most samples, they move ahead of the proteins in the gel.

After turning off the electric current and removing the gel from the glass plates that hold it in place, the gel is stained. The stain used in this technique was originally developed to dye wool, which, like your own hair, is composed of protein. This stain binds specifically to proteins and not to other macromolecules such as DNA or lipids. After destaining, distinct blue bands appear

on the gel, each band representing on the order of $10^{12}$ molecules of a particular protein that have migrated to that position; the larger the amount of protein, the more intense the blue staining.

In this Preliminary Activity, you will (1) extract proteins from muscle tissue, (2) conduct electrophoresis of the resulting protein extracts, (3) analyze the results using Logger *Pro*, and (4) construct a cladogram using the results.

After completing the Preliminary Activity, you will first use reference sources to find out more about protein gel electrophoresis before you choose and investigate a researchable question. Some topics to consider in your reference search are:

- protein
- amino acid
- gel electrophoresis
- fish
- evolutionary tree
- cladogram
- phylogenetic relationships
- SDS-PAGE
- denature
- molecular weight
- dalton
- kilodalton (kD)

## PART I PROTEIN EXTRACTION FROM MUSCLE

Your first task is to extract proteins from muscle tissue, unfold and denature them, and give each protein an overall negative charge using Laemmli sample buffer, mechanical forces, and heat. In this investigation you will add tiny pieces of muscle to Laemmli sample buffer and manually disrupt the tissue by flicking the tubes. This will release muscle specific proteins from the cells, unfold them, and add an overall negative charge to each protein. You will then pour off the extract and heat the extracted proteins to 95°C, which will complete their denaturation.

## MATERIALS

| Materials: Student Workstation | Quantity |
|---|---|
| 1.5 mL fliptop microtubes | 3 |
| 1.5 mL screwcap microtubes | 3 |
| 1 mL transfer pipette | 1 |
| fish samples (shark, catfish, salmon), labeled 1–3 | 3 species |
| marking pen | 1 |
| Laemmli sample buffer (SB) | 1.5 mL |
| knife or scissors to cut fish samples | 1 |

| Materials: Common Workstation | Quantity |
|---|---|
| water bath set to 95°C | 1 |

## PART I PROCEDURE

1. Obtain and wear laboratory gloves and protective eyewear.

2. Assign letters (e.g., SH, CA, SA ) to each fish sample to be investigated. Your instructor has a record of how each sample was labeled.

3. Label 1.5 mL fliptop microtubes with the number of the fish species to be analyzed. There should be one labeled tube for each fish sample being prepared for electrophoresis.

4. Add 250 µL of Laemmli sample buffer to each labeled tube.

5. For each sample, obtain a piece of fish muscle (avoid skin, fat, and bones) approximately 0.25 x 0.25 x 0.25 cm, and transfer it to the appropriately labeled microtube. Close the lid.

6. Gently flick the microtube 15 times with your finger to agitate the tissue in the sample buffer.

7. Incubate the samples for five minutes at room temperature to extract and solubilize the proteins.

8. Pour the buffer containing the extracted proteins, but not the solid fish piece, to a labeled 1.5 mL screwcap tube. **Note**: It is not necessary to transfer all of the fluid to the screwcap tube, since only a small volume (<20 µL) is actually needed for gel loading.

9. Heat your fish samples in their screwcap tubes for five minutes at 95°C to denature the proteins in preparation for electrophoresis.

10. Store the samples at room temperature if they are to be loaded onto gels within 3–4 hours, or store them at –20°C for up to several weeks.

