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Comparative Proteomics Kit I: Protein Profiler Module

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Protein Profiler Kit

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Is There Something Fishy About Teaching Evolution?

Explore Biochemical Evidence for Evolution

Why Teach Protein Electrophoresis?

- Powerful teaching tool
- Real-world connections
- Laboratory extensions
- Tangible results
- Link to careers and industry
- Standards-based

Scientific Inquiry

- Use of gel electrophoresis to fingerprint proteins
- Use of experimental controls
- Creation of standard curves and cladograms
- Interpretation of experimental results

Chemistry of Life

- Chemical and physical properties of proteins
- Protein structure (1°, 2°, 3°, 4°) and function
- Protein extraction techniques
- Chemistry of protein electrophoresis

Genetics

- DNA > RNA > protein > trait
- Molecular vs. morphological classification
- Posttranscriptional and posttranslational modification
- Phylogenetic and cladistic analyses

Evolution

- Cladistics and phylogenetic relationships
- Biodiversity and natural selection
- Evolution of adaptive traits
- Physical environment as selective force

Cell and Molecular Biology

- Eukaryotic cell structure and function
- Muscle structure and function

Environmental and Health Science

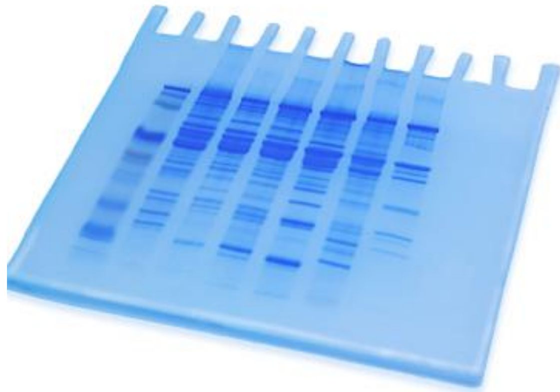
- Interdependence of organisms
- Detecting and analyzing variations in proteins
- Comparative proteomics

Comparative Proteomics I: Protein Profiler

Kit Advantages

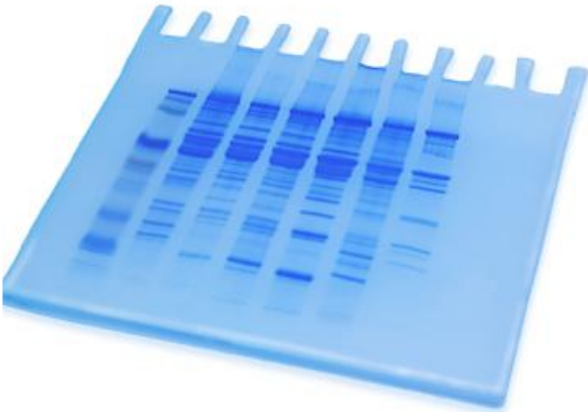
- Analyze protein profiles from a variety of fish
- Study protein structure/function
- Use polyacrylamide electrophoresis to separate proteins by size
- Construct cladograms using data from students' gel analysis
- Compare biochemical and phylogenetic relationships. Hands-on evolution wet lab
- Sufficient materials for 8 student workstations
- Can be completed in three 45 minute lab sessions

Workshop Timeline



- Introduction
- Sample Preparation
- Load and electrophorese protein samples
- Compare protein profiles
- Construct cladograms
- Stain polyacrylamide gels
- Laboratory Extensions

Traditional Systematics and Taxonomy



- **Classification**
  - Kingdom
  - Phylum
  - Class
  - Order
  - Family
  - Genus
  - Species
- **Traditional classification based upon traits:**
  - Morphological
  - Behavioral

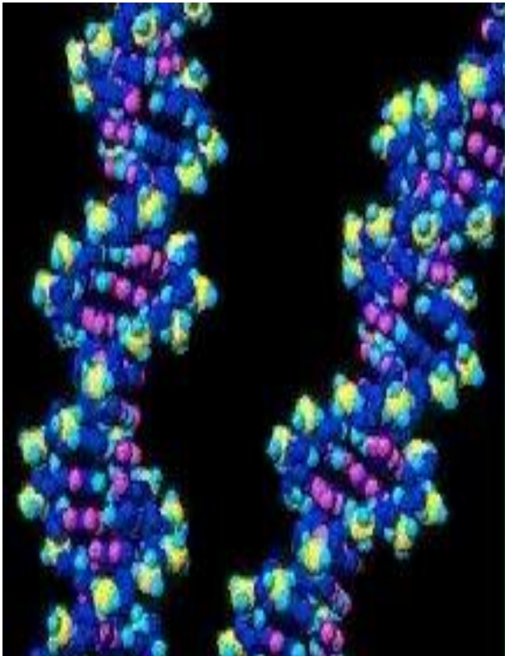
Can biomolecular evidence be used to determine evolutionary relationships?

Biochemical Similarities

- Traits are the result of:
  - Structure
  - Function
- Proteins determine structure and function
- DNA codes for proteins that confer traits

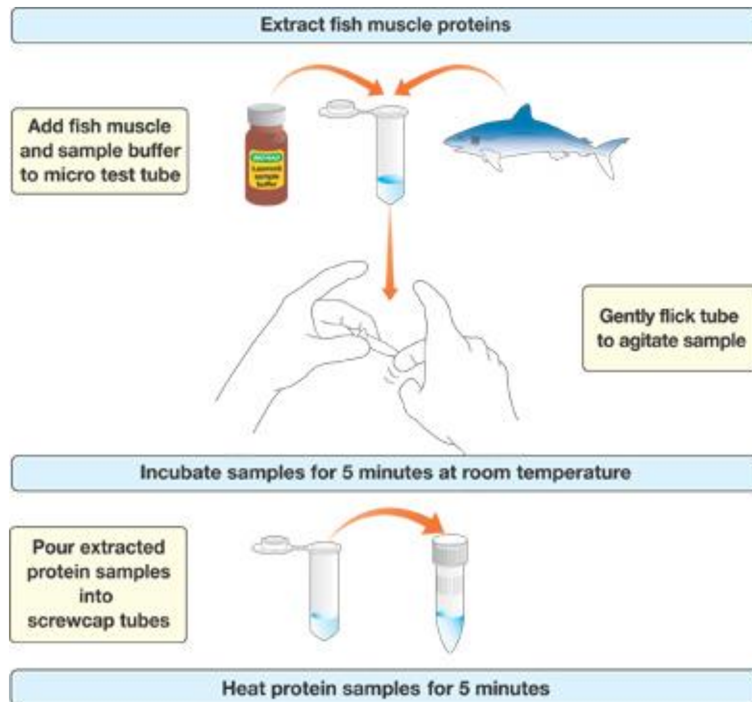
DNA→RNA→PROTEIN→TRAIT

Biochemical Differences

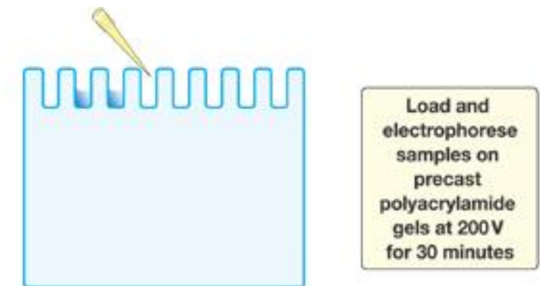


- **Changes in DNA lead to proteins with:**
  - Different functions
  - Novel traits
  - Positive, negative, or no effects
- **Genetic diversity provides pool for natural selection = evolution**

