**Protein Fingerprinting with Agarose Gel Electrophoresis**

**Introduction:** Every cell in an organism has the recipe to make every single protein in that organism's body, but what proteins does each cell actually make? During this lab investigation, you will isolate and then separate the proteins from different bovine tissues (skeletal muscle, heart muscle, liver, etc.) using gel electrophoresis. The result will be a “protein fingerprint” for different cell types that you can examine to see if they may be making the same or different proteins.

**Equipment and Materials:**

*For the class:*

Hot water bath (70°C) or microwave

Hot water bath or heat block (95°C)

3% agarose in 1X Tris-glycine buffer

1X Tris-glycine-SDS buffer

Coomassie blue gel stain

Bovine tissue samples

*For each group:*

Horizontal gel electrophoresis apparatus, electrodes, and power supply

P200 or 50 µL fixed volume micropipette and 3 micropipette tips per sample

4 microcentrifuge tubes with 500 µL of sample buffer

4 empty 1.7 mL microcentrifuge tubes

Gel staining tray

**Pouring an Agarose Gel:**

1. Melt the bottle of 3% agarose in 1X Tris-glycine buffer by heating the bottle in the microwave until the solution is completely clear (i.e., no solid agarose remains). Alternatively, your teacher may have pre-melted the agarose, in which case, it will be kept melted in a 70°C water bath.
2. Seal both ends of the gel caster with stoppers or masking tape.
3. Slowly pour the melted agarose into the sealed caster – pour a thicker gel than you would for DNA gel electrophoresis because you will need to load more sample than normal.
4. Insert the comb near one end of the caster (the black/negative electrode if pouring inside the gel box).
5. Allow the gel to cool and solidify (10-30 minutes depending on gel size and thickness). In the meantime, prepare your samples.

**Sample Preparation:**

1. Label one sample buffer tube for each of the four tissues your group will be using.
2. Cut a small sample of each tissue (approximately the size of a pencil eraser or one of your fingernails) and put it into the corresponding sample buffer tube.
3. Gently shake the sample tubes and let them sit at room temperature for 5 minutes – make sure the tissue samples are submerged in the buffer before starting your timer (you may need to use a pipette tip to force the sample down into the buffer; you can also use the tip to try and macerate the sample a little).
4. Label four new 1.7 mL tubes – one for each tissue sample.
5. Pipette as much of the liquid as you can (not the tissue!) from your sample buffer tube to the corresponding new tube.
6. Incubate the new sample tubes at 95°C for 5 minutes in order to denature the extracted proteins before loading them on your gel.

**Gel Electrophoresis:**

1. When your gel has solidified, remove the stoppers/tape from the caster. Carefully remove the comb from the gel by pulling it straight up (do not pull at an angle as this will break the wells).
2. Add enough 1X Tris-glycine-SDS buffer to the gel box so that it covers the top of the gel, filling the wells without overflowing the gel box. Position the gel box in a suitable spot before loading your samples because once you start loading, you cannot move the gel box.
3. Use a micropipette to load as much of the first sample as you can into the well without overflowing the well (40-50 µL would be best). Repeat the process for each of your samples. Include a diagram of your gel below, labelling where you loaded each sample on the gel.
4. Connect the red/positive and black/negative wires to the appropriate electrodes of your gel box (black-to-black and red-to-red) and to their corresponding location on the power supply.
5. Set the power supply to 125 volts and run your gel for 20-30 minutes. The dye should travel 6-8 cm from the wells during this time.
6. Turn off the power supply and unplug the electrodes from the gel box.
7. Carefully remove the gel from its caster and place the gel in a staining tray.
8. Pour enough Coomassie blue stain into the tray to cover the gel completely. Be careful with the gel stain as it will also stain your skin, clothes, etc.
9. Cover your gel and stain it for at least 30 minutes; if you cannot see any protein bands after 30 minutes, continue to stain your gel until you do. You can also leave your gel in the stain overnight.
10. After staining, your protein bands may be robust enough to view on a white light box for analysis without needing to destain the gel (if so, proceed to the next step). However, you can increase the contrast between the protein bands and the surrounding gel for easier analysis by destaining the gel in water. To do so, use gloved hands to prevent your gel from coming out of the staining tray while pouring off the Coomassie blue stain (the stain is drain safe). Rinse the gel and the tray with water and pour off the rinse. Add enough water to the staining tray to cover your gel completely (the more water, the faster the gel will destain, but be careful not to overfill the tray, especially if you will be using an orbital shaker to make the destaining process more efficient). Your gel should be sufficiently destained overnight, but you can leave it in the water for several days without affecting the protein bands.
11. Place your gel on a white light box (or white paper) for easy viewing. Draw a picture of your gel bands below and label which sample was loaded into which well:



**Analysis:** Agarose gel electrophoresis separates proteins based on size. Each band (line) in the fingerprint represents a protein of a given size. There may actually be several different proteins of similar enough size that they are found in what appears to be one band on the gel; however, without further analysis with more advanced separation techniques (e.g., that use both size and electrical charge), we cannot tell how many proteins are really found in one band. For this activity, we will assume that each band represents a single protein.

1. Compare the protein fingerprints from the different tissues. What can you conclude about what proteins each type of cell makes?
2. Which tissues had the most similar protein fingerprints? Which tissues had the most different protein fingerprints?
3. Why would protein fingerprints from different types of cells look different? Why would they look the same?