## PART II ELECTROPHORESIS: GEL LOADING, RUNNING, AND STAINING

## MATERIALS

| Materials: Student Workstation | Quantity |
|---|---|
| fish protein extracts from Part 1 | 3 species |
| actin and myosin standard, 12.5 µL (AM) | 1 |
| Precision Plus Protein™ Kaleidoscope™ prestained standards, 6 µL (Stds) | 1 |
| 4–20% Mini-PROTEAN® TGX™ | 1 |
| 1–20 µL adjustable-volume micropipet | 1 |
| Prot/Elec™ pipet tips for gel loading | 7 tips |
| Mini-PROTEAN Tetra cell electrophoresis module | 1 per 2 gels |
| 1X Tris-glycine-SDS (TGS) running buffer | 700 mL per gel box |
| power supply (200 V constant) to be shared between workstations | 1 |
| sample loading guide for 10-well comb (optional) | 1 per gel box |
| buffer dam (only required if running 1 gel/box) | 1 |
| staining trays | 1 per 2 gels |
| Bio-Safe™ Coomassie stain | 50 mL per 2 gels |

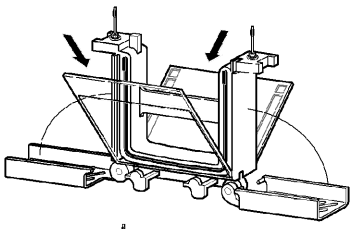
| Materials: Common Workstation | Quantity |
|---|---|
| water bath set to 95°C | 1 |
| water for gel destaining (tap water is fine) | |

## PART II PROCEDURE

1. Reheat frozen fish samples and actin and myosin standard at 95°C for 2–5 minutes to redissolve any precipitated detergent. Note: If you have prepared your fish samples in this session, there is no need to reheat them.

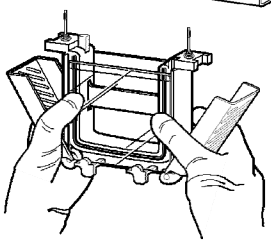
2. Obtain a Mini-PROTEAN Tetra gel box.

3. Prepare a TGX cassette by peeling off the green tape on the bottom of the cassette.



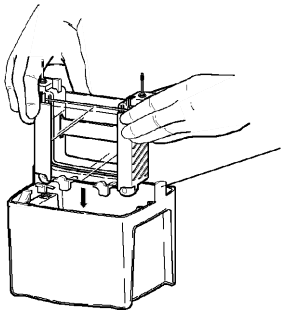
4. Remove the comb from the TGX cassette.

5. Place TGX cassette into the electrode assembly that has the banana plugs with the short plate facing inward. Place a buffer dam or another TGX cassette on the opposite side of the electrode assembly, with notch on buffer dam facing inward.



6. Push both gels towards each other, making sure that they are against the green gaskets that are built into the clamping frame; make certain that the short plates sit just below the notch at the top of the green gasket. Slide the green arms of the clamping frame over the gels, locking them into place.

7. Lower the electrode assembly with the gels in it into the mini tank on the side of the tank with the plastic tabs. Make sure that the red banana plug goes on the side of the tank with the red oval. **CAUTION**: When running 1 or 2 gels only, DO NOT place the Companion Running Module in the tank. Doing so will cause excessive heat generation and prevent electrophoretic separation.



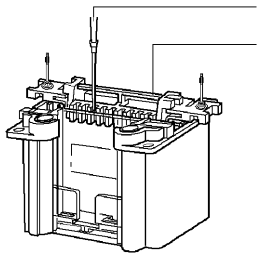
8. Completely fill the inner chamber with 1x TGS electrophoresis buffer, making sure the buffer covers the short plate (~150 mL).

9. Double-check that the buffer in the inner buffer chamber is well above the top of the smaller plate. If it is not, you may have a leak; consult with your instructor. **Note:** If you do have a leak, the outer chamber of the gel box can be filled to above the inner small plates, to equalize the buffer levels in both reservoirs.

10. If available, place a yellow sample loading guide on the top of the electrode assembly. The guide will direct your pipet tip to the correct position for loading each sample in a well.

| Lane | Volume | Sample |
|---|---|---|
| 1 and 2 | empty | empty |
| 3 | 10 µL | actin and myosin standard (AM) |
| 4 | 10 µL | fish sample 1 |
| 5 | 10 µL | fish sample 2 |
| 6 | 10 µL | fish sample 3 |
| 7 | 5 µL | Precision Plus Protein Kaleidoscope prestained standards |

11. Load 5 µL of Precision Plus Protein Kaleidoscope prestained standard gently into well # 3 using a thin gel loading tip.
**Note**: The fine barrel of the gel loading tips means liquid is slower to go into the tip than normal tips. You must therefore release the plunger of the micropipet very slowly, otherwise you will not pipet the correct volume.