Protein Fingerprinting Procedures

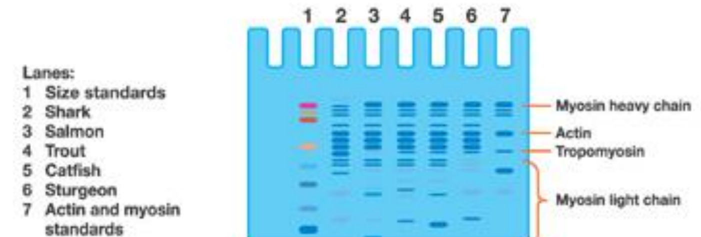


Day 1



Stain with Bio-Safe™ Coomassie and destain with water

Day 2



Analyze results and dry gels (optional)

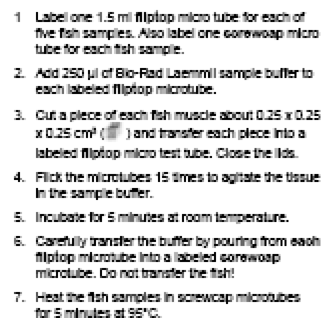
Compare molecular data to evolutionary tree

Day 3

Laboratory Quick Guide

Comparative Proteomics Kit I: Protein Profiler Module – Quick Guide

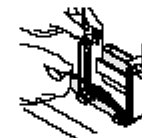
Lesson 1 Quick Guide



1. Label one 1.5 ml fliptop micro tube for each of five fish samples. Also label one screwcap micro tube for each fish sample.
2. Add 250 µl of Bio-Rad Laemmli sample buffer to each labeled fliptop microtube.
3. Cut a piece of each fish muscle about 0.25 x 0.25 x 0.25 cm³ ( ) and transfer each piece into a labeled fliptop micro test tube. Close the lids.
4. Flick the microtubes 15 times to agitate the tissue in the sample buffer.
5. Incubate for 5 minutes at room temperature.
6. Carefully transfer the buffer by pouring from each fliptop microtube into a labeled screwcap microtube. Do not transfer the fish!
7. Heat the fish samples in screwcap microtubes for 5 minutes at 95°C.

Lesson 2 Quick Guide

1. Set up Mini-PROTEAN 3 gel box and add 1x TGS electrophoresis buffer to the chamber.
2. Prepare a Ready Gel cassette 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.
3. Remove the comb from the Ready Gel cassette.
4. 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.



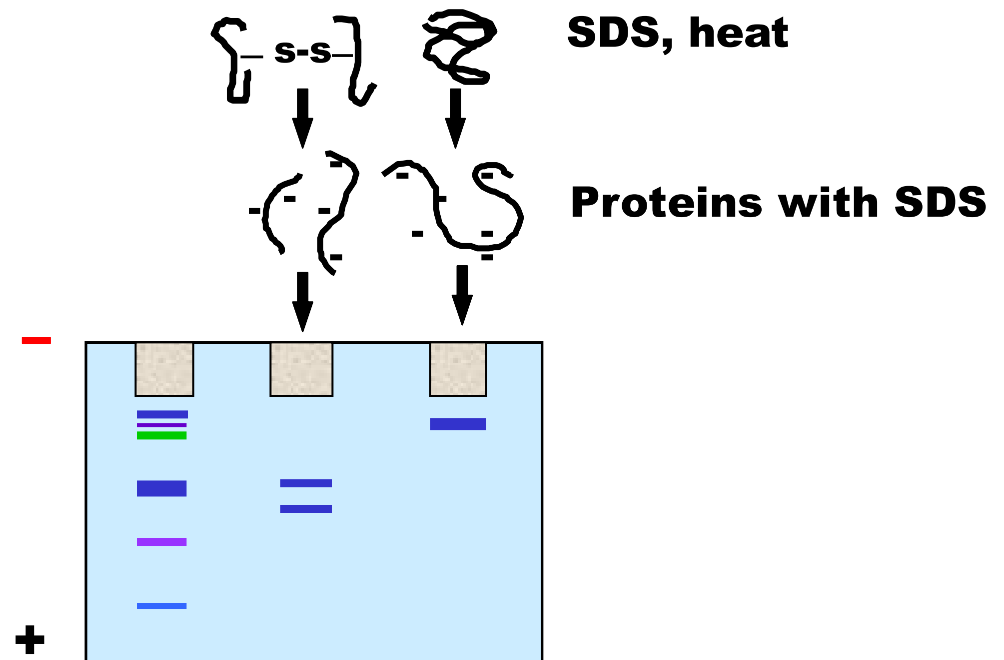
QUICK GUIDE

What's in the Sample Buffer?

- **Tris** buffer to provide appropriate pH
- **SDS** (sodium dodecyl sulfate) detergent to dissolve proteins and give them a negative charge
- **Glycerol** to make samples sink into wells
- **Bromophenol Blue** dye to visualize samples

Why **Heat** the Samples?

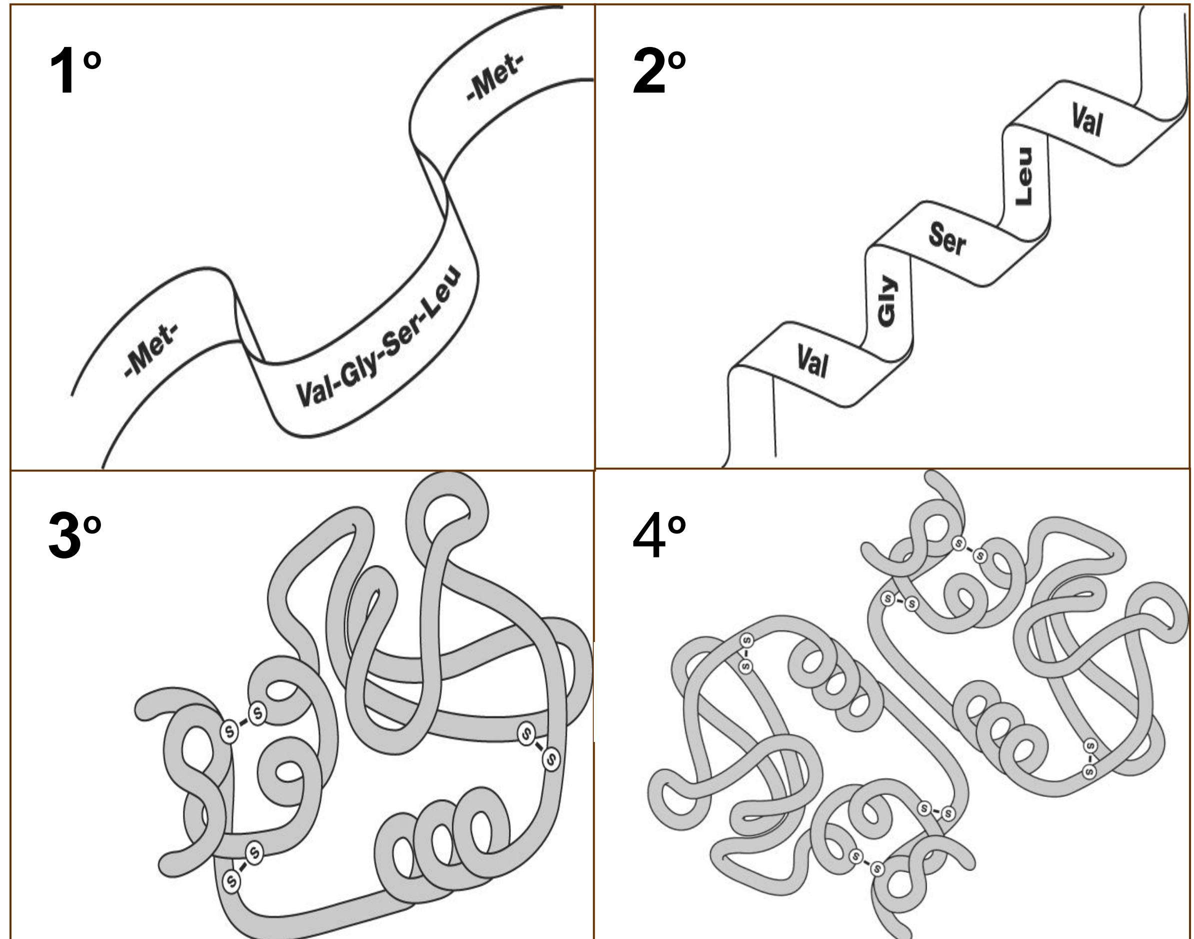
- **Heating** the samples **denatures** protein complexes, allowing the separation of individual proteins by size



