Isoelectric Focusing

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Isoelectric Focusing

- Electrophoresis in a pH gradient
- Separation method that resolves protein markers on the basis of their isoelectric points
- Successfully used in clinical, research, and agricultural fields
  - Blood
  - Serum
  - Muscle Extracts
  - Seed Extracts

Isoelectric Focusing

- Attributes of IEF
  - Ability to separate on total protein marker profiles or enzyme protein marker profiles
  - Proteins with same molecular weights will separate out by pH
  - Testing time is quick (~2- 3days)
  - Ability to have permanent record of the gel
  - Pre-cast gels available
  - Versatile to a large number of crops for the agriculture industry
  - Seed or tissue extraction

Isoelectric Focusing Sample Preparation

- Load seed or tissues into a 48 or 96-well microplate
- Utilize a seed crusher or cutter to extract the seed proteins

Isoelectric Focusing Sample Preparation

- A specific quantity of extraction solution is added into the wells of the microplate with a multi-channel pipette
- Incubate samples overnight at 4C
- Centrifuge samples with the resulting solution ready to be applied to an IEF gel
- The microplate containing the sample solution can be frozen for sample retesting or future evaluation

Isoelectric Focusing of Samples

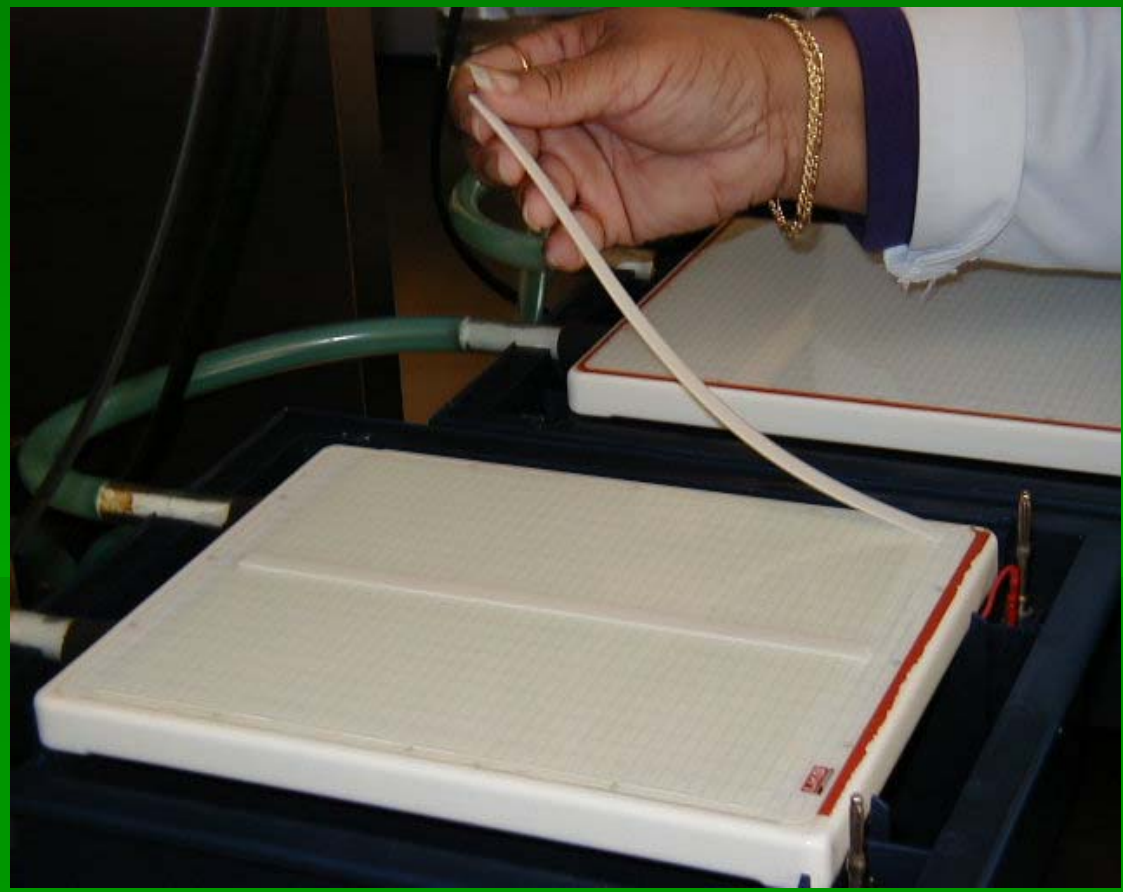
- Prepare Multiphor unit
  - Turn on circulating water bath 12-15C
  - Level unit
  - Clean cooling plate

