Who’s Jeans Are These?

INTRODUCTION
Did you know that scientists believe the birds you see outside today may be related to dinosaurs!? How was this link made between 2 animals that look so different? Can a heart be grown in a lab and used in a human body? Today we are going to explore how these discoveries are made possible. But first, let’s play a game! 1) Get into groups of 3. 2) Designate someone to be a) DNA, b) mRNA/tRNA, c) rRNA, d) the environment and e) a hormone.

The student instructions for this game are included in the cover pages. The game uses linking toys to demonstrate DNA giving coding information to mRNA/tRNA. The mRNA/tRNA then gives the code and “amino acids” to the rRNA to make the protein. The “environment” (box hole size) changes after each successful protein to demonstrate how protein needs to change based on changing environment. Once a successful fish has been made, start the environment back to the beginning for the next fish. During the down time, DNA may practice with pipette skills using the protocol in the cover pages.

Once all groups have successfully sequenced two proteins (two life fish), have all groups return for general over-view discussion of the process and function of DNA, RNA and proteins.

- Below is a diagram that shows genomic and proteomic views of protein synthesis and modification.

![Diagram](image-url)

**Genomics**

- Genetic Code mutation
- Posttranscriptional Modifications: RNA Editing
- Alternative Splicing
- mRNA Synthesis/Degradation
- (DNA → RNA → Protein → Physical property)

**Proteomics**

- Posttranslational Modifications
- Protein: Gradient
- Protein interaction
- Cellular Location
- Muscle Composition and Contraction

- The genomic view has DNA alterations as the major source for hypothesizing changes in physical property.
- The proteomic view has posttranscriptional and posttranslational alterations as the major source for evaluation of genetic alterations resulting in changes in physical property.

Student Focus questions

1. If DNA is not directly interacting with the environment how does it know when to make changes? Messenger, such as some hormones, must go to the DNA and inform of change
2. Do similar protein profiles confirm that animals are related? (Students may assume yes however
profiles alone cannot confirm relatedness. Proteins can only provide support to additional data such
as fossil location, physical characteristics, etc.)

Yes, because protein comes from DNA.

No, because protein profile changes are caused by the environment and since environmental
changes are not based on a continuous function of time, profile similarities cannot be viewed as such.

3. Does a change in a protein always result in a benefit to the species? (Hint: Dead fish)

No, though the proteins fit the environment certain combinations in the “profile” did not prove
successful.

EXPLORATION-Part A  ***NOTE: The answers below will change based on which fish
you choose to use for the experiment. This is just an example of what may be readily
available at your local grocery store. If you change fish, make sure to change the
student guide to match.***

Materials:
• Computer with internet access

Procedures:
1. As a group, go to the FishBase website [www.FishBase.net] to research the following 5 different
species of fish (see attached data sheet).
2. Record data in the Fish Data Sheet below.

Hints:
1. For taxonomic classification: a) The 1st name is the class, c) The 2nd name is the order, and d)
The 3rd name is the family.
2. Anadromous – migrates to fresh water to spawn. For this example, these are labeled Fresh.
Student answers may vary, making variation in responses below.
3. Demersal & Benthopelagic – lives and feeds on bottom of water floor
4. Brackish – mixture of salt water and fresh, but more salt than fresh
5. Oceanodromous – fish that migrate within salt water only
6. Marine = Saltwater
7. Nekton = any freely swimming organism which can swims against the current.

Fish Data Sheet

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Rainbow TROUT</th>
<th>SALMON</th>
<th>TILAPIA</th>
<th>HALIBUT</th>
<th>RED SNAPPER</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mexico</strong></td>
<td><strong>Salmon</strong></td>
<td><strong>Ecuador</strong></td>
<td><strong>Alaska</strong></td>
<td><strong>Indonesia, wild</strong></td>
<td></td>
</tr>
<tr>
<td>Scientific Name</td>
<td>Orconhynchus mykiss</td>
<td>Salmo salar</td>
<td>Oreochromis mossambicus</td>
<td>Hippoglossus stenolepis</td>
<td>Lutjanus Semicinctus</td>
</tr>
<tr>
<td>Taxonomic Classification</td>
<td>Class: Actinopterygii</td>
<td>Class: Actinopterygii</td>
<td>Class: Actinopterygii</td>
<td>Class: Actinopterygii</td>
<td>Class: Actinopterygii</td>
</tr>
<tr>
<td></td>
<td>Order: Salmoniformes</td>
<td>Order: Salmoniformes</td>
<td>Order: Perciformes</td>
<td>Order: Pleuronectiformes</td>
<td>Order: Perciformes</td>
</tr>
<tr>
<td></td>
<td>Family: Salmon</td>
<td>Family: Salmonidae</td>
<td>Family: Cichlidae</td>
<td>Family: Pleuronectidae</td>
<td>Family: Lutjanidae</td>
</tr>
<tr>
<td></td>
<td>Genus: Orconhyncus</td>
<td>Salmo</td>
<td>Oreochromis</td>
<td>Genus: Hippoglossus</td>
<td>Genus: Lutjanus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>120 cm</td>
<td>120-150 cm</td>
<td>39 cm</td>
<td>258 cm</td>
<td>35 cm</td>
</tr>
</tbody>
</table>
Name _________________________ Date ____________________________________