Making Proteins

DNA	TAC	GGA	TCG	AGA	TGA
mRNA	AUG	CCU	AGC	UCU	ACU
tRNA	UAC	GGA	UCG	AGA	UGA
Amino Acid	Tyr	Gly	Ser	Arg	STOP

Levels of Protein Organization

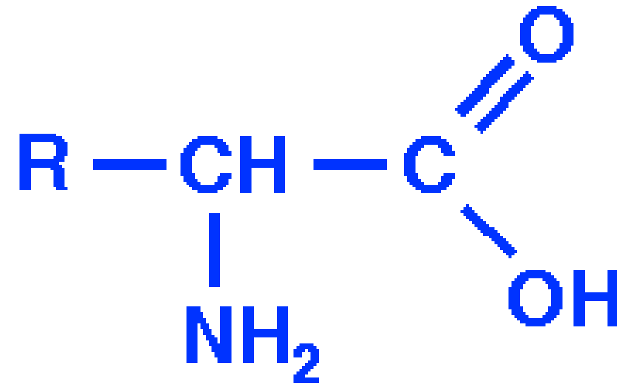


Protein Size Comparison

- Break protein complexes into individual proteins
- Denature proteins using detergent and heat
- Separate proteins based on size

Protein Size

- **Size measured in kilodaltons (kD)**
- **Dalton = approximately the mass of one hydrogen atom or 1.66 x 10^{-24} gram**
- **Average amino acid = 110 daltons**

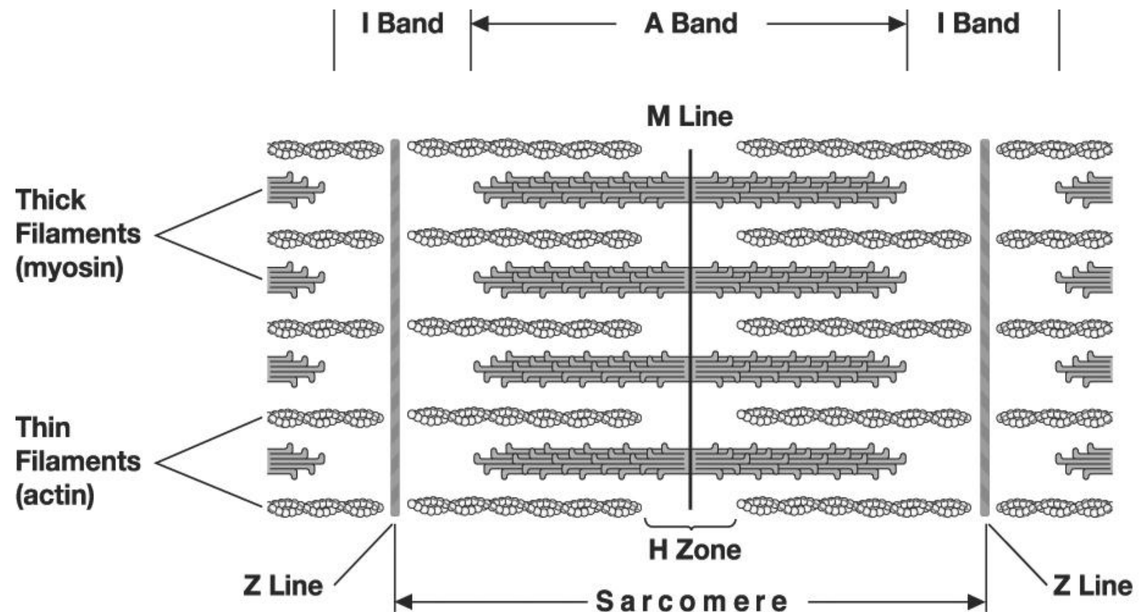


Muscle Contains Proteins of Many Sizes

Protein	kD	Function
Titin	3000	Center myosin in sarcomere
Dystrophin	400	Anchoring to plasma membrane
Filamin	270	Cross-link filaments
Myosin heavy chain	**210**	**Slide filaments**
Spectrin	265	Attach filaments to plasma membrane
Nebulin	107	Regulate actin assembly
α-actinin	100	Bundle filaments
Gelosin	90	Fragment filaments
Fimbrin	68	Bundle filaments
Actin	**42**	**Form filaments**
Tropomysin	35	Strengthen filaments
Myosin light chain	**15-25**	**Slide filaments**
Troponin (T.I.C.)	30, 19, 17	Mediate contraction
Thymosin	5	Sequester actin monomers

