

# College of the Canyons Introduction to Biotechnology: Custom Lab



## Protein Analysis: Gel Electrophoresis

Version 8-12-12

- Protein electrophoresis makes a student appreciate DNA. Unlike DNA's constant charge to mass ratio, proteins are infinitely more varied and complex than DNA (or RNA).
- Small proteins may have strong charges, larger proteins have no charge, endless combinations of charge to mass ratios make separating proteins comparatively very complex.
- As if this were not enough, the properties a protein exhibits in an "electrophoretic field" (AKA gel box) can vary according to how the protein was isolated, pH and solubility, etc.
- So, to approach protein electrophoresis, we are going to look at a very specific set of factors.
- The types of proteins examined will be muscle proteins samples from aquatic organisms. The relative ease of extraction and purity of muscle tissue makes it ideal for this analysis.
- The use of different solutions to solubilize and extract the proteins will be investigated. Triton and SDS are two common detergents that impart very specific properties to the protein as they extract and/or solubilize.
- Using various gel polymers (agarose and acrylamide), the resulting proteins will be separated and the various strengths and weaknesses of the detergents and polymers revealed.
- Via actual tissue isolation and gel preparation the student can appreciate the effectiveness of each technique in providing meaningful gel data. For comparison purposes, a set of professionally prepared "fish standards" will be visualized to help students better understand the limits of resolution possible with PAGE technology. Both protein mass and quantity can be assessed provided gel images are of sufficient quality.
- Further analysis of data can be used to reveal forensic information, evolutionary relationships and countless other features regarding protein chemistry.
- The length of these bullets reflects the density of what you about to investigate!

## I. Objectives

1. To become familiar with operation of vertical gel electrophoresis rigs and observe the higher resolution afforded over horizontal rigs.
2. To understand why vertical rigs offer the researcher this greater levels of resolution.
3. Extract proteins from tissues using a variety of detergents and understand effects of detergent on protein properties, gel image, buffer system used, etc.
4. Examine gel images and extract as much information as possible. Compare personally prepared gel images to “textbook” quality gel images in order to assess effectiveness of technique and limits of technology with respect to quantitative and qualitative analysis of gel data.

## II. Background:

### ➤ A Forensic Examination Using Protein Diversity

Proteins, separated by gel electrophoresis, can be used to determine similarities and differences between tissue samples from various organisms. Due to the range of amino acids found in proteins, the process of isolating and separating proteins using gel electrophoresis is very complex with many mitigating factors. While there are additional factors, three primary factors that affect the gel image investigated in this lab are: a. tissue type analyzed, b. extraction / buffer system utilized, c. gel polymer. Again this is not a complete list: voltage, run time, protein quality and quantity, staining and other visualization techniques are but a few of the more compounding factors. Suffice to say, proteins make students often reflect happily on the relatively simple analysis of DNA (see previous lab). Here are few considerations regarding the system we have decided to look at.

**A: Tissue type:** Muscle tissue is examined as it relatively easy to extract from a variety of samples (fish, chicken, crab, etc). It is also relatively homogenous (not too mixed with other tissue) and easily obtained. Since many creatures have readily available muscle tissue it is commonly used in taxonomic analysis (blood is often used, but not animals have blood, sponges, sea jellies, etc.) as well as looking at the relatively simple range of muscle proteins in a typical tissue. While there is some diversity, most muscle tissue samples contain between 12-15 individual proteins. Depending on the technique used, sample freshness, etc, sometimes as few as 4 bands are visible to as many as a dozen plus bands. So when assessing the samples, see how many protein bands are visible with in a given sample. Also, compare the sample (say cod fish) in different gel types to see what each gel can help you visualize (band #, size, position, etc.).

*The following is a partial list of the proteins that may be apparent in your gel image.*

Tropomyosin	Spectrin	Troponin (T)
Actin	C protein	Myosin (light chain)
Titin	Nebulin	Troponin (I)
Dystrophin	Alpha-actinin	Troponin (C)
Filamin	Gelsolin	Thymosin
Myosin (heavy chain)	fimbrin	

**B: Buffers:** You are not alone in your frustration over buffers! There are numerous buffers, each with its own abbreviation, use, cost, etc. In addition to the range of buffers specific to a particular protocol, there are often a number of buffers used with in one protocol. To make things even more confusing, some buffers have multiple names, uses, and abbreviations. One persons’ running buffer will be another person’s

electrophoresis buffer. Also, many buffers have supplemental chemicals added to them, that affect the name of the buffer (and thus the protocol where the buffer is used). For instance, TG buffer (Tris / Glycine) may have SDS added to it (Sodium Dodecyl Sulfate). This TG/SDS buffer is commonly used in protein analysis where the size of the protein is being investigated.