<table>
<thead>
<tr>
<th>Size in cm</th>
<th>Fresh</th>
<th>Fresh</th>
<th>Fresh</th>
<th>Salt</th>
<th>Salt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh or Salt Water</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td># of dorsal spines</td>
<td>3-4</td>
<td>3-4</td>
<td>15-18</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Food source</td>
<td>Insects Mollusks Other fish</td>
<td>Other fish, mollusks, crustations, bugs</td>
<td>Other fish, phytoplankton</td>
<td>Other fish, crab, clam</td>
<td>nekton</td>
</tr>
</tbody>
</table>

3. Rank the fish in order of size from smallest to largest:
   1. Red Snapper
   2. Tilapia
   3. Salmon/Trout
   4. Halibut

4. List:
   Salt water fish:
   Halibut
   Red Snapper
   Fresh water fish:
   Tilapia
   Salmon
   Trout

5. Rank the fish in order of fewest to most dorsal spines:
   1. Halibut
   2. Red Snapper
   3. Salmon/Trout
   4. Tilapia

5. Try to sort the fish based on the type of food they eat. Record the type of food that these fish eat beside the word "Food", and place the name of the fish that eat the same type of food in the appropriate circle.
7. Use the previously collected data to determine the number of characteristics each fish has in common with the other and record your findings in the following table. There may be up to 7 common characteristics: 1) Class, 2) Order, 3) Family, 4) Size (within 10-15 cm of each other is considered common), 5) fresh/salt, 6) # of dorsal spines (within 4 is considered common), 7) Food source (at least 1 common food source is considered common). ADD up the total of commonalities.

<table>
<thead>
<tr>
<th></th>
<th>RAINBOW TROUT</th>
<th>SALMON</th>
<th>TILAPIA</th>
<th>HALIBUT</th>
<th>RED SNAPPER</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAINBOW TROUT</td>
<td></td>
<td>7</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>SALMON</td>
<td>7</td>
<td></td>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>TILAPIA</td>
<td>2</td>
<td>3</td>
<td></td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>HALIBUT</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>RED SNAPPER</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>12</td>
<td>14</td>
<td>10</td>
<td>9</td>
<td>7</td>
</tr>
</tbody>
</table>

8. Based on the data you collected, predict which fish species seems to be the LEAST likely to be related to any other species. WHY do you think this?
   Red Snapper; Has the least amount of similar characteristics.

9. Based on the data you collected, predict which two fish species are the MOST likely to be related. WHY do you think this?
   Salmon and Trout; These have the most amount of similar characteristics.

Before the students begin making their own cladogram, make a fake cladogram using something they have shown interest in and use this to help explain how to design a cladogram. For example, classify dogs based on number of wrinkles:

If using the above example, note that this is not an accurate cladogram for these dogs. It is simply a prediction based on 1 physical characteristic. Explain to the students about sub branches for those animals that show a close relationship with only one other animal who has a relationship with others on the trunk. In the example here, the Cane Corso is a type of bullmastiff. Also explain that it may be
unlikely their fish will have a sub-branch, but encourage thinking about the relationships. For the student fish cladograms, this is a prediction and not based on all the facts. If they can defend their cladogram, accept the diagram.

10. Now we are going to design a diagram to help us see the relationship of these animals. Follow the step-by-step instructions and draw your diagram below:
   a) Draw a “trunk”: diagonal line across the page from left to right in the upward direction.
   b) Identify the fish with the least in common with any other fish. ________________________
   c) Draw a branch 90° with the trunk near the bottom of the trunk and label with this fish’s name.
   d) Identify the 2 fish with the most in common. _____________________ and _________________
   e) Draw another branch near the top of the trunk and label the branch with one of the fish names and the very top with the other fish name.
   f) Determine the how closely related the last two fish are to the branches already placed. Make branches in the middle to show this relationship. An example is on the board to help and you are encouraged to ask for help if need.
   g) Once your diagram has been approved by the teacher, draw it on the board to share your predictions with others in the class.