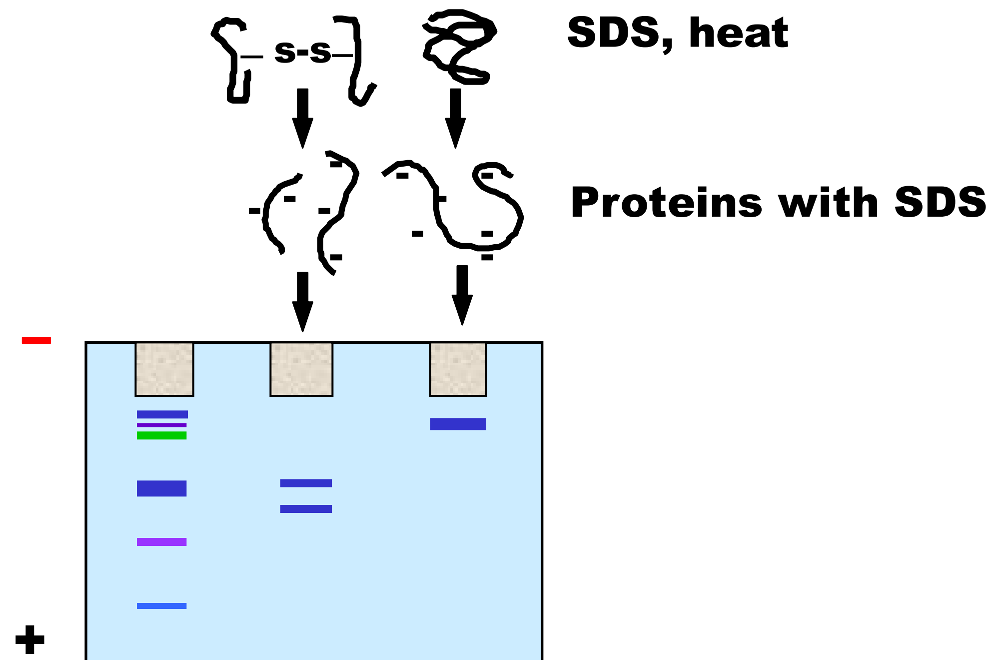
Actin and Myosin

- **Actin**
  - 5% of total protein
  - 20% of vertebrate muscle mass
  - 375 amino acids = 42 kD
  - Forms filaments
- **Myosin**
  - Tetramer
  - two heavy subunits (220 kD)
  - two light subunits (15-25 kD)
  - Breaks down ATP for muscle contraction



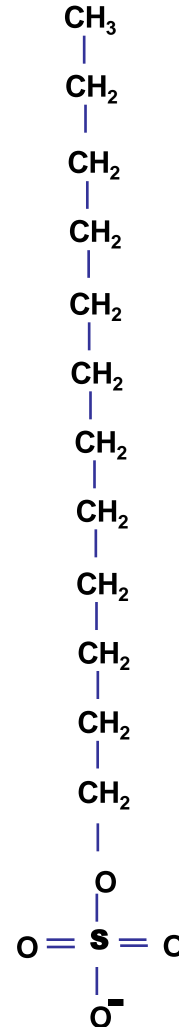
How Does an SDS-PAGE Gel Work?

- **Negatively charged proteins** move to positive electrode
- **Smaller proteins** move faster
- **Proteins** separate by size



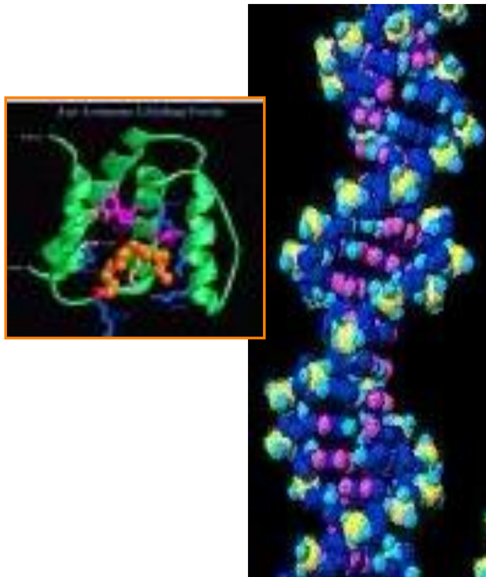
SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

- **SDS detergent** (sodium dodecyl sulfate)
  - **Solubilizes and denatures proteins**
  - **Adds negative charge to proteins**
- **Heat denatures proteins**



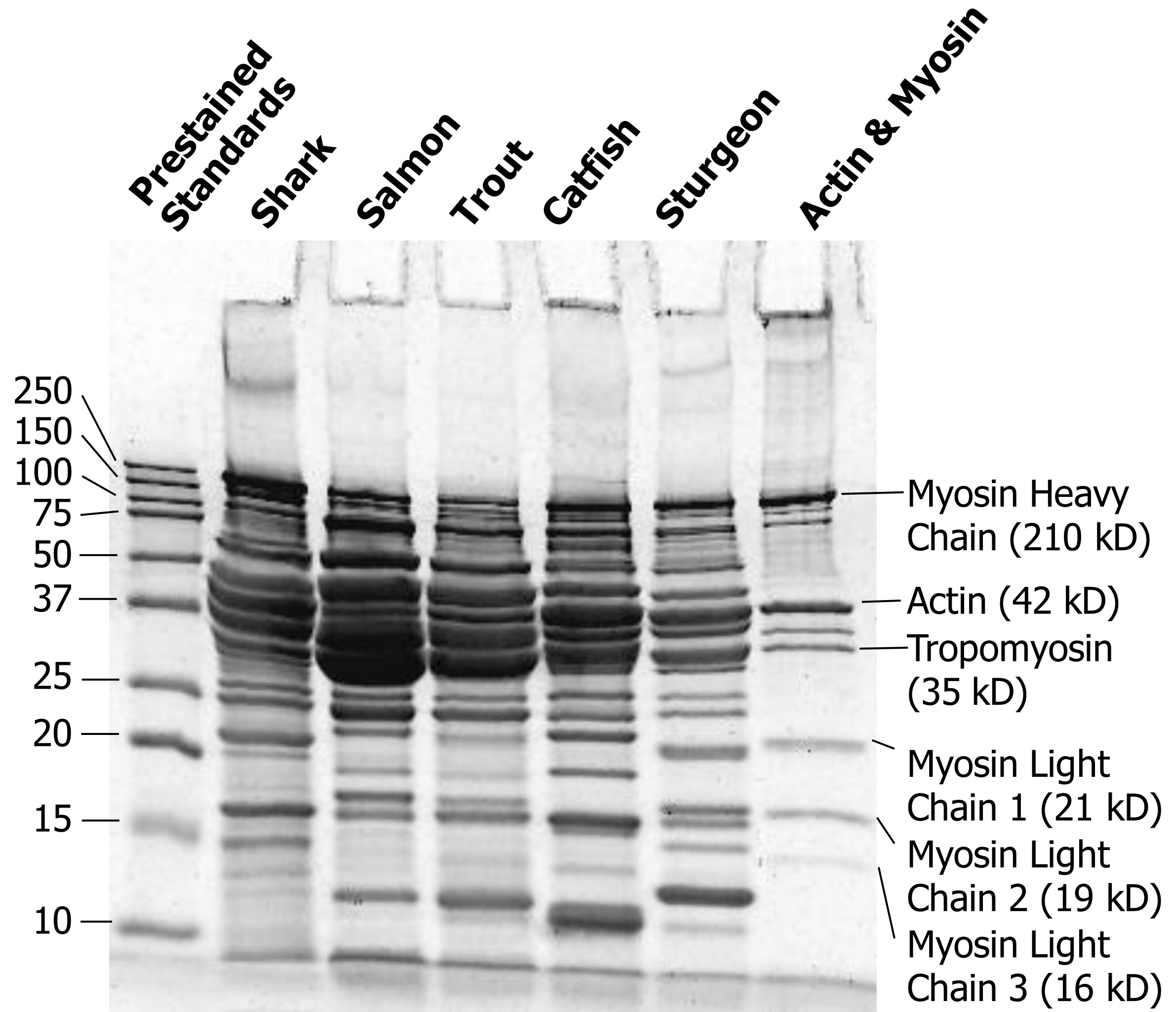
SDS

Why Use Polyacrylamide Gels to Separate Proteins?