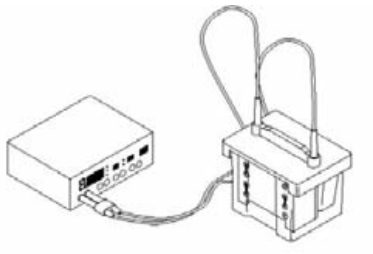


12. Using a fresh tip each time, load 10 µL of each of your protein samples gently into the wells designated in the table above.

13. Using a fresh tip, load 10 µL of the actin and myosin standard gently into well # 3.

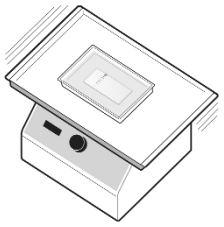
14. After loading all samples, remove the yellow sample loading guide (if used), place the lid on the tank, and insert the leads into the power supply, matching red to red and black to black. Set the voltage to 200 V and run the gels for 30 minutes. Watch for the separation of the standard.

15. When gels are finished running, turn off the power supply and disconnect the leads. Remove the lid and lift out the electrode assembly and clamping frame.



16. Pour out the running buffer from the electrode assembly. Open the cams and remove the gel cassettes.

17. To stain the proteins in your gel, lay your gel cassette flat on the bench with the short plate facing up. Carefully pry apart the gel plates, using the gel opening key. The gel will usually adhere to one of the plates. Transfer the plate with the gel adhering to it to a tray containing tap water allowing the liquid to detach the gel from the plate. The gel may also be lifted directly (and gently!) from the plate and placed into the water. If there is sufficient time, rinse the gel three times with tap water for five minutes by carefully pouring out the water and replacing it. Rinsing the gel will improve the intensity of the protein bands.



18. Carefully pour out the water and replace with 50 mL of Bio-Safe Coomassie stain per two gels.

19. Allow the gels to stain for at least one hour, with shaking if available. Gels can also be stained overnight. In this case, seal the container to reduce evaporation.

20. After at least one hour discard the stain and replace it with a large volume of water. This will destain the gel. Allow the gel to sit overnight with rocking action if available. Change the water 2–3 times if possible. Bands will become visible after a few hours of destaining.

## PART III ANALYSIS

## MATERIALS

computer
Logger *Pro*
Vernier White Digital Bioimaging System

## PART III PROCEDURE

1. Connect the White Light Transilluminator to AC power and turn it on.

2. Prepare the ProScope for use.
   a. Connect the 1–10x lens to the ProScope.
   b. Connect the ProScope to a USB port of your computer.
   c. Mount the ProScope to the stand and position the stand next to the transilluminator, opposite the side with the hinge.
   d. Level the ProScope so that its lens is parallel to the surface of the transilluminator.

3. Prepare Logger *Pro* for use.
   a. Start Logger *Pro*.
   b. Choose Gel Analysis ▶ Take Photo from the Insert menu.
   c. Check Close Window and Auto Arrange as the Photo Actions.

4. Take a photo of the gel.
   a. Transfer the gel to the central portion of the transilluminator platform.
   b. Orient the gel and platform so that the wells are at the top of the picture in Logger *Pro*.
   c. Orient and focus the ProScope so the bands, lane numbers, and rule are clear and sharp.
   d. Place the Imaging Hood over the ProScope and the transilluminator. Reach through the flap of the hood to make final adjustments for best position, focus, and resolution.
   e. Once satisfied with the image, click Take Photo. The screen should now resemble Figure 5. **Note:** You may want to re-size the photo and graph to increase the size of the photo for ease of analysis.