11. What is this diagram called? Cladogram
12. How does your diagram compare to others in the class? Do they all agree?
   Answers will vary depending on how each group organized their information.
13. If they do not agree, why do you think they do not agree?
   Answer will vary and may reflect differences in data collection and interpretation.
14. Do you think these physical characteristics alone can be used to accurately classify animals into related groups? WHY or WHY NOT?
   Answer may vary and should reflect that physical characteristics may be up to interpretation and may not be accurate if used alone.

EXPLORATION - Part B

BEFORE THE LAB PREPARE THE FOLLOWING:

Prepare student reagents
1. **Fish:** Cut each fish sample into roughly 0.5 cm square chunks (one chunk for each group), place on card or plastic and label A–E for a blind study. Be sure to keep a record of what kind of fish is A–E. Place a pair of tweezers for each kind of fish. See the cover sheet for picture example.
2. **Laemmli sample buffer:** For each group, add 1.5 ml of Laemmli sample buffer into a 1.5 ml flip-top microtubes and label SB. Store at room temperature.
3. **Rehydrate Actin & Myosin Standard:** Add 500 μl of Laemmli sample buffer to the vial of actin & myosin standard and incubate at room temperature for 5 minutes. Transfer 12.5 μl of the
rehydrated actin and myosin sample to an “AM” labeled screw-cap tube (one tube for each group) and heat for 5 minutes at 95°C. Store at −20°C if it will not be used right away.

4. **Tris-Glycine-SDS (TGS) Running Buffer**: You will need ~850 ml of 1x running buffer per Mini-PROTEAN 3 gel box (one gel box runs 2 gels). Thus for 1 gel box you will require .5 liters. Mix 85 ml of 10x TGS with 765 ml of distilled water. Tip: you may want to prepare extra 1x TGS buffer in case your gel boxes leak after assembly. 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. This requires approximately 900 ml of 1x TGS buffer per gel box and is a more convenient fix than reassembling the apparatus mid-lesson. Store at room temperature.

5. **Precision Plus Protein Kaleidoscope**: Label 1.5 ml flip-top tubes “K”, one for each group. Add 6 µl of prestained protein standard Kaleidoscope into each tube. Store at −20°C.

A group’s set up might look like this:

**Protein Extraction from Muscle**

**Materials:**
Each of the following is required per class:
- Water bath set to 95°C
- 5 pairs of tweezers

Each of the following is required per group:
- 5 fish samples labeled A-E
- 5 screw cap microtubes (1.5 ml)
- Micropipette set at 250 µl (or 125 µl if larger pipette not available)
- 5 flip-top microtubes (1.5 ml)
- Permanent marking pen
- 1.5 ml Laemmli sample buffer in microtube
- Timer
- Mini-PROTEAN 3 gel box
- Ready Gel precast polyacrylamide gel
- 850 ml of 1x tris-glycine-SDS (TGS) running buffer in 1000ml beaker
- Gloves

**Procedures**: *(NOTE: The procedures with an X have been done by your teacher.)*

1. Check off each step in the box provided as you complete that step.

2. Label 5 - 1.5 ml flip-top microtubes with the letters A-E. There should be one labeled tube for each fish sample being prepared for electrophoresis and put gloves on.

3. Using the micropipette, add 150 µl of Laemmli sample buffer (blue fluid in microtube labeled “SB”) to each labeled flip-top microtube.

4. For each sample, obtain a piece of fish muscle (avoid skin, fat, and bones), and transfer it to the appropriately labeled microtube. Be sure to use different tweezers to pick up each.
5. Close the lid.

6. Make sure the muscle is submerged in the buffer and gently flick the microtube 15 times with your finger to agitate the tissue in the sample buffer.

7. Incubate the samples (let them sit) 5 minutes at room temperature to extract and solubilize proteins.

8. While you are waiting, label 5 – 1.5 ml screw cap tubes with the letters A-E.

9. After the 5 minutes, pour the buffer containing the extracted proteins (this is the liquid part in the flip-top microtubes), but not the solid fish piece, into a labeled 1.5 ml screw cap tube. Note: It’s not necessary to transfer all of the fluid to the screw cap tube, since only a small volume (<20 µl) is actually needed for gel loading. A micropipette may make this easier.