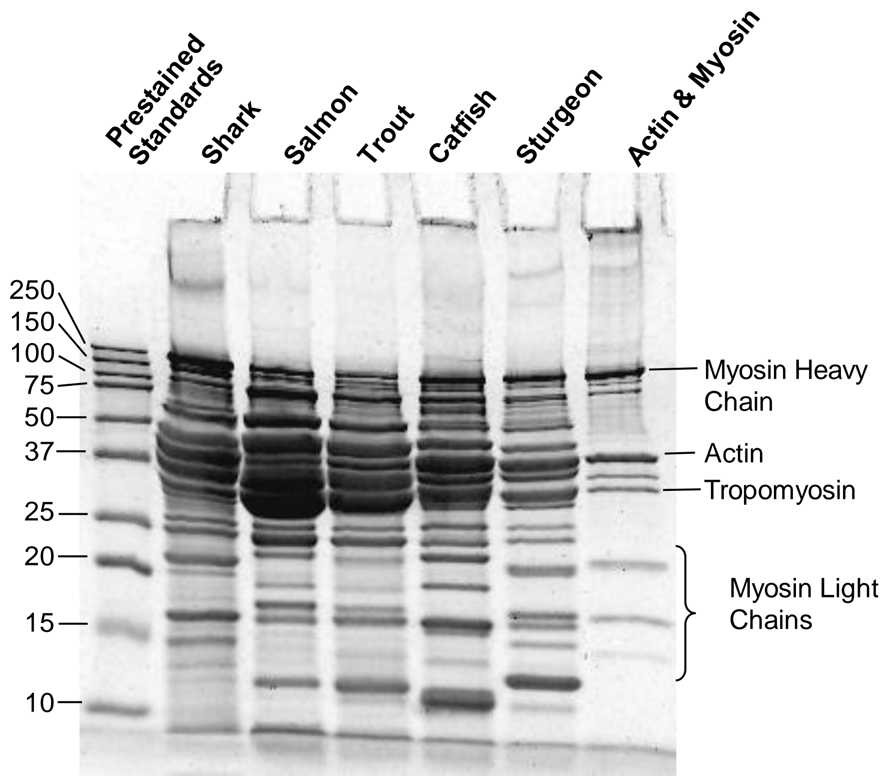


- **Polyacrylamide gel has a tight matrix**
- **Ideal for protein separation**
- **Smaller pore size than agarose**
- **Proteins much smaller than DNA**
  - Average amino acid = 110 daltons
  - Average nucleotide pair = 649 daltons
  - 1 kilobase of DNA = 650 kD
  - 1 kilobase of DNA encodes 333 amino acids = 36 kD

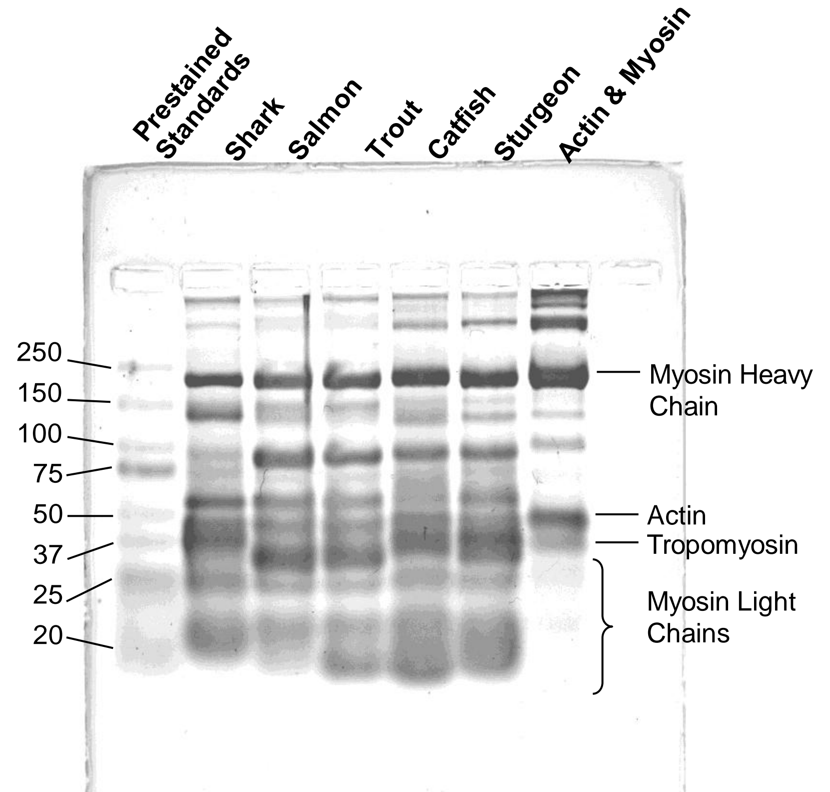
Polyacrylamide Gel Analysis



Can Proteins be Separated on Agarose Gels?