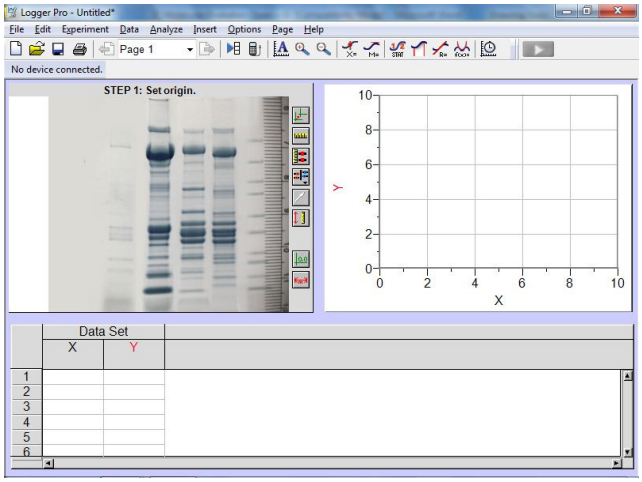



**Figure 5** *Sample Logger* Pro *screen with gel image*

5. Indicate the position of the wells on the photograph.
   a. Click Set Origin, .
   b. Click the photograph just to the left of the first well. A yellow coordinate system will appear on the photograph.
   c. Position the x-axis directly along the bottom edge of the wells. You can move the origin by clicking either axis and dragging it to the desired location. The axis can be rotated by clicking the round handle on the x-axis.

6. Convert the units of distance from pixels to millimeters.
   a. Click Set Scale, .
   b. Click and drag to draw a line that is several centimeters long using the rule along the side of the gel image as your guide. For example, click and drag between the 1 cm mark and the 5 cm mark to create a line that is five centimeters long.
   c. Enter the distance value and units in *millimeters* and click OK.