10. There is an extra microtube labeled “AM”. This is actin/myosin proteins. Since all fish have this protein, it will serve as a control to compare against.

11. Push all microtubes down into the holder so that the lids are level with the top of the holder and heat your fish samples and the actin & myosin (AM) standard in their screw cap tubes for 5 minutes at 95°C to denature the proteins in preparation for electrophoresis.

12. While you are waiting, assemble the Ready Gel cassette for electrophoresis as follows:
A. Set up Mini-PROTEAN 3 gel box and review the name of each component to the box. If it is already set up, make sure you know what each piece is called.

B. Prepare a Ready Gel cassette (polyacrylamide gel) by cutting along the black line on the bottom of the cassette with a razor blade and pulling off the plastic strip, as indicated on gel cassette.

C. Remove the comb from the Ready Gel cassette.

D. Place Ready Gel cassette into the electrode assembly with the short plate facing inward. Place a buffer dam or another Ready Gel cassette on the opposite side of the electrode assembly, with notch on buffer dam facing inward.
E. Slide gel cassette, buffer dam, and electrode assembly into the clamping frame.

F. Press down the electrode assembly while closing the two cam levers of the clamping frame.

G. Lower the inner chamber into the mini tank.

H. Completely fill the inner chamber with 1x TGS electrophoresis buffer, making sure the buffer covers the short plate.

I. Fill mini tank approximately 200 ml of 1x TGS electrophoresis buffer.

J. Place the yellow sample loading guide on top of the electrode assembly.
Preparing for the SDS-PAGE

General Principles of SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

This technique is widely used in molecular biology research to examine proteins to answer a variety of questions. For this lesson the molecular technique will answer the following…

• How many proteins are in my sample?
• What differences are there in the proteins from different organisms?
• How much protein do I have?

Materials: Electrophoresis - Gel loading, running, and staining

Each of the following is required per group:

- 5 fish protein extracts from exploration B1
- 1 actin and myosin standard (12.5 µl)
- Precision plus protein kaleidoscope prestained standard (6 µl)
- 1 adjustable-volume micropipette
- 7 pipette tips for gel loading
- Mini-Protean Tetra cell electrophoresis module (1 per 2 gels)

- 1x tris-glycine-SDS (TGS) running buffer (700 ml per gel)
- 1 Power supply (200 V constant) to be shared
- Staining/drying tray (1 per 2 gels)
- 1 small cup for trash

Procedure:

1. 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.

2. The yellow sample loading guide on the top of the electrode assembly will direct your pipette tip to the correct position for loading each sample in a well.

3. Record in which well of your gel you will load which of your samples in the table below:

<table>
<thead>
<tr>
<th>Well</th>
<th>Volume</th>
<th>Sample Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>empty</td>
<td>none</td>
</tr>
<tr>
<td>2</td>
<td>empty</td>
<td>none</td>
</tr>
<tr>
<td>3</td>
<td>5 µl Stds</td>
<td>Precision Plus Protein Kaleidoscope prestained standard (Stds)</td>
</tr>
<tr>
<td>4</td>
<td>*10 µl sample A</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>*10 µl sample B</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>*10 µl sample C</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>*10 µl sample D</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>*10 µl sample E</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>*10 µl AM</td>
<td>actin &amp; myosin standard (AM)</td>
</tr>
<tr>
<td>10</td>
<td>empty</td>
<td>none</td>
</tr>
</tbody>
</table>
4. Make sure your micropipette is set at 5 µl.
5. Load **5 µl** of Precision Plus Protein Kaleidoscope Prestained standard (labeled “K”) gently into well # 3 using a thin gel loading tip.
   
   **Note:** You must release the plunger of the micropipette very slowly, otherwise you will not pipette the correct volume.
6. Put the used tip in the small cup provided.
7. Change your micropipette to 10 µl.
8. Using a fresh tip each time, load **10 µl** of each of your protein samples gently into the wells designated in your table above.
9. Using a fresh tip, load **10 µl** of the actin & myosin standard gently into well # 9.
10. 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.

**STOP!**
Let’s talk about it…

This next section gives a direction for discussion while the samples are running...

This technique is widely used in molecular biology research to examine proteins to answer a variety of questions. For this lesson the molecular technique will answer the following:

- How many proteins are in my sample?
- What differences are there in the proteins from different sources?
- How much protein do I have?