Isoelectric Focusing of Samples

- Place 2 mL of water onto the center of the cooling plate
- Mark a reference point on the edge of gel
- Place the gel on the cooling plate, distribute the bead of water being careful to not trap any air bubbles
- Blot excess water from edge of gel
- Use blotting paper to remove moisture from the gel

Isoelectric Focusing of Samples

- Prepare three precut electrode wicks
- Saturate two wicks with anode solution
- Place each anode wick parallel along the long edges of the gel



Isoelectric Focusing of Samples

- Saturate the remaining wick with cathode solution
- Place the cathode wick along the center of the gel between the two anode wicks
- Prefocus the gel if this step is required
  - Place the electrode wires directly on the anode and cathode wicks
  - Lower electrode cover connect to power source, run electric current at specific wattage for 10 minutes

Isoelectric Focusing of Samples

- Place paper or plastic template on each side of the gel at its designated position (1cm from the anode wicks)

Isoelectric Focusing of Samples

- Use application tool to take off frame of paper template

Isoelectric Focusing of Samples

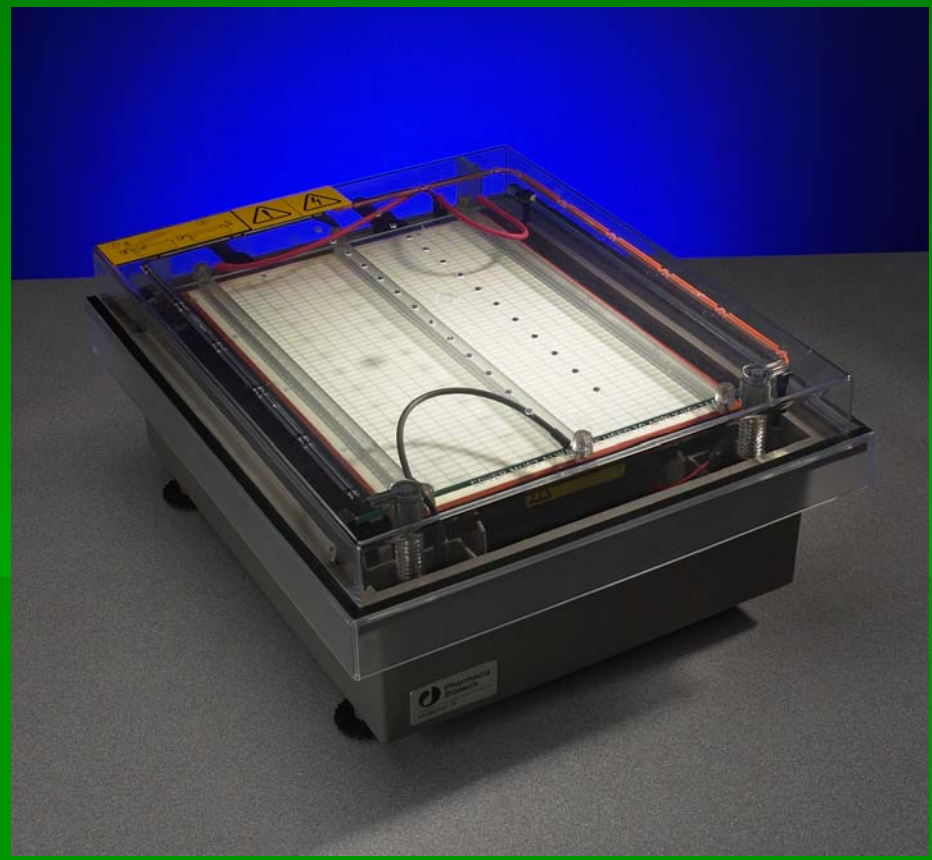
- Final layout of the gel

Isoelectric Focusing of Samples

- Pipette the prescribed quantity of sample solution into each sample well

Isoelectric Focusing of Samples

- Focusing
  - Electrode wires are placed onto wicks
  - Safety cover is placed on unit
  - Connect electrode wires to power supply
  - Gel is run at constant voltage for a determined amount of time



Isoelectric Focusing Silver Staining

- Place the recently run gel into a staining dish, pour 200 ml of fixative solution onto the gel, rock for 15 minutes
- Wash the gel while rocking for one hour in 500 ml of deionized water, repeat wash step with fresh water
- Allow the gel to completely dry overnight at room temperature