So, for our lab...two buffer systems will be used. These two buffers come in one of two “flavors”. The buffers are enriched with Triton or SDS. Triton helps to dissolve the proteins, cell membrane, etc, but otherwise does not significantly change the protein’s chemistry. By comparison SDS dissolve proteins, cell membranes, etc, and also imparts a strong negative charge on the protein. Furthermore, the negative charge is roughly proportional to the size of the protein. In addition larger proteins take on lot of SDS, smaller proteins take in less SDS. Each of the below buffers is then enriched with the detergent (SDS or Triton) and therefore the protein is constantly bathed in the correct detergent (as well as other things that make the buffer specific to its function). The other name in the buffer helps one to understand what they are used for. Electrophoresis buffers allows for current to flow in the electrophoresis chamber. Sample buffer allow for initial protein extraction and solubilization. Loading buffer is colored and heavy. These features make the sample easy to handle and see when loading into the gel well. Here are some details that may help.

<b>Gel Set Up</b>	<b>Loading/sample buffer may be enriched w/</b>	<b>Electrophoresis Buffer</b>
Agarose w/comb @ end	SDS	Tris/Glycine Buffer w./SDS
Agarose w/comb in middle	Triton 100X	Tris/Glycine w/ Triton 100X
PAGE w/vertical gel rig	SDS	Tris/Glycine Buffer w./SDS

**C. Gel Type:** As you know, agarose can be used in a variety of concentrations. In additional to increasing the concentration, we will change the position of the gel wells in agarose gels to allow for proteins to migrate according to their initial charge. The PAGE gels have many more elements to it, and as a result, we will practice with this device before running our actual gel. As we read the practice exercises, we will get some more information on PAGE gels. Some elements of agarose gel electrophoresis are covered in previous labs FYI (DNA lab).

➤ **Vertical Gel Rigs**

Vertical gel rigs afford the researcher a greater level of resolution than can be generated in horizontal rigs. One reason for this is the relative ease and precision with which the gels can be loaded. As a result of gravity, the sample enters the gel in a more uniform manner, and as a result the bands that appear are clearer. In addition to this precision associated with loading, the gels can be prepared in a stratified manner. While stratified gels can be prepared for use in a horizontal gel rig, by pouring and using the gel in the same orientation, associated imperfections can be minimized. Our particular vertical gel has even greater resolution due to the chemical it is prepared from. Agarose gels have a maximum concentration of about 5%. To put this another way, one can only dissolve so much agarose in a buffer. The upper limit is about 5 grams per 100 mls of solution or 5%. PAGE gels (short for polyacrylamide gel ) can have polymer concentrations of up to 30%. The reason for this high percentage is that the gel is polymerized in the gel rig (as

opposed to agarose, where the preformed polymer is just dissolved.) As a result of this polymerization, greater concentrations can be achieved.

As mentioned, the resolving power of PAGE gels is partially due to the fact that they may be stratified. A 5 % gel could be poured atop a 10% gel, which is then atop a 20 % gel. As a result, it is like having three gels in one! Many polyacrylamide gels (and the one we are using in this class) are pre-cast. This makes for better gels (as they can be done professionally under very exacting conditions). An additional benefit comes from the fact that the gel, once polymerized, is *relatively non-toxic*. Unpolymerized acrylamide is a potent neuro-toxin, and hence great care must be taken in lab when casting a PAGE gel.

➤ **Gel Electrophoresis Ideas**

After a practice run with the vertical gel rig, three gels will be used (1 set per team). Two gels are 4% agarose. One will have the comb placed near the side of the gel, and the other will have the comb placed near the middle of the gel. A third set of samples will be prepared for the vertical (PAGE) gel rig. See table two for clarification.