1. **Inform** students that proteins must be treated in order to be evaluated through SDS-PAGE techniques. (denatured to linear chains and negatively charged)
2. Three factors used to treat proteins – **heat**, ionic detergent, and **reducing agents**. These completely disrupt the 2°, 3°, and 4° structures of proteins and protein complexes into the desired negatively charged primary 1° structure Figure 5. (You may use the linker tools from the pre-lesson game to display the protein confirmations below.)
• **Primary structure** = denatured linear chain of amino acids
• **Secondary structure** = domains of repeating structures, such as β-pleated sheets and α-helices
• **Tertiary structure** = 3-dimensional shape of a folded polypeptide, maintained by disulfide bonds, electrostatic interactions, hydrophobic effects
• **Quaternary structure** = several polypeptide chains associated together to form a functional protein

---

3. Once proteins have been treated and loaded into the gel, a current can be applied and the proteins will migrate as if they have equivalent negative charge densities. Mass then becomes the main variable affecting the migration rate of each protein. (Hint: $F=ma$)

4. SDS-PAGE reagents and process used to denature and evaluate proteins are illustrated below. (You may use the linker tools from the pre-lesson game to display the process below. The green tools are amino acids and the red represent disulfide bonds.)
Student Focus Questions

1. What was the purpose of heating the samples? (Tell student that other chemical such as DTT or BME may also be used to insure complete disulfide breakage through reduction reactions.)

Heat was used to unravel proteins into single strands.

2. How is SDS used to selectively separate proteins? (Hint: SDS coats proteins will negative charge.)

Because proteins are negatively charged the current that passes through the gel moves them in the negative cathode to positive anode direction.

3. Since proteins vary in size how do you expect them to move through the gel based on speed? (Hint: F = ma)

Since the current applied to the gel is constant (not changing) the smaller pieces will accelerate faster than the larger protein pieces.

☐ 11. 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.

☐ 12. Pour out the running buffer from the electrode assembly. Open the cams and remove the gel cassettes and place in staining tray.

☐ 13. Add 50 ml of Bio-Safe Coomassie Stain for 1 hour. If available place on a rocking platform during this.
14. After 1 hour discard stain and replace with 50 ml of tap water overnight (you may see bands within an hour).

15. Set in a tray to dry.

**FROM the BioRad Protein Profiler Guide:** The information from gels can be preserved in many ways. The wet gels themselves can be scanned or photocopied (taking care both not to tear the delicate gel and not to damage electrical equipment with wet gels). Alternatively, the gels can be dried between two sheets of cellophane (a cellophane sandwich). In contrast to delicate wet gels, dried gels are quite tough, which makes analysis much easier. In addition, they last for years, can be much more easily scanned and photocopied, and also give your students a physical record of their experiment. The GelAir gel drying assembly table and drying frames allow you to very easily put together a cellophane sandwich with 4 gels per sandwich. Additionally, if you have the GelAir drying oven, you can dry the gels in 2–3 hours.

The alternative to using the GelAir apparatus is to make your own cellophane sandwiches using plastic tubs and rubber bands. This method is more cumbersome, and without the gel drying oven, gels take 1–2 days to dry; however, this method gives perfectly good results. With regards to gel analysis, in the laboratory, scientists often use specialized scanning equipment to analyze gels, such as the GS-800™ densitometer, with specialized software such as Quantity One® 1-D analysis software which scans wet gels and determines the size, position, and intensity of every band on the gel. Your students will mimic the work performed by computers by performing post-lab analysis of their gel.

The GelAir Drying system has clear instructions on how to use this apparatus.

**LUNCH TIME!**
**CONCEPT DEVELOPMENT**

Using the ruler provided measure the distance each band for each species has traveled beginning 25 mm from the well. Place an X to indicate a band at a specific distance for each species. *The first several mm represent the actin and myosin in the samples and should be relatively similar. Starting measurements beyond this will help ensure data which will differentiate between species.*

<table>
<thead>
<tr>
<th>Distance Migrated (mm)</th>
<th>Species A</th>
<th>Species B</th>
<th>Species C</th>
<th>Species D</th>
<th>Species E</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>38</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>39</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>41</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>42</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>43</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>44</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>46</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>47</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>49</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>51</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>52</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>53</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1. By looking at the bands from SDS-PAGE molecular techniques a scientist can get a profile of the proteins in the sample. This is called Protein Profile.

2. In the table below both record how many bands each species has in common. This is similar to what you did for the fish characteristics earlier in the lesson.