Isoelectric Focusing Silver Staining

- Clean all staining glassware and stir bars with reducing wash solution
- Formulate the silver staining solution
- Prepare gel for staining by washing for 5 minutes in 200 ml deionized water
- Pour silver stain mixture over the gel's surface and begin rocking the gel
- Allow the gel to stain until bands reach desired intensity

Isoelectric Focusing Silver Staining

- Discard the staining solution and rinse gel in deionized water
- Pour stop solution onto the gel, soak for 10 minutes
- Place the gel in 200 mL of deionized water, soak for 10 minutes
- Allow gel to air dry

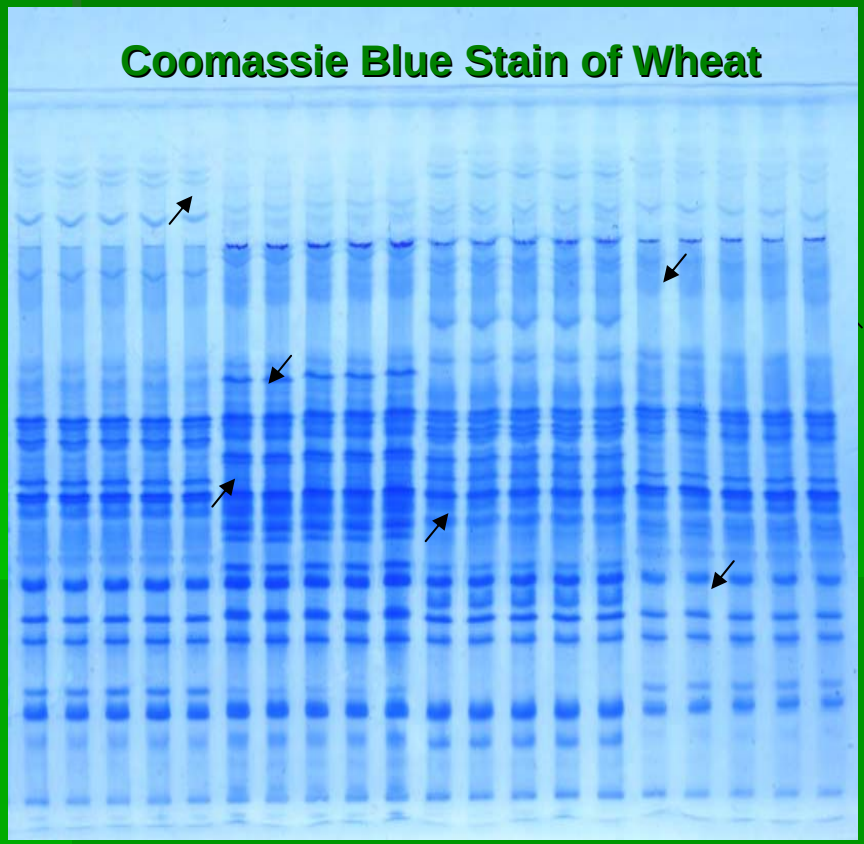
Isoelectric Focusing Enzyme Staining

- Thirty minutes before the completion of the gel run prepare the enzyme stain
- Pour the stain over the gel surface immediately after focusing
- Rock and heat the gel during staining