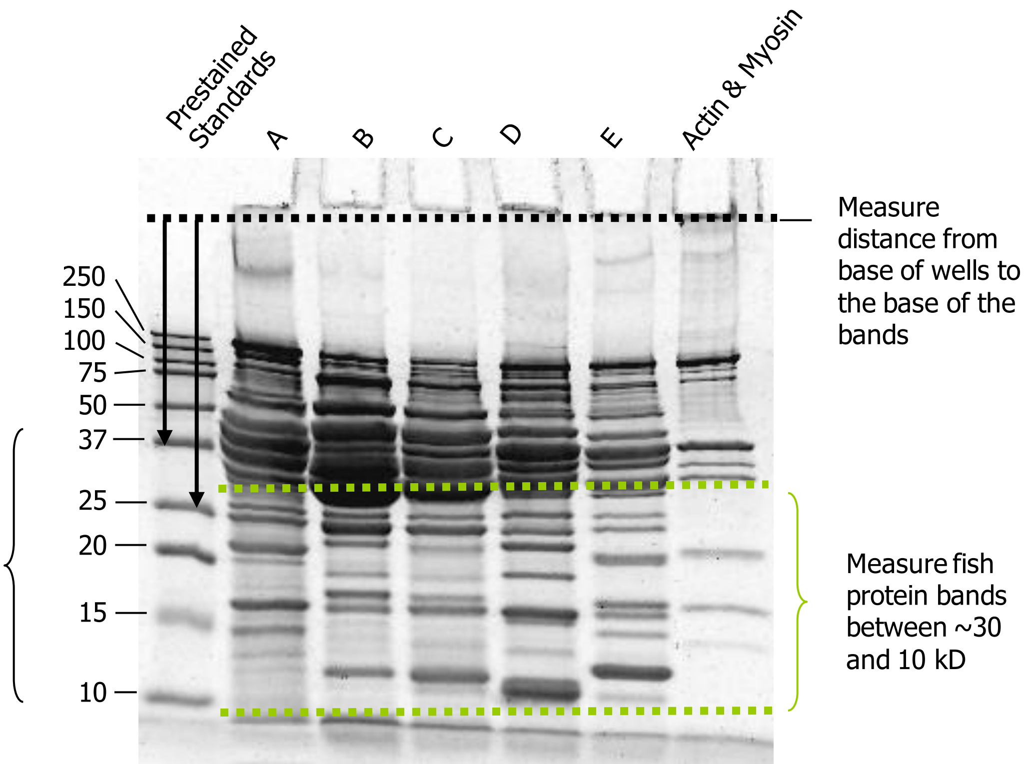


Polyacrylamide

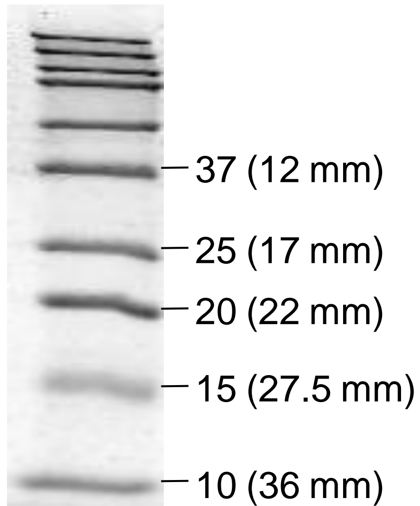


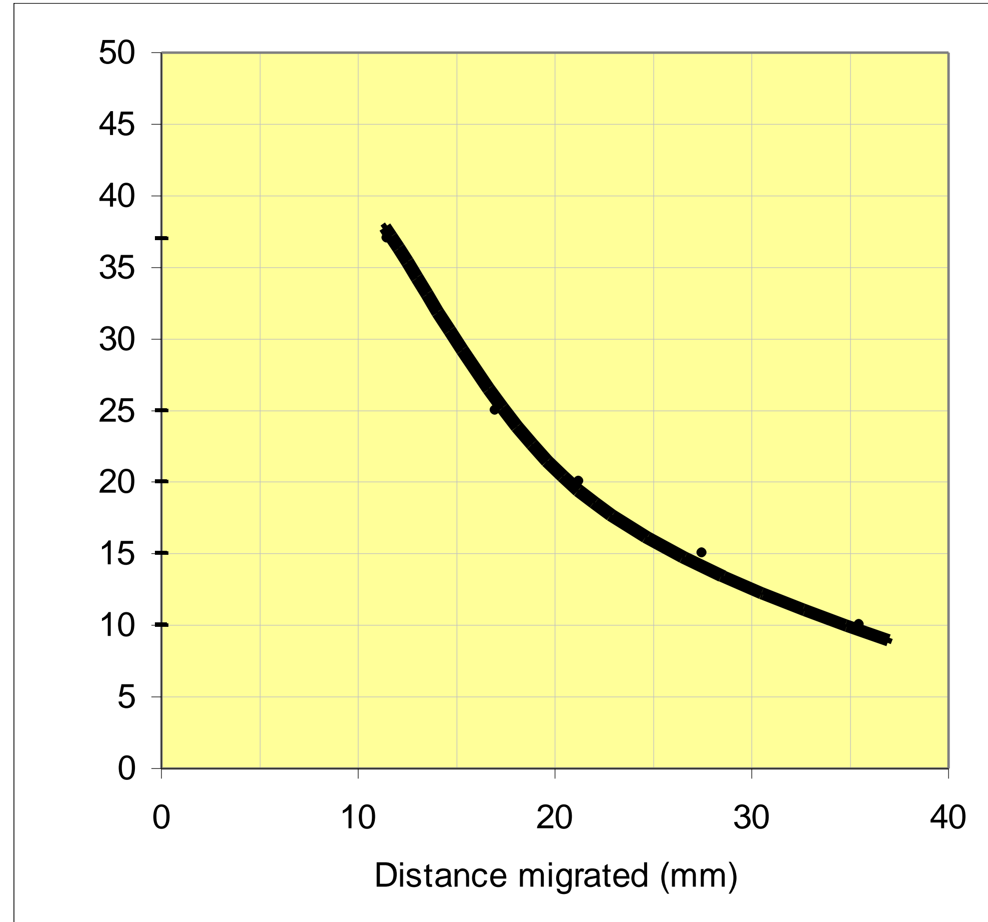
Agarose

Determine Size of Fish Proteins

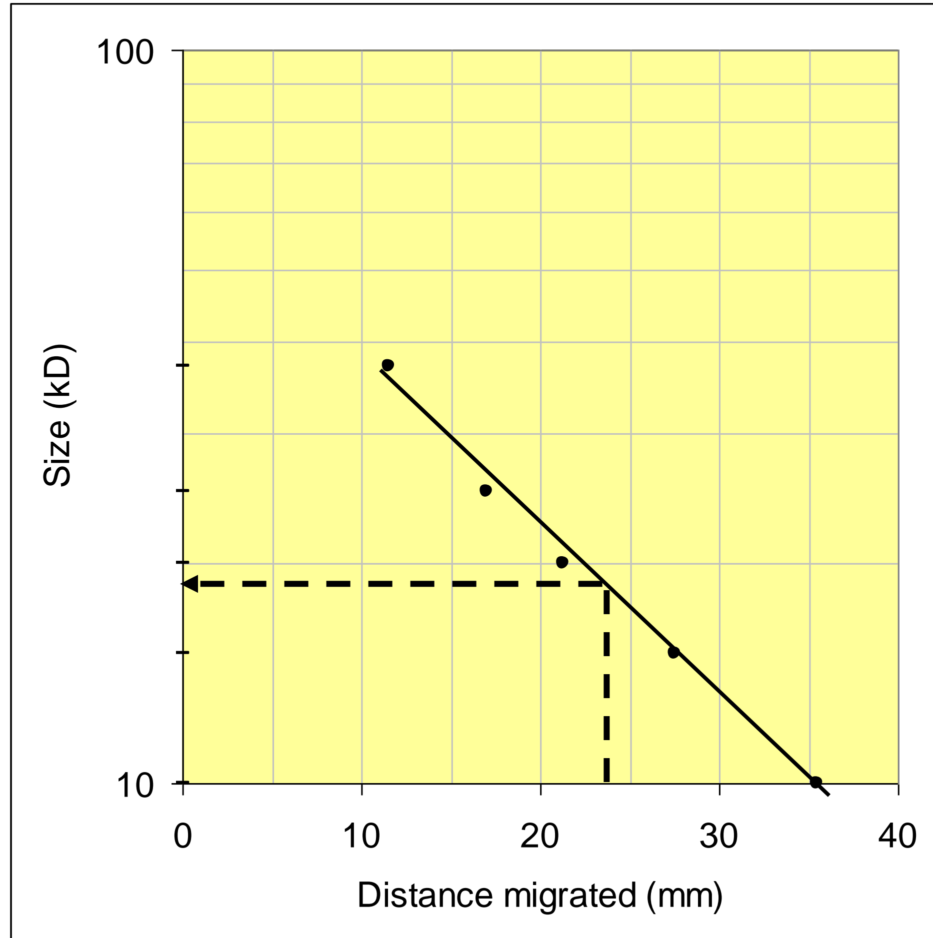


Molecular Mass Estimation

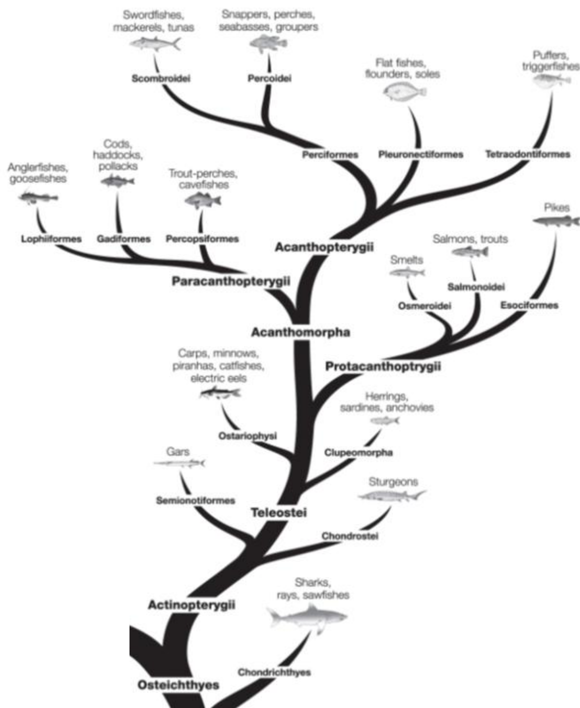


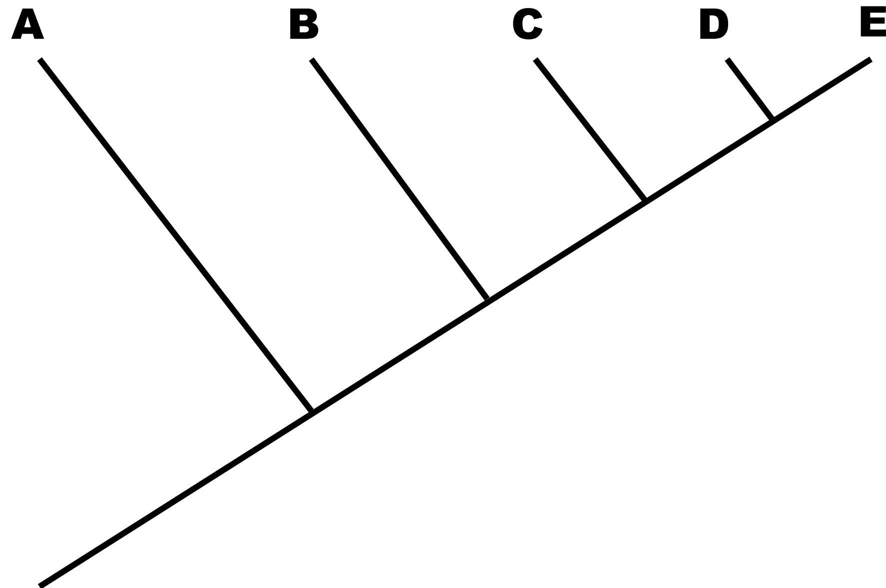


Molecular Mass Analysis With Semi-log Graph Paper



Using Gel Data to Construct a Phylogenetic Tree or Cladogram





Each Fish Has a Distinct Set of Proteins

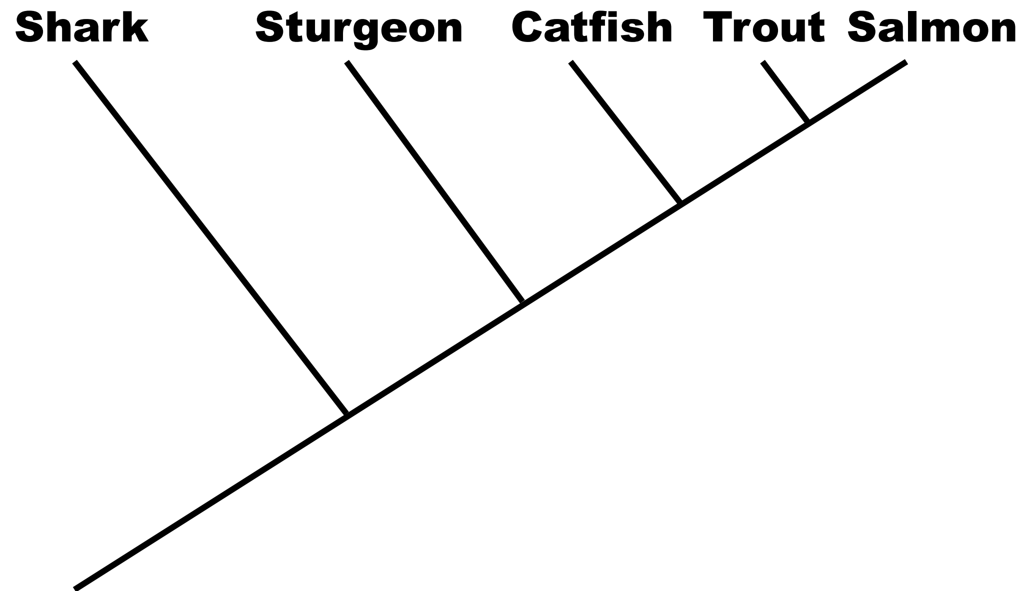
	Shark	Salmon	Trout	Catfish	Sturgeon
Total # proteins	**8**	**10**	**13**	**10**	**12**
Distance proteins migrated (mm)	25, 26.5, 29, 36, 36.5, 39, 44, 52	26, 27.5, 29, 32, 34.5, 36.5, 37.5, 40.5, 42, 45	26, 