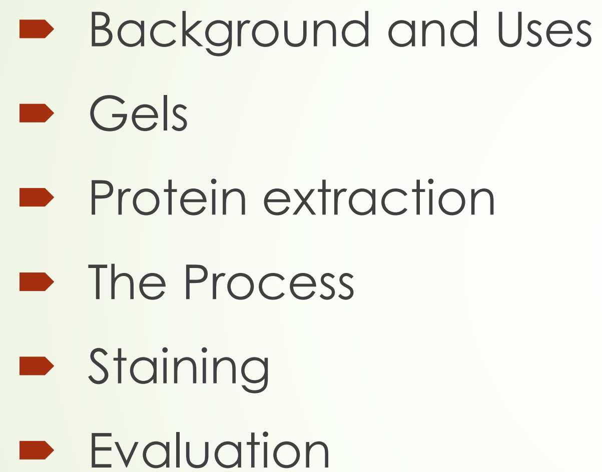
Isoelectric Focusing (IEF)

Kalyn Brix, SoDak Labs, Inc.

The Agenda



- Background and Uses
- Gels
- Protein extraction
- The Process
- Staining
- Evaluation

Proteins

- Net charge of protein is the sum of the positive and negative charges of the amino acid side chains

pH and pI

- pH gradient in the gel
- pI = isoelectric point in which the net charge of the protein is zero
- Protein that is moved by electrophoresis moves to the location on the gel where the net charge is 0

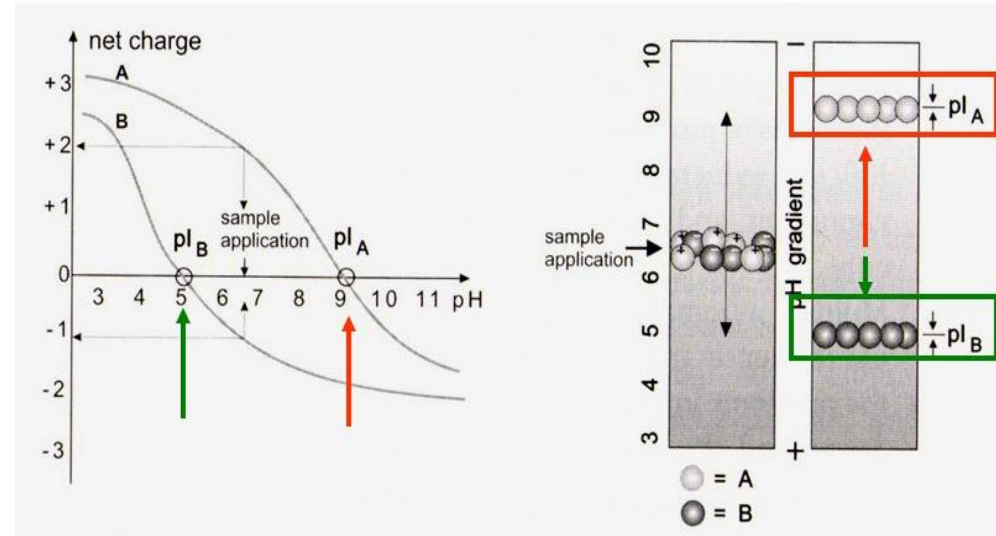