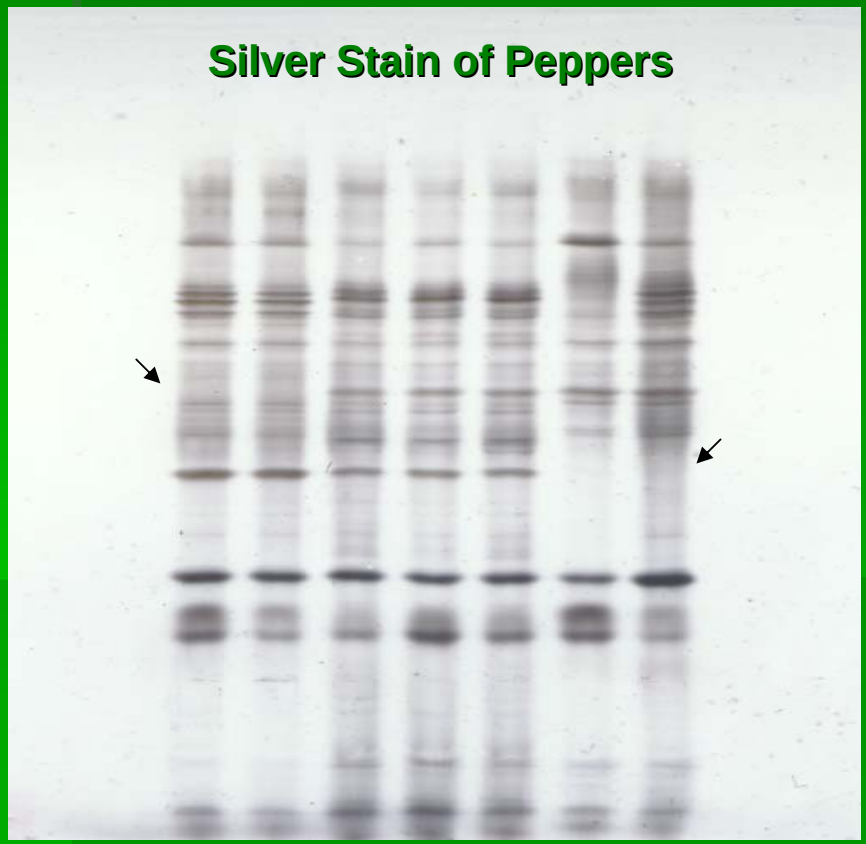
Isoelectric Focusing Enzyme Staining

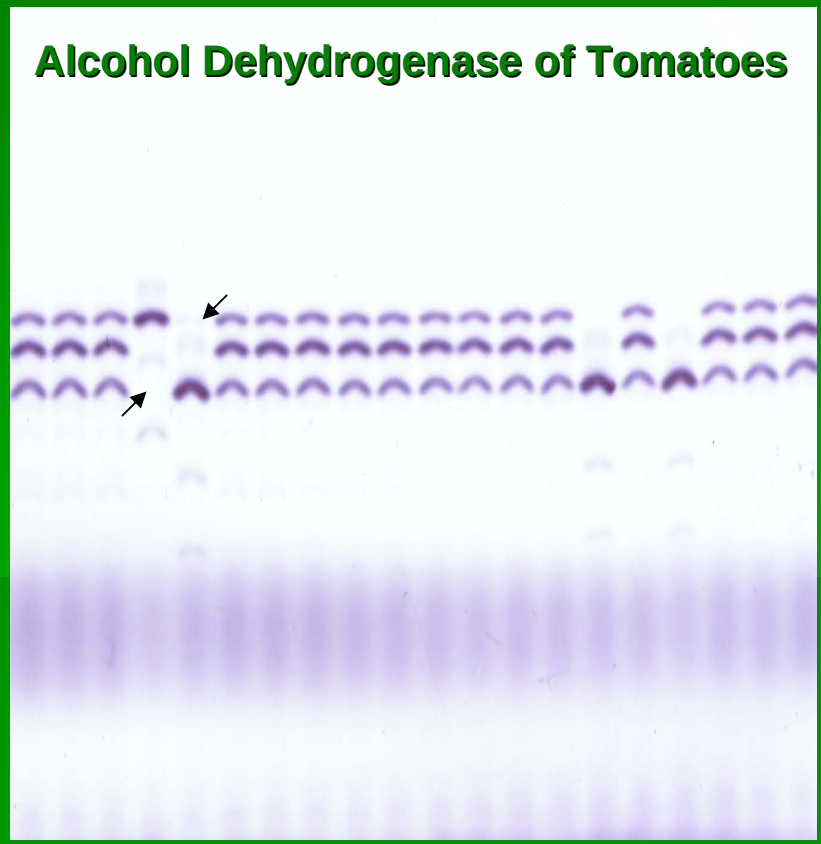
- Allow the staining to continue until the bands reach strong intensities
- Stop the reaction with 200 mL enzyme stop solution for 3 minutes
- Wash the gel with two cycles of 500 mL deionized water for one hour
- Allow the gel to dry
- The gel can now be analyzed and interpreted

