

# PROTEIN GEL ELECTROPHORESIS

## INTRODUCTION

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In this lab, you will explore fish diversity by use of SDS-PAGE (Sodium dodecyl sulfate polyacrylamide gel electrophoresis). Based on the results of SDS-PAGE, you will construct an evolutionary tree that shows the relationship of five different fish. In the past, only visible traits were used for classification of species. Now, modern taxonomy (systematics) uses molecular biology as another tool to classify and show evolutionary relationships among organisms. DNA, RNA, and protein diversity are all used. For this lab, you will create a protein “fingerprint” of the five fish to create your evolutionary trees. The first part of this lab will involve extraction of protein from fish muscle. You will be given frozen tissue from five different fish to prepare samples for electrophoresis. The second part of this lab will involve loading and running the gels, followed by staining.

SDS-PAGE is similar to DNA gel electrophoresis in that it resolves different size proteins through a gel matrix as they move towards a positive electrode. Some of the differences are that 1) SDS is used to denature proteins and coat them so that they all carry negative charges. Recall that DNA is already negatively charged. Proteins, however, can have a variety of net charge, so in order for it to move through a gel, they must all be coated with SDS. SDS is included in Laemmli sample buffer, the type of buffer you will dissolve your proteins in. 2) The type of gel is a thin polyacrylamide layer sandwiched by two glass plates. Your gels are already cast and will be set up for you. The gels are vertical and have wells at the top end. This is where you will load your samples. 3) The staining procedure uses a blue stain called Coomassie Blue. It will require an overnight destain to visualize the gel. No ethidium bromide is used.

## PROTEIN EXTRACTION

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### MATERIALS

- 5 fliptop microfuge tubes
- 5 screwcap microfuge tubes
- 1 ml transfer pipet
- Fish samples
- Laemmli sample buffer
- 95 °C water bath

### PROCEDURE

1. To make this a blind study, assign a letter (e.g. A-E) to each fish sample to be investigated. Keep a record of which fish was assigned to which number. Label 5 fliptop microfuge tubes A-E.
2. Add 250  $\mu$ l of Laemmli sample buffer to each tube.
3. For each sample, obtain a piece of fish muscle approximately  $0.25 \times 0.25 \times 0.25 \text{ cm}^3$  and transfer it to the appropriately labeled tube.
4. Gently flick the microfuge tube 15 times with your finger to agitate the tissue in the buffer.
5. Incubate the samples for 5 minutes at room temperature to extract and solubilize the proteins.
6. Label screwcap tubes A-E.
7. Pipette out the liquid from fliptop tubes and transfer to screwcap tube with same letter. Screw down the cap. Heat your fish samples and the actin and myosin (AM) standard for 5 minutes at 95 oC to denature proteins and prepare for electrophoresis.

## **PROTEIN GEL ELECTROPHORESIS**

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### MATERIALS

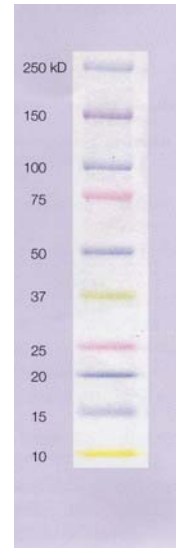
Fish protein extracts from lab one - 5 species  
Actin & myosin standard, 12.5  $\mu$ l - 1 vial  
Precision Plus Protein Kaleidoscope prestained standards, 6  $\mu$ l - 1 vial  
15%, 10-well, Ready Gel precast gel  
1–20  $\mu$ l adjustable-volume micropipet  
Prot/Elec pipet tips for gel loading  
Mini-PROTEAN 3 electrophoresis module (gel box)  
1x Tris-glycine-SDS (TGS) running buffer 500 ml per gel box  
Power supply (200 V constant) to be shared between workstations  
Sample Loading Guide – for 10 well comb  
Buffer dam (only required if running 1 gel/box)  
Staining trays - 1 per 2 gels  
Bio-Safe Coomassie stain for proteins- 50 ml per 2 gels  
Water bath set at 80–95°C 1  
Water for gel destaining (tap water is fine)

### PROCEDURE

1. Reheat frozen fish samples and actin and myosin standard at 95°C for 2–5 minutes to redissolve any precipitated detergent.
2. Using a fresh tip each time, load 10  $\mu$ l of each of your protein samples gently into the middle 5 wells.
3. Using a fresh tip, load 10  $\mu$ l of the actin & myosin standard gently into an available well.
4. After loading, 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 about 30 minutes. Watch for the separation of the standard. Run gels until blue dye front is at the end of the gel.
5. When gels are finished running, turn off the power supply and disconnect the leads.
6. Remove the lid and lift out the electrode assembly and clamping frame.
7. Pour out the running buffer from the electrode assembly. Open the cams and remove the gel cassettes.
8. Now it's time to stain the proteins in your gel. Lay your gel cassette flat on the bench with the short plate facing up. Cut the tape along the sides of the gel cassette. Carefully pry apart the gel plates, using your fingertips. 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.
9. If there is sufficient time, rinse the gel 3 times with tap water for 5 minutes by carefully pouring out the water and replacing it.
10. Carefully pour out the water and replace with 50 ml of Bio-Safe Coomassie stain per 2 gels.
11. Allow the gels to stain for 1 hour, with shaking if available.
12. After 1 hour discard the stain and replace it with a large volume of water to destain the gel overnight with rocking action if available.
13. Your gels will be photographed and posted online. You will have a chance to view your gels next lab period.