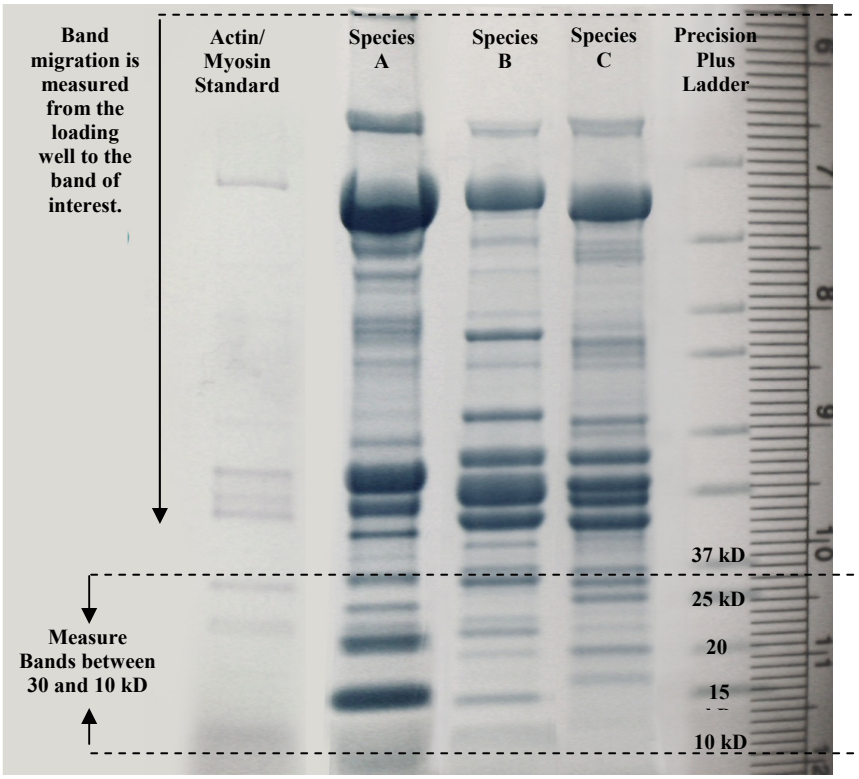


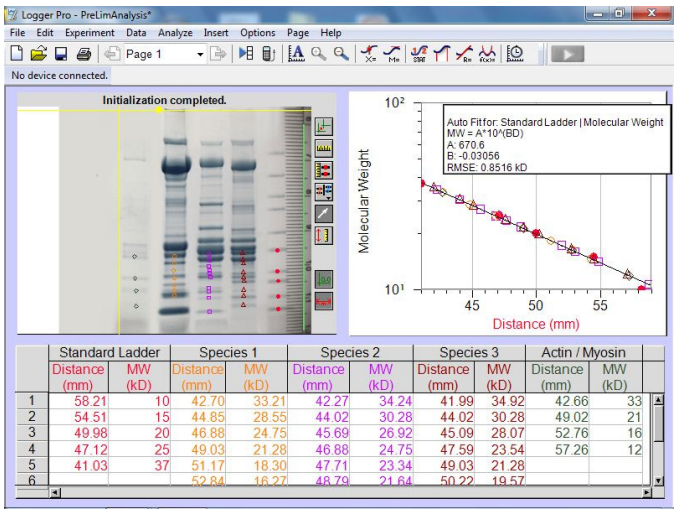

**Figure 6** *Image of fish muscle proteins from three different species separated by SDS-PAGE using a 4–20% TGX gel stained with Bio-Safe Coomassie stain*

The 4–20% TGX gel is designed to separate small proteins—proteins less than 40 kD. As shown in Figure 6, your gel analysis will concentrate on the range between 30–10 kD. **Note**: If a different percentage acrylamide gel or an agarose gel has been run, analyze the section of the gel that has the best separation.

7. Identify the bands and molecular weight values of the standard ladder using the Precision Plus Protein Kaleidoscope prestained standards lane molecular weight values in Table 1.
   a. Click Set Standard Ladder, .
   b. Click the center of the 37 kD band in the Precision Plus Protein Kaleidoscope prestained standards lane.
   c. Enter the molecular weight of this band using the values in Table 1. Click OK.
   d. Click the center of the next band in this lane and enter the molecular weight value. Click OK.
   e. Repeat this process for the visible bands of the standard ladder (25 kD to 10 kD). Logger *Pro* will automatically create a standard curve on the graph.

| Table 1: Molecular Weights from Standard Ladder | |
|---|---|
| Band number | Molecular weight (kD) |
| 1 | 37 |
| 2 | 25 |
| 3 | 20 |
| 4 | 15 |
| 5 | 10 |

8. Identify the bands in the remaining lanes. Logger *Pro* will record the distance migrated and the molecular weight of each band. Each band will also be plotted.
   a. Click Add Lane, , and choose Add Lane.
   b. Click the center of the first band in the first experimental lane. Notice that when you click, three things happen: a marker with a distinct shape and color is placed on the photograph, a matching marker is placed on the standard curve of the graph, and the distance and molecular weight are added to the data table.
   c. Click the center of the next band in this lane.
   d. Continue this process for each visible band in the experimental lane.
9. Repeat Step 8 for each remaining lane.
10. Double-click the base pair column of the Standard Ladder data table. A Column Definitions window will appear. Change the Name to **Molecular Weight.** Change the Short Name to **MW**, and the Units to **kD**. Click Done.
11. Double-click the base pair column for Lane 2 in the data table. A Column Definitions window will appear. Change the Name to **Molecular Weight.** Change the Short Name to **MW**, and the Units to **kD**. Now select the Options tab at the top of this window. Change the displayed precision to 2. Click Done.
12. Repeat Step 11 for the remaining lanes.
13. Double-click the Column Heading, Lane 2. Rename the Data Set with the name of the sample (e.g., Catfish). Repeat this process for the remaining lanes. The screen should now resemble Figure 7.




| | Standard Ladder | | Species 1 | | Species 2 | | Species 3 | | Actin / Myosin | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Distance (mm) | MW (kD) | Distance (mm) | MW (kD) | Distance (mm) | MW (kD) | Distance (mm) | MW (kD) | Distance (mm) | MW (kD) |
| 1 | 58.21 | 10 | 42.70 | 33.21 | 42.27 | 34.24 | 41.99 | 34.92 | 42.66 | 33 |
| 2 | 54.51 | 15 | 44.85 | 28.55 | 44.02 | 30.28 | 44.02 | 30.28 | 49.02 | 21 |
| 3 | 49.98 | 20 | 46.88 | 24.75 | 45.69 | 26.92 | 45.09 | 28.07 | 52.76 | 16 |
| 4 | 47.12 | 25 | 49.03 | 21.28 | 46.88 | 24.75 | 47.59 | 23.54 | 57.26 | 12 |
| 5 | 41.03 | 37 | 51.17 | 18.30 | 47.71 | 23.34 | 49.03 | 21.28 | | |
| 6 | | | 52.84 | 16.27 | 48.79 | 21.64 | 50.22 | 19.57 | | |

