

Can biomolecular evidence be used to determine evolutionary relationships?

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Biochemical Similarities

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



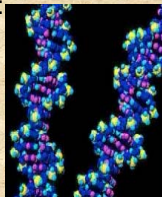
DNA RNA Protein Trait

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Biochemical Differences

- Changes in DNA → proteins with:
 - different functions
 - novel traits
 - positive, negative or no effects
- Genetic diversity provides pool for natural selection = **evolution**



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Outline of Experiment

- Add fish muscle to Laemmli Buffer
- Incubate for 5 minutes or longer
- Heat to 95°C for 5 minutes
- Load 10 ul
- Run 200 volts for 30 minutes
- Rinse 3 x 5 minutes in dH₂O
- Stain for 1 hour in BioSafe Coomassie
- Destain overnight in water
- Dry gels/photograph

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What's in the sample buffer?

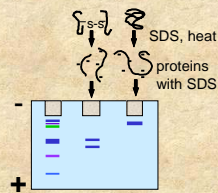
- **Tris** buffer to provide appropriate pH
- **SDS** (sodium dodecyl sulfate) detergent to dissolve proteins and give them a negative charge
- **Glycerol** to make samples sink into wells
- **Bromophenol Blue** dye to visualize samples

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Why heat the samples?

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



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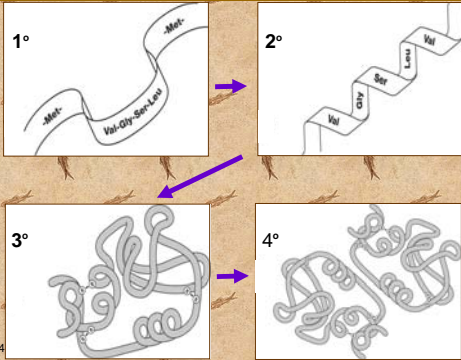
Making Proteins

- **DNA** TAC CGA TCG TGA ACT
- **mRNA** AUG GCU AGC ACU UGA
- **tRNA** UAC CGA UCG UGA ACU
- **amino acid** Met - Ala - Ser -Thr - Stop

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Levels of Protein Organization



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Protein Size Comparison

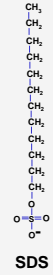
- Break protein complexes into individual proteins
- Denature proteins using detergent and heat
- Separate proteins based on size

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SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

- **SDS** (Sodium Dodecyl Sulfate) detergent
 - solubilizes and denatures proteins
 - negative charge to proteins
- Heat denatures proteins

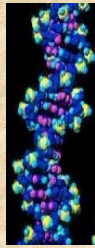


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Why Use Acrylamide Gels to Separate Proteins?

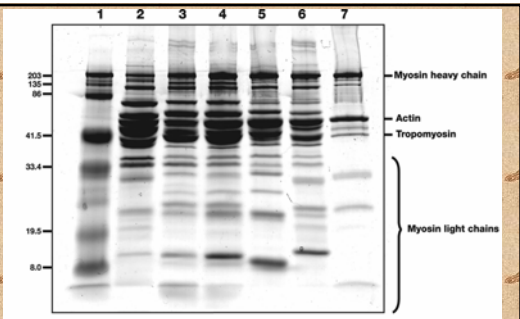
- Acrylamide gel → tight matrix
- Ideal for protein separation
- Smaller pore size than agarose
- Proteins much smaller than DNA
 - average amino acid = 110 Da
 - average nucleotide pair = 649 Da
- 1 kilobase of DNA = 650 kDa
- 1 kilobase of DNA encodes 333 amino acids = 36 kDa



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Gel Analysis

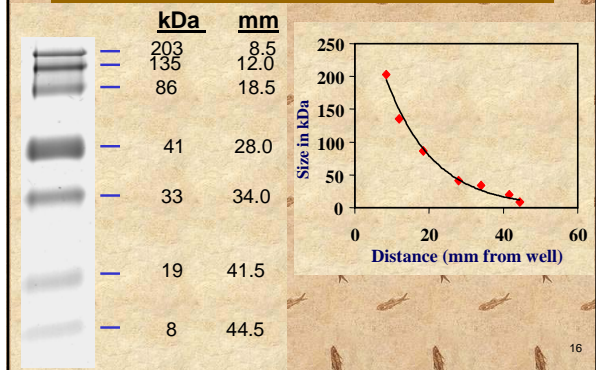


Lane 1. Kaleidoscope Markers
 2. Shark
 3. Salmon
 4. Trout
 5. Catfish
 6. Sturgeon
 7. Actin and Myosin Standard

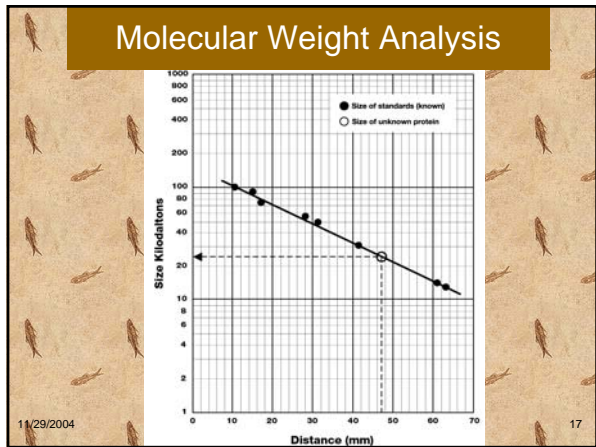
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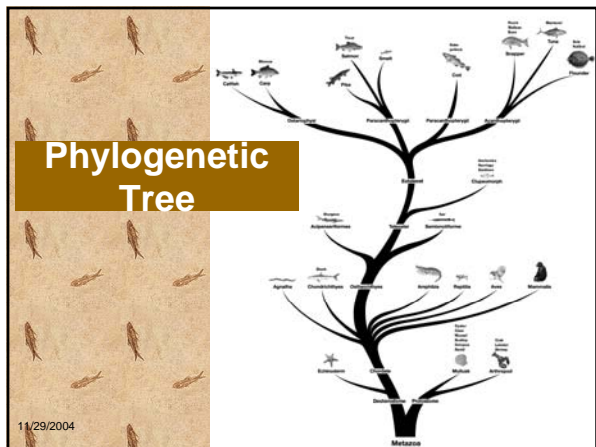
Molecular Weight Estimation



Molecular Weight Analysis



Phylogenetic Tree



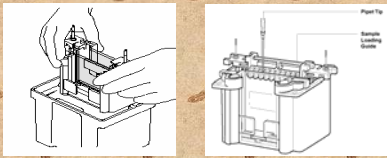
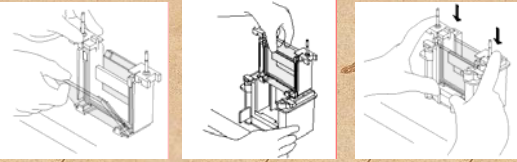
Extensions

- Independent Study
- Western blot analysis

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Ready Gel Assembly



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