**Final Technical Note:** The two types of buffer systems are designed to treat the proteins in fundamentally different manners. Table one shows composition of the loading buffer for a Triton system verse Laemmli system. First we will comment on the fundamental difference between the two systems and lastly, as a review we will cover the remaining elements of the buffer to help review their roles.

The Laemmli system was devised to denature or “break down” the protein partially. Specifically, the proteins’ “higher-level structures” are destroyed as a combined result of a reducing agents (often beta mercaptoethanol or dithio-threitol DTT) will break many of the sulfide bonds that hold the more complex protein structural levels together. Additionally the SDS (sodium dodecyl-sulfate) inserts itself into the protein. The non-polar parts of the detergent insert themselves into the non-polar parts of the protein. Sticking out are the negatively charged sulfate groups. This imparts a negative charge. The protein’s higher-level structure (tertiary and quaternary levels) are now largely destroyed via the combined action of the reducing agent and the SDS undoing a number of the bonds that hold together the tertiary and quaternary bonds seen in many complex proteins. The original sequence of amino acids and some of the secondary structures (alpha helix and beta pleats) are still intact. So the protein will now act a lot like a piece of DNA. The protein now migrates towards the positive pole, as it is being pushed by the steady streams of electrons traveling from the black electrode to the red positive electrode. Larger pieces will move slower, smaller pieces, more quickly. This is all a results of the simplification of its structure and the stable charge to mass ratio imparted by the SDS. Subsequently, the mass of the protein can be accurately determined using SDS/PAGE gel electrophoresis with a Laemmli buffer system.

By comparison, the Triton system is more gentle on the protein. The non-ionic detergent only slightly alters the protein structure and has little if any effect on the protein charge. As a result, the protein can migrate in either direction (towards positive or negative pole). This type of protein configuration is called “native” (as opposed to the SDS treated proteins being “denatured”). The Triton system, thus leaves the protein much more intact, but isolation can be difficult as the protein is often only slightly different from many other proteins, and as a result, the purification process is often only

a fraction of what SDS can attain. As for the remaining parts of the loading buffer, the glycerol adds weight to help it sink into the gel well. The blue dye makes it easier to follow while loading and handling and the Tris is to help keep the pH stable and create an isotonic solution.

**TABLE ONE:**

<p><b>Laemmli Loading Buffer</b>            4% SDS            20% glycerol            10% 2-mercaptoethanol            0.004% bromphenol blue            0.125 M Tris HCl</p>	<p><b>Triton Loading Buffer:</b>            4 % Triton            20% glycerol            0.004% bromphenol blue            0.125 M Tris HCl</p>
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- **Loading buffer, the “gel rig” and electrophoresis buffer (Note: this information should be used LATER when you prepare the gels as needed).**

The first prepared gel will be an agarose gel poured with the wells at the end of the gel and use SDS electrophoresis buffer to dissolve the agarose.

A second agarose gel will be prepared using Triton 100 X electrophoresis buffer. The wells are located in the middle of the gel.

A third gel (PAGE gel) is prefabricated. Samples will be prepared with SDS sample buffer and ran with a SDS /reducing agent enriched electrophoresis buffer.

Please ensure that the fish samples are prepared with the correct sample buffer (and clearly labeled) as seen in sample preparation Step 1.9 in part 2. The following table may be helpful.

### **III.SOP/Lab Activities:**

Important: Always note any addenda that are posted by the instructor.

- **Practice run:**

- Using the information gathered from the demonstration, set up your vertical gel rig. Remember to add a small film of silicone to the rubber seal on the gel rig prior to inserting the gel cartridge. Be sure to remove the comb and the plastic tape/strip at the bottom front of the gel rig (before positioning the gel cartridge. This side (with tape) of the gel cartridge “faces out” or towards you. You can remove the tape after the gel rig is installed, but it is easier if the gel cartridge is in your hand. Fill the top chamber (AKA the one in the back) and check for leaks using TG/SDS electrophoresis buffer. Once no leaks have been confirmed (wait about 5 minutes), fill the bottom well and the top well to the appropriate levels (about 2-3 mm over the lower back plate in the “upper reservoir” and about 1 cm below the top of the “bottom reservoir”. Have the instructor come by and inspect your rig prior to loading samples. If you are interested, you can ask the instructor for a special PAGE gel loading pipet tip. Do not change the tips between samples (as this is only practice, but you can appreciate how these tips may be useful).