27.5, 29, 29.5, 32, 34.5, 36.5, 37.5, 40.5, 42, 45, 46.5, 51.5	26, 27.5, 29, 32, 36.5, 38, 38.5, 41, 46, 47.5	26, 27.5, 30, 30.5, 33, 35.5, 37, 39, 39.5, 42, 44, 47

Some of Those Proteins Are Shared Between Fish

Distance (mm)	Size (kD)	Shark	Salmon	Trout	Catfish	Sturgeon
25	32.5	x				
26	31.5		x	x	x	x
26.5	31.0	x				
27.5	30.0		x	x	x	x
28.5	29.1					
29	28.6	x	x	x	x	
30	27.6			x		x
30.5	27.1					x
32	25.6		x	x	x	
33	24.7					x
34.5	23.2		x	x		
35.5	22.2					x
36	21.7	x				
36.5	21.2	x	x	x	x	
37	20.7					x
37.5	20.2		x	x		
38	19.7				x	
38.5	19.3				x	

Character Matrix Is Generated and Cladogram Constructed

	Shark	Salmon	Trout	Catfish	Sturgeon
Shark	8	2	2	2	2
Salmon	2	10	10	5	3
Trout	2	10	13	5	4
Catfish	2	5	5	10	2
Sturgeon	2	3	4	2	12