Analysis of Protein Markers

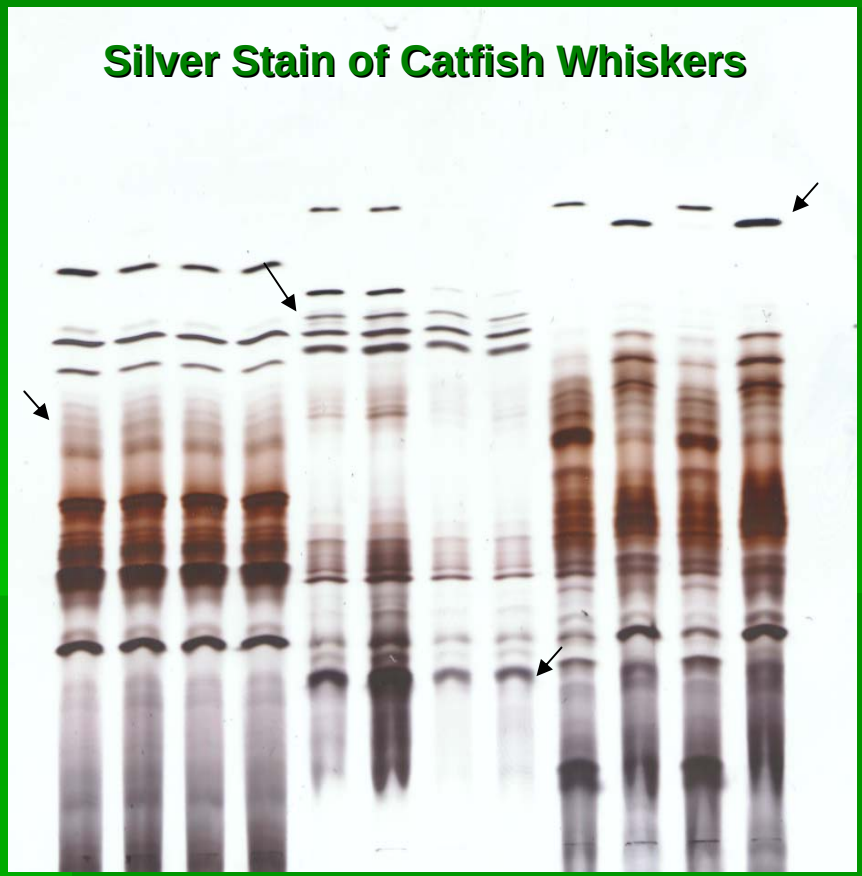


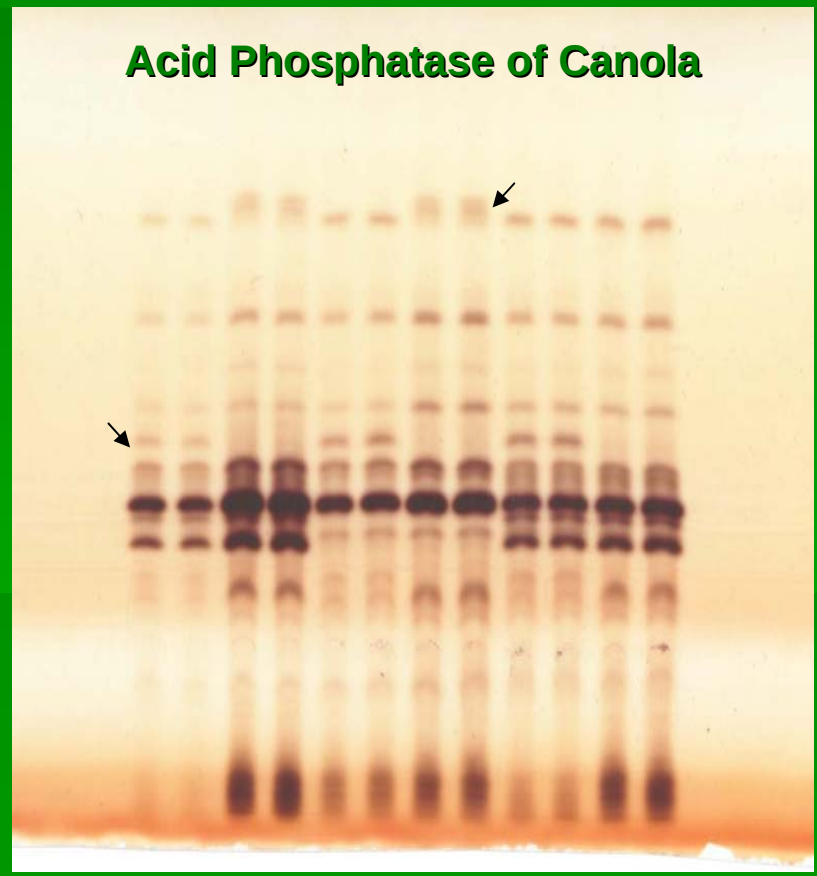
Analysis of Protein Markers, cont.





Analysis of Protein Markers, cont.





Analysis of Protein Markers, cont.

Applications of Isoelectric Focusing

- Agricultural Industry
  - Genetic Purity
  - Variety identification, including trait variety identification.
  - Parental Line Maintenance
  - Breeding Programs

Questions?