2. Into the wells created at the top of the gel, using the pipette tip, “wash” the well by aspirating up the electrophoresis buffer and expelling it back into the well. This will not only clean out the well, but also serve for practice for controlling the pipet tip as it sits between the two plates of the PAGE cartridge. Excess pressure can split the plates apart, and putting the pipet tip too far from the gel well while loading the sample may result in the sample not being loaded properly.
3. Using the practice dyes, load all of the wells with different samples and with different amounts of dyes (ranging from 5-40  $\mu$ l). *Caution: be sure not to press the pipette tip into the well at all (just rest the tip atop the edge of the gel cartridge, and the fluid will sink into the well as it is expelled) as this will cause separation of the gel plates!* This practice lab will only be mentioned briefly in your lab notebook, so exact sample loading information is not crucial.
4. Start the gel device at 50 volts. After 5 minutes, check to see if the samples are all even, no leaks, etc. If this is the case, increase voltage to 100 volts. As the sample runs, note any anomalies in the pattern (i.e. are all of the lanes running at the same rate?) Run the rig for about 1/2 an hour or until the samples have run out of the bottom of the gel.
5. Turn off the power supply and unplug the chamber. With a 10-25 ml serological pipette and pump, remove and save the buffer from the lower chamber. Once most of the buffer has been removed, loosen the bolts on the rig and the buffer in the back chamber will leak into the front chamber. Depending on time, use the pipet to remove the buffer from the bottom chamber. If you want, you can also pour the remaining buffer into the get buffer bottle (use a funnel and be prepared for some spillage). Dismantle the device (wash thoroughly with distilled water) and invert to dry. Replace the comb and tape, rewrap in plastic bag and store gel for later use.

➤ **A Protein Diversity Examination Using Gel Electrophoresis**

**Table 2: Needed Supplies / Equipment**

Materials	
Reagents	Equipment
Running Buffers (**)	Sample Buffer
4% Agarose or PAGE Gel	P-20 & P-200 Micropipettes
Pink or Blue Loading Buffer (**)	Pipette Tips
Crushed Ice	1mL Transfer Pipette
Electrophoresis Buffers (**)	Electrophoresis Chamber
3 sets of 0.25 cm <sup>3</sup> piece of Salmon, Cod, Krab, Shrimp, Swordfish, Tilapia. 2 with SDS and 1 with Triton	70 °C water bath 95 °C Water Bath
Clinical Micro centrifuge	Kalidoscope Protein Marker
Floating microfuge rack	1.5mL Microfuge Tubes w/locking caps when noted, very important!
1.5mL Microfuge Tubes	
White light box (with gel imaging system)	Staining trays, Code Blue Protein Stain Waterproof Marker

*Note: The following SOP pertains to both the vertical and horizontal gel rigs unless otherwise noted. (\*\*) refers to which buffers, which are specific to gel used and detergent.*

*## Fish samples may be changed, so see addenda for updates.*

## 1. Sample preparation (remember 2 sets in SDS and 1 in Triton 100 X)

- 1.1 Cut a piece of fish muscle about 0.25 cm<sup>3</sup> in size. Make three of each fish type for each student team. Make sure there is pure muscle tissue (no skin, scales, etc).
- 1.2 Into **labeled** (fish and detergent) 1.5 ml locking microfuge tubes, Add 200µl of sample buffer (**SDS or Triton**, NOT blue in color) and then the appropriate fish sample (label tubes accordingly) and vortex to mix.
- 1.3 Incubate for 5 minutes at room temperature. Flick the tube a few times during this time to help solubiize the sample. Next place samples at 95 degrees water bath for 3 minutes. Flick the tube a few times at the end of the incubation to help solubiize the sample (samples may be hot, so exercise caution).
- 1.4 Label appropriate # of 1.5 ml microfuge tubes with your initials and sample identification.
- 1.5 Take tubes with solubilized fish samples and spin at high RPM using clinical centrifuge (so use ones that got to 14,000 RPMs).
- 1.6 From each of the above tubes, aliquot 20 of sample buffer/ protein. **Take care to ONLY get supernatant! Into labeled, microfuge tubes.**
- 1.7 Change pipette tips to avoid contamination and avoid disturbing the pellet.
- 1.8 To each of the above tubes add 6 ul of the CORRECT loading buffer. The loading buffer has color and the reducing SDS buffer is blue and has a reducing agent (mercaptoethanol added) and is STINKY. The Triton buffer is pink and had no such smell. Note, if you are the first students to reach this step, please confer with the instructor and ensure that they add the reducing agent in the fume hood while wearing gloves.
- 1.9 Repeat steps 1-6 for each sample of tissue. A total of 18 samples (12 SDS and 6 Triton) will be prepared. Make sure to label both sample name and preparation (e.g. Salmon, SDS). Note: **This step is a reminder in case you forgot to do this for all samples!**
- 2.0 Check to see that the microfuge tubes are tightly capped. Place your samples in the 70°C water bath for 3 minutes to further denature the proteins. Cool to room temperature and freeze.
- 2.1 Each team should also prepare 10 µl of a protein standard by adding 7 µl of SDS or Triton loading buffer to 3 µl of “Kaleidoscope” Protein Standard. You will need 2 SDS standards and 1 Triton standard. For Triton sample, use Triton sample buffer.