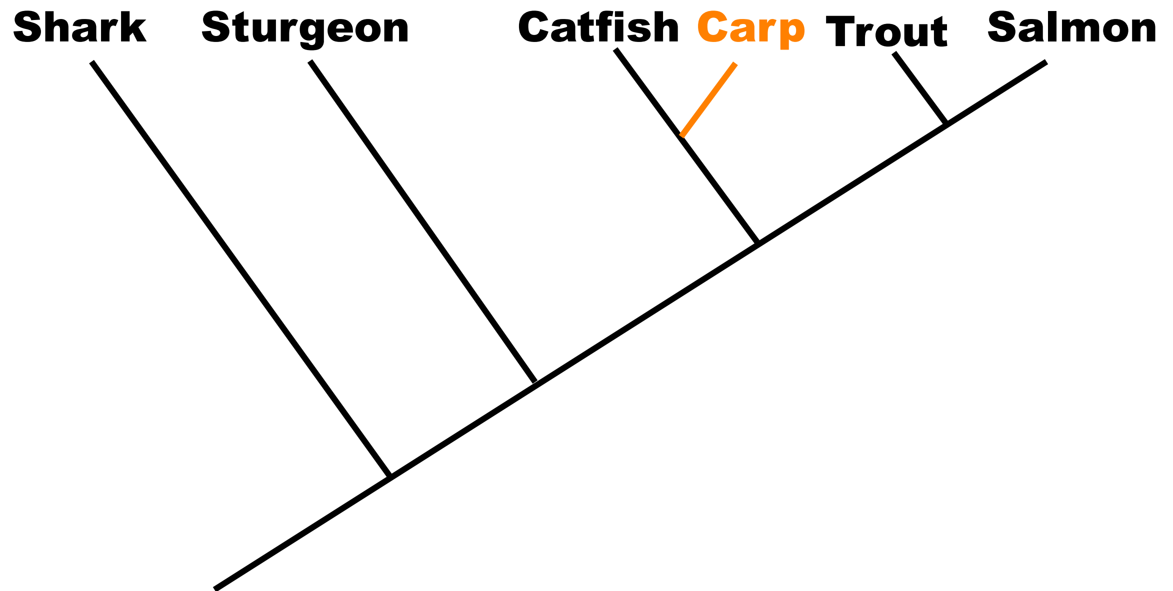


Phylogenetic Tree

Evolutionary tree showing the relationships of eukaryotes. (Figure adapted from the tree of life web page from the University of Arizona (www.tolweb.org).)

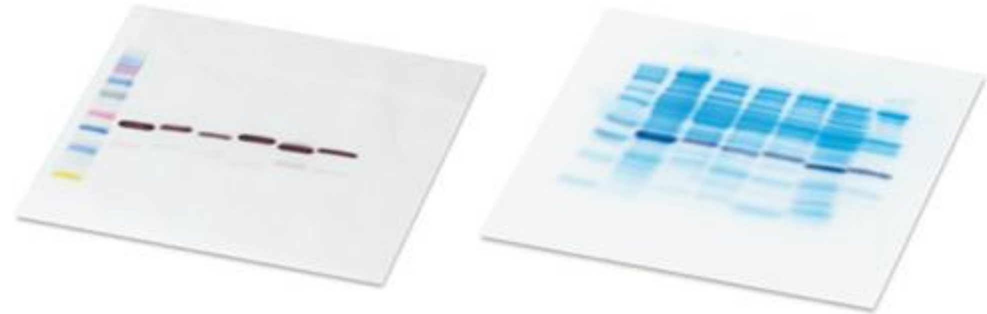
Pairs of Fish May Have More in Common Than to the Others

	Shark	Salmon	Trout	Catfish	Sturgeon	Carp
Shark	8	2	2	2	2	2
Salmon	2	10	10	5	3	5
Trout	2	10	13	5	4	5
Catfish	2	5	5	10	2	8
Sturgeon	2	3	4	2	12	2
Carp	2	5	5	8	2	11

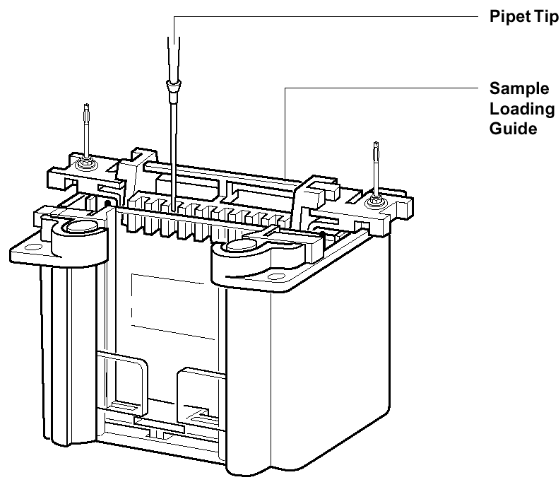


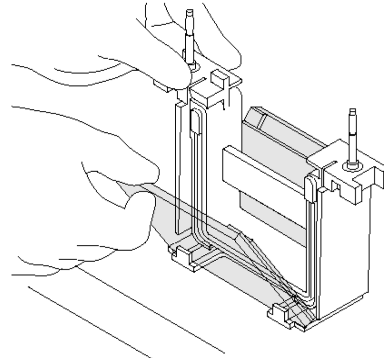
Extensions

- Independent study
- Western blot analysis

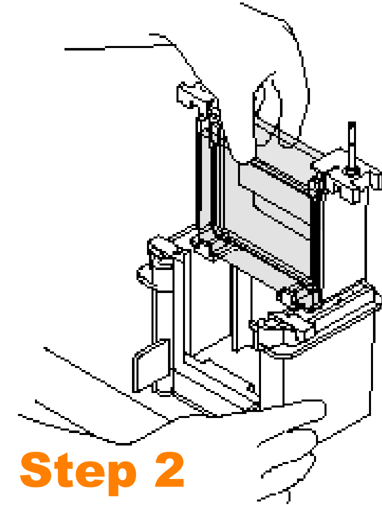


Ready Gel® Precast Gel Assembly

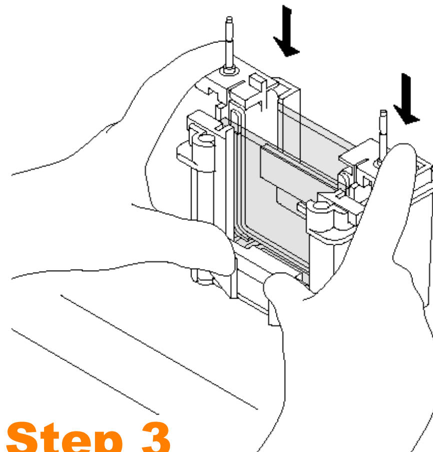




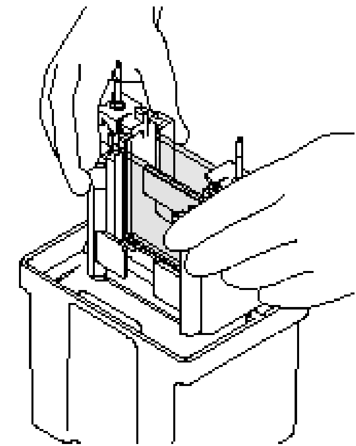
Step 1



Step 2



Step 3



Step 4