**Figure 7** *Sample Logger* Pro *screen with SDS-PAGE gel analysis*

14. Save and print the results of the gel analysis (optional).
15. Record the distance and molecular weight values in your data table. See the example below.

| Table 2: Example Data for Catfish | |
|---|---|
| Distance Migrated (mm) | Molecular Weight (kD) |
| 42.70 | 33.21 |
| 44.85 | 28.55 |
| 46.88 | 24.75 |
| 49.03 | 21.28 |
| 51.17 | 18.30 |
| 52.84 | 16.27 |
| 54.33 | 14.65 |
| Total number of bands | 7 |

## PART IV CONSTRUCTING A CLADOGRAM

From your gel you can create a cladogram based on proteins that the fish have in common. You can then determine whether your cladogram supports your predictions and/or matches the evolutionary relatedness of the fish species determined by morphological analysis in the evolutionary tree provided. Each protein band that a fish has in common with another fish is a shared characteristic. Cladistic analysis assumes that when two organisms share a characteristic, they had a common ancestor that had that characteristic, and this can be represented as a node on a cladogram with two branches coming from that node representing the descendent organisms.

In this exercise you will define the shared characteristics (i.e., make a list of all the different proteins in fish muscle), find which proteins (characteristics) are shared between fish, and construct a cladogram based on your data.

# PART IV PROCEDURE

**Determine which fish have each characteristic (protein)**

1. Create a data table that resembles Table 3.
   a. Find the first band that is equal to or less than 30 kD. Then find the distance this band migrated in mm. Next, find the band that is closest to or greater than 10 kD and find the distance this band migrated in mm.
   b. Create a column for distance migrated. The first row in this column should start at the distance that the band closest to 30 kD migrated. Each row should increase by 0.5 mm increments until it reaches the distance that band closest to 10 kD migrated.
   c. Create two columns for each fish sample. Label one column Band and the other column MW with units kD.
   d. For each sample, find the distance that each band migrated and mark it on the table. You may need to round to the closest distance. List the molecular weight for each band.

| Distance Migrated (mm) | Table 3: Example Data for Three Fish Species | | | | | |
|---|---|---|---|---|---|---|
| | Shark | | Catfish | | Salmon | |
| | Band | MW (kD) | Band | MW (kD) | Band | MW (kD) |
| 45 | X | 28.07 | X | 28.55 | | |
| 45.5 | | | | | X | 26.92 |
| 46 | | | | | | |
| 46.5 | | | | | | |
| 47 | | | X | 24.75 | X | 24.75 |
| 47.5 | X | 23.54 | | | | |
| 48 | | | | | X | 23.34 |
| 48.5 | | | | | | |
| 49 | X | 21.28 | X | 21.28 | X | 21.64 |
| 49.5 | | | | | | |
| 50 | X | 19.57 | | | | |
| 50.5 | | | | | | |
| 51 | | | X | 18.3 | | |
| 51.5 | | | | | | |
| 52 | | | | | X | 17.25 |
| 52.5 | X | 16.40 | | | | |
| 53 | | | X | 16.27 | X | 16 |
| 53.5 | | | | | | |
| 54 | | | | | | |
| 54.5 | X | 14.46 | X | 14.65 | | |
| 55 | | | | | X | 14.1 |
| 55.5 | | | | | | |
| 56 | | | | | | |
| 56.5 | | | | | | |
| 57 | X | 12.02 | | | | |
| 57.5 | | | | | | |
| 58 | | | | | | |
| 58.5 | | | | | | |
| 59 | | | | | X | 10.69 |

**Find the number of characteristics shared by each of the fish**

2. Compare the number of bands (Xs) in common with every other fish sample. You can find this using your data table (e.g., bands in common with other fish species are highlighted in Table 3). Write down the total number of bands that each fish species has between 30–10 kD. Then write down the number of bands that each species has in common with every other species.

3. Create a table that resembles Table 4 below.
   a. Make sure the row and column headings correspond to the species of fish that you used in this exercise (e.g., Shark, Catfish, Salmon).
   b. Find the total number of bands that each species of fish had in the 30–10 kD range (e.g, count the number of Xs in each Band column).
   c. Write this number in the cell that compares each species to itself (e.g., Shark *vs.* Shark). Highlight these cells in your table.
   d. Fill in the rest of the cells by listing the number of bands that each species has in common with every other species. (e.g, Shark has 3 bands in common with Catfish and 1 band in common with Salmon).

Table 4: Number of Bands in Common

| | Shark | Catfish | Salmon |
|---|---|---|---|
| Shark | **7** | 3 | 1 |
| Catfish | 3 | **6** | 3 |
| Salmon | 1 | 3 | **9** |

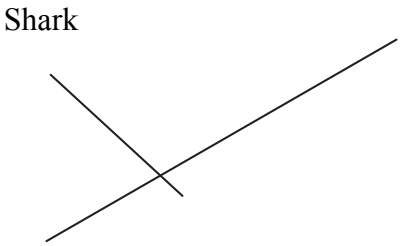
4. Convert each cell to the percentage of bands in common with each species (optional).
   a. Create a table that resembles Table 5.
   b. Make sure the row and column headings correspond to the species of fish that you used in this exercise (e.g., Shark, Catfish, Salmon).
   c. For each column in Table 4, divide the number in each cell by the number that you highlighted. Multiply this value by 100 to convert to percentage.
   d. You have now converted raw band numbers to the percentage of bands that are in common with each species.
   e. Write these percentages down in the appropriate cells in your new data table.

Table 5: Percentage of Shared Characteristics

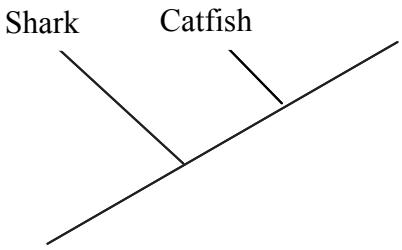
| | Shark | Catfish | Salmon |
|---|---|---|---|
| Shark | **100%** | 50% | 11% |
| Catfish | 43% | **100%** | 33% |
| Salmon | 14% | 50% | **100%** |

**Construct your cladogram**

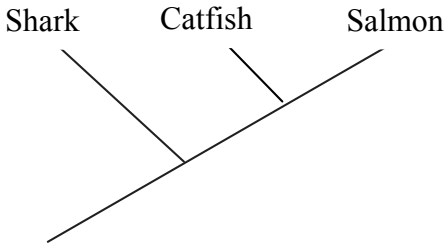
5. Now you are ready to construct your cladogram. First draw a line to form the trunk of your cladogram. We know that the shark is an ancestral species since it has only 1 band in common with the salmon and just 2 bands in common with the catfish. Draw a side branch off the line near the bottom of the trunk and label that branch shark. The node (where the side branch meets the trunk) represents an ancestor that is common to all the fish in this analysis.




4. Find the fish with the most bands in common with the other two fish. In this case it should be the catfish, since it has 3 bands in common with the salmon and 3 bands in common with the shark. Draw a side branch off the trunk near the middle and label this branch catfish. The node represents a common ancestor of the catfish.




5. Determine where the Salmon belongs on the Cladogram. In the data provided, the salmon has 3 bands in common with the catfish, but only one band in common with the Shark. So the salmon is more closely related to the catfish than the shark. This suggests that the salmon is more derived than the Shark or Catfish. Place the Salmon at the top of the main branch of your cladogram.



## QUESTIONS

1. Compare your cladogram with your original predictions.

2. What was the purpose of heating the samples?

3. How were the proteins extracted from the fish samples?

4. Why do SDS-coated proteins move when placed in an electric field?

5. What is the purpose of the actin and myosin standards and the Precision Plus Protein Kaleidoscope prestained standard?

6. What is the purpose of the stain?

7. List at least three common sources of the proteins actin and myosin, in addition to fish muscle.

8. List at least one researchable question dealing with the evolution of muscle proteins and gel electrophoresis.