### STOP POINT

Following protein preparation, samples may be stored at -20°C indefinitely. Make sure all samples are labeled with fish type and buffer type (SDS or Triton).

## 2. Casting the agarose gels

- 2.1 Prepare two gel trays for casting by pushing the gates into the *up* position. The screws holding the gates to the tray should be snug *but not tight*. If screws are too tight, the gates will leak.
- 2.2 Position the 8-tooth comb into the slots nearest the one end of the tray and one in the middle of the other(\*\*). Make certain the comb is seated on both sides. Set the tray flat on the table.

**NOTE:**

When preparing gels with SDS, Triton or any other detergent, you must take great pains to avoid any bubbles. As the gels contain a detergent, the bubbles formed from boiling will stay for a long time. Start and stop the microwave frequently. Swirl the liquefied agarose very gently and pour very slowly into the rig. Prior to pouring the gel, wait a few minutes to let the solution clarify. The bubbles will rise to the top and burst in the solution. So treat gently and watch closely.

- 2.3 Make the 4 % agarose in a microwave by dissolving 1.6 grams in 40 mls of electrophoresis buffer with SDS. Create another flask with Triton buffer and 1.6 grams of agarose. Keep them labeled and proceed to microwave. Set on high for 2 minutes and then heat at 30-second intervals. Again, watch for boil over and add 40 mls of SDS / agarose to one rig (with comb near side) and 40 mls Triton / agarose to the other rig (with comb in middle.)
- 2.4 Pour the agarose into the tray without creating any bubbles. If there are a few bubbles that have formed, try to remove the bubbles with a *clean* pipette tip. Bubbles and other inconsistencies in the gel can disrupt the flow of electrical current through the gel. Allow about 20 minutes for the gel to solidify. While the gels are solidifying, start work on the vertical gel rig (**step 3**).
- 3. Loading samples for electrophoresis: Remember to record what samples went where. Make sure EVERY partner in the team has this information. Add the fish samples in the same order, left to right, as listed on the previous page (salmon, cod, etc). Realize the PAGE gel can easily be confused as it looks very similar when viewed from either side.)**

**Vertical Gel Rig Loading:**

**When handling the PAGE gel directly, use gloves. The cartridge alone is not a problem.**

- 3.1. Attach vertical gel to box (remember to add a small film of silicone to the rubber seal on the gel rig prior to inserting the gel cartridge). Fill front and back chambers with Tris / glycine/SDS electrophoresis buffer and remove comb and tape.
- 3.2 Rinse all of the wells three times with 150  $\mu$ l of SDS electrophoresis buffer using a micropipette (it is not necessary to change the tip between samples).
- 3.3 For continuity's sake, add the samples in the same order that the agarose gel samples will be added. Ensure that every student has a copy of this information.
- 3.4 Load samples (20  $\mu$ l per lane) and the 10  $\mu$ l of protein Kalidoscope standard. Fill any unfilled lanes with 20  $\mu$ l of sample buffer. Run the gel @ 80 volts for 5 minutes and then 125 volts for 50 minutes, or until the dye is 1 cm from the bottom of the gel.