<table>
<thead>
<tr>
<th>Fish Name:</th>
<th>Salmon</th>
<th>Trout</th>
<th>Tilapia</th>
<th>Halibut</th>
<th>Red Snapper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species A</td>
<td>Species B</td>
<td>Species C</td>
<td>Species D</td>
<td>Species E</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>A</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>C</td>
<td>A</td>
<td>C</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>D</td>
<td>D</td>
<td>A</td>
<td>D</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>E</td>
<td>E</td>
<td>E</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>TOTAL</td>
<td>TOTAL</td>
<td>TOTAL</td>
<td>TOTAL</td>
<td></td>
</tr>
</tbody>
</table>

3. Predict which two of your fish species are MOST likely to be related. Why do you think this?

All answers will depend on the results of the electrophoresis and the type of fish used. Here it should be Salmon & Trout

4. Which species is the LEAST likely to be related to the other fish species?

All answers will depend on the results of the electrophoresis and the type of fish used. Here it should be red snapper

5. In the table above, write the actual names of each species (given to you by your teacher) and make a cladogram in the space provided based on the protein profile. (Draw it on your wet/dry board also).

All answers will depend on the results of the electrophoresis and the type of fish used.

6. Does your cladogram using the protein profile match the cladogram you made using the physical characteristics of the fish species?

All answers will depend on the results of the electrophoresis and the type of fish used.

7. If not, how does it differ and why do you think this occurred?

All answers will depend on the results of the electrophoresis and the type of fish used.

8. Does your protein profile cladogram agree with others in the class? If not, why might this be the case?

There should be a significant amount of agreement between groups as the proteins are the same for each group. Band distance may vary between groups, but the same basic pattern should be observed.
9. Do you think the protein profile alone can be used to accurately classify animals into related groups? WHY or WHY NOT?

Answers will vary, but should reflect that the protein profile would be more reliable than observation of characteristics alone.

10. Do you think the protein profile and the physical characteristics that we looked at first could be used TOGETHER to accurately classify animals into related groups? WHY or WHY NOT?

Answers will vary, but should reflect that the use of both together would make the most accurate predictions.

11. Comparisons of **physical** characteristics and biological characteristics, such as **protein profiles**, can be demonstrated in a **Cladogram** to **predict** the lineage of organisms.
CONCEPT APPLICATION

Field Trip!! We are now going to see how protein profiles, such as the one we discovered to predict the relatedness of fish species, can be used in an engineering lab. On the way back, answer the following questions…

1. How were protein profiles used in the lab we observed? Be SPECIFIC in your description (i.e. not just ‘to make heart arteries’ or ‘tissue engineering’).

2. How does this compare with how we used protein profiles? (Think…the engineering lab did not compare species, but what did it compare?)

3. What other potential uses might tissue engineering have in our health? Be CREATIVE.
AUTHENTIC ASSESSMENT

You are a famous zoologist. While on a field study in Africa, you discover a new species of snake and want to add this new species to the current snake cladogram. Below is the article about the new species.

A new species of spitting cobra (Naja) from north-eastern Africa

(Serpentes: Elapidae)

Wolfgang Wuster1* and Donald G. Broadley2
1 School of Biological Sciences, University of Wales, Bangor LL57 2UW, Wales, U.K.
2 Biodiversity Foundation for Africa, P.O. Box FM 730, Famona, Bulawayo, Zimbabwe
(Accepted 26 July 2002)

Abstract
A new species of spitting cobra Naja nubiae sp. nov. is described from north-eastern Africa. The distinctiveness of the new species is confirmed by multivariate analysis of pattern and scalation data. Phylogenetic analysis of mitochondrial DNA sequences reveals the new species to be the sister taxon of N. pallida, but with considerable levels of sequence divergence relative to that species. The populations concerned had previously been assigned to N. pallida. The new species differs from N. pallida principally in having more than one dark band across the neck and under the throat, as well as a pair of spots under the throat. It occupies a disjunct range across Egypt, the Sudan, Chad, Niger and Eritrea, where it seems to occupy primarily relatively mesic habitats. Naja mossambica is more closely related to N. nigricollis than to N. pallida and the new species. A key to the African species of Naja is presented.

1. What will you name your new species? Answer will vary

2. Using complete sentences, give a description on how you will determine the classification of your new species and how you would display it.

Answers here will vary, but assess that 3 main concepts are present: 1) Review of physical characteristics, 2) review of molecular biological characteristics such as protein profiles, 3) displayed on a cladogram.