**ANALYSIS**

Find your Kaleidoscope standard lane on your gel and locate the bands based on the figure to the right. Locate your actin and myosin standards. These muscle proteins are highly conserved and should be the same in all of your fish lanes. Myosin heavy chain is 210 kD and actin is 42 kD. Myosin light chains vary from 15-25 kD. These may appear as a smear. If you do not see these proteins in your lanes, you may have not properly extracted your muscle proteins. You will use other muscle proteins that are more variable to construct your cladogram. These proteins tend to fall in the small molecular weight range of 10-30 kD.



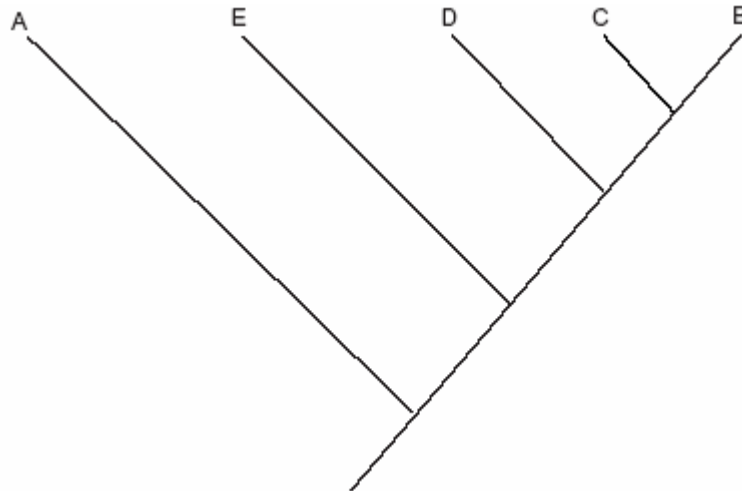
Since you will be focusing on low weight proteins from 10-30 kD, be sure that you can identify the standard bands 10 kD to 37 kD. Measure all bands in each lane of your gel between 10 kD and 30 kD and put distances in the table below. Create a standard curve using your Kaleidoscope standard data. Use a log scale. Insert a trendline with a formula so that you can estimate band sizes for other lanes. Calculate estimated fragment sizes and input in the table below.

Based on the similarity/differences of bands in each of the 5 fish lanes, you will construct a cladogram.

**Table 1. Estimating band sizes**

Kaleidoscope standard		A		B		C		D		E		Actin and Myosin	
Dist. (mm)	Actual kD	Dist. (mm)	Est. kD	Dist. (mm)	Est. kD	Dist. (mm)	Est. kD	Dist. (mm)	Est. kD	Dist. (mm)	Est. kD	Dist. (mm)	Est. kD
	37												
	25												
	20												
	15												
	10												

A cladogram shows the relationship among several species. Traditionally, only physical characteristics were used, but now molecular data has become commonly used. A well-constructed cladogram has branchpoints that only give two possibilities (dichotomous). The lower the branchpoint, the earlier the split occurred in evolutionary time. So think of time as on a vertical axis. This is also why organisms that exist today are all listed on top. The very top can be thought of as present time. For example, in the cladogram below, A, B, C, D, and E are all listed on top. C and B are more closely related to each other than D and B because the branchpoint that connects C and B is much higher than the branchpoint that connects D and B. The most distantly related organisms to all of the others would be A.



Based on your data, you will construct a cladogram similar to above. Your cladogram may have more branching than the one above and that is acceptable. Your goal is to best represent the relationship among all of your 5 fish. The best way to start is to use the table below to place each species in order of similarity to each of the other species. For instance, you want to know the order of similarity of A to all of the others. Use your gel and data in table 1 to compare the presence/absence of bands in each fish. Do this for one fish at a time. Once table 2 is complete, you can begin to construct a cladogram that satisfies all of the similarities and differences in the table. Use the actual name of the fish in your cladogram, not letters A-E.

Table 2: Similarity between fishes				
Fish	Most Similar	2 <sup>nd</sup> Most Similar	3 <sup>rd</sup> Most Similar	4 <sup>th</sup> Most Similar
A				
B				
C				
D				
E				

**QUESTIONS FOR YOUR REPORT**

1. (2 pts) Present an abstract of your work. Your abstract should include a brief description (1/2 page) of 1) what you set out to learn, 2) what procedures you performed, and 3) a summary of your data or discoveries.
2. Present your gel with all lanes properly labeled.
3. (2 pts) Present Table 1 and your Standard Curve.
4. (2 pts) Present Table 2 and your Cladogram.
5. In words, describe the logic you used to construct your cladogram based on Table 2.
6. (2 pts) If you wanted to know for sure you had myosin in each of the fish lanes, what technique would you perform? Describe the technique and how you would perform it to specifically identify myosin.