**While gel is running proceed to agarose gel system (next page...).**

**NOTE: If your group is familiar with running PAGE gels, please see the instructor for setting up a second PAGE gels with some prepared fish samples available from vendors.**

- 3.5 After gel has run the required amount of time, shut off the power and remove the electrophoresis buffer from the bottom chamber using the aforementioned technique.



3.6 Pour the buffer from the top chamber (using a funnel) directly into the container with the electrophoresis buffer (or use a pipet if you prefer). Once most of the buffer has been removed, remove the gel cartridge from the rig.

3.7 Rinse the rig using soapy water first and then distilled water, and invert it to allow it to air dry. As you prepare to touch the actual PAGE gel, remember to use gloves.

3.8 With the instructor's help, remove the gel from the casing (by inserting a spatula between the plates and twisting). Carefully separate the plates and note orientation. Nick the lower right corner (to help identify the gel lane's orientation, assuming the gel is in the same position as it is when it is in the cartridge, taped side facing you). **MAKE SURE YOU UNDERSTAND THIS IDEA!** Label a piece of tape with your group's names, gel information, date and affix to the **bottom** of a gel staining tray. To the tray, add 50 mls of Code Blue stain. Place lid on the tray and put tray onto oscillating shaker table as directed by instructor. The instructor will then stain and de-stain the samples.

#### 4. Agarose Gel Rig Loading

- 4.1 After the agarose gel has solidified, push the gates into the *down* position and carefully place the gel and rig it into the electrophoresis chamber so that the comb-end is nearest the negative (black) electrode (for SDS sample). For Triton 100 X sample, center gel comb between both electrodes as evenly as possible (remember to place gates in down position).
- 4.2 Using both hands, carefully pull the comb straight out of the gel being careful to avoid tearing the sides or bottom of the wells. Sometimes it helps to hold the gel down with a finger while pulling up on the comb.
- 4.3 For the gel with wells located near one end, use SDS Laemmli buffer, and for the gel with the wells located in the middle, use the Triton X 100 Laemmli buffer. Fill the chamber with buffer (\*\*\*) until the level is about 1-2 mm *above* the surface of the gel. Check the buffer level to be certain it is still covering the gel. *There should be no observable "dimples" above the wells.* Add additional buffer if necessary.
- 4.4 Place a new tip on a P-20 micropipette and adjust the pipette to 20  $\mu$ L. Depress the plunger to the *first stop* and aspirate up 20  $\mu$ L of the protein sample (SDS or Triton). Place tip above well on gel and slowly depress the plunger to expel the sample. Note lane position of the sample in your notebook, and repeat with remaining samples and other gel (be consistent with all three gels with respect to sample / lane loading and note any addenda on the dry erase board). Load any empty lanes with 15  $\mu$ L of sample buffer (with dye; Triton or SDS as needed). Do not forget to add Kalidoscope standards as needed.
- 4.5 Close the cover tightly over the electrophoresis chamber. Connect the electrical leads to the power supply. Be certain that both leads are connected properly with the anode (-) to anode (black to black) and cathode (+) to cathode (red to red).
- 4.6 Before turning the power supply "on," rotate the voltage adjustment *counter-* clockwise to set the voltage to zero. Turn on the power supply. Set the voltage to 100v.
- 4.7 When the blue tracking dye has moved to within 1-2 cm of the far edge (+ end, red plug) of the gel, set the voltage back to zero, and turn the power supply off. The box will be foggy, so to see the blue dye, look through the side of the gel box. The dye in the gel will be visible as you look through the buffer solution. The Triton gel will be done in roughly half of the time of the SDS gel FYI. Unplug the electrical leads *from the power supply* by grasping the plugs, not the cords. Remove the cover of the electrophoresis chamber. Sometimes it is hard to see the gel through the lid (foggy,

- soap bubbles, etc). This said, look through the side of the gel box, where you can usually see the dye as it sits in the gel box.
- 4.8 Carefully remove the gel and rig, using the rig to support the gel. Slide the gel off the rig and place your gel into the plastic tray designated for staining. Label with tape as done for the vertical gel. Take the gel and tray to your instructor for staining.
  - 4.9 Stain the gels for 4 hours in Code Blue gel dye.
  - 4.10 De-stain all of the gels in D.I. water. Put the destain solution into appropriate waste container. Change the water episodically and keep gel tray on a revolving table, spinning at very low RPMs (n do not damage the gel, especially the PAGE gel).
  - 4.11 Use **gloves when handling** PAGE gels. Once the destaining is complete, place PAGE gel into tray and cover with about 50 ml of gel-drying solution (45% ethanol, 5% glycerol). **Do this step in the fume hood.**
  - 4.12 Wet two cellophane sheets in the above “gel-drying” solution. Set one of the sheets on the drying rig and carefully place the PAGE gel atop this sheet. Use a spatula and never lift the gel by its edge. Try to center the gel on the drying rig and BE CAREFULL as gel is quite fragile. Place the second cellophane sheet atop the gel. “Burp” the air bubbles from between the sheets by rolling with a pencil or otherwise burping the bubbles out. Place the other half of the rig onto the sheets and secure with 3 to 4 clamps.
  - 4.13 Label rig with your group name, and place in the fume hood overnight to dry. CAUTION: THE FUMES ARE NOXIOUS, SO DO NOT DRY IN THE SAME INCUBATORS THAT CULTURES ARE STORED IN.
  - 4.14 Take the dried PAGE gel and both agarose gels to the gel imaging system. The white light filter is needed, and take a few minutes to familiarize yourself with the gel imaging system (there are at least two different gel imaging systems FYI). By adjusting the contrast, light source, exposure time, etc. a reasonable quality gel image can be produced. Make enough copies for all of the members in your group. Put the gel images into you lab notebook and **completely** label the gel image. Email copies of ALL gel images to each student member of the team, and a copy to the instructor.

**IV. Post-Lab Questions/Activities:** The following post lab questions are for your benefit. The questions will help you to address a range of topics relating to the lab activity. Along with the post lab handouts, these questions will help to ensure that you have both correct information regarding the lab data and crucial lab processes. Complete the post lab questions at the end of the lab and post lab handouts (keys for both of these are available from your instructor) before making any lab-notebook entries.

1. Which gel, horizontal or PAGE, gave you the best resolution? Why?
2. What does Triton do to the proteins? How does this relate to the comb position in the agarose gels? What does SDS do to the proteins?
3. Why was the reducing agent added to the SDS/ sample buffer? What two specific chemical reactions are occurring and how do these alter the protein’s movement in the gel?
4. Decide which samples (fish, shrimp) are more closely related to the “Krab” (AKA Surimi, a fish product). Cite one piece of evidence from each agarose gel and three pieces of evidence from the PAGE gel to support your assertion.
5. Pick a high quality lane from the PAGE gel. You may want to use the image produced from the prepared fish samples on the PAGE gel. Using graph paper and the colored protein standards (mass available from the instructor) make a standard curve of protein mass verse distance migrated. Using interpolation, determine the molecular weight of the proteins in the lane you have chosen to analyze. Referencing the anatomy/physiology texts in class (or the list of proteins included at

the front of this lab manual) try to identify some of the proteins by name. Lastly, a graph of distance migrated versus molecular mass for protein is often less accurate than a similar graph created for DNA. Name three factors that may affect the distance migrated of a protein that would not expected to be a factor influencing DNA. (Hint, DNA has a stable charge to mass ratio, i.e. a piece of DNA twice as big will have twice the charge and it is always negative).

**V. Notebook Entries:** Data from the lab should be the focus of this section and if there are any incorrect results, you should discuss this, as well as expected results. Section V should be addressed in both your results and discussion. Your data should drive the discussion. An informed discussion is dependent on understanding the post lab questions/activities.

**Your intro should:**

- Role of detergents/gels in protein analysis.
- Muscle tissue rational as a model tissue.
- Muscle tissue proteins see in various fish samples (3-4).
- Gel resolution, types of gels, protein prep and affect on gel image.

**Results should be:**

- Three gel images, completely labeled (gel type, run time, lane info, etc).

**Discussion should consider the following:**

- What are the strengths and weaknesses of each gel image?
- On PAGE gel comment on strengths and weakness of gel image / PAGE technique. Type of fish used for Krab, evidence in each gel. SDS / molecular mass idea.

The previous lab protocol can be reproduced for educational purposes only. It has been developed by Jim Wolf, and/or those individuals or agencies mentioned in the references.

References:

Professor Martin Ikkanda, Pierce Community College, Woodland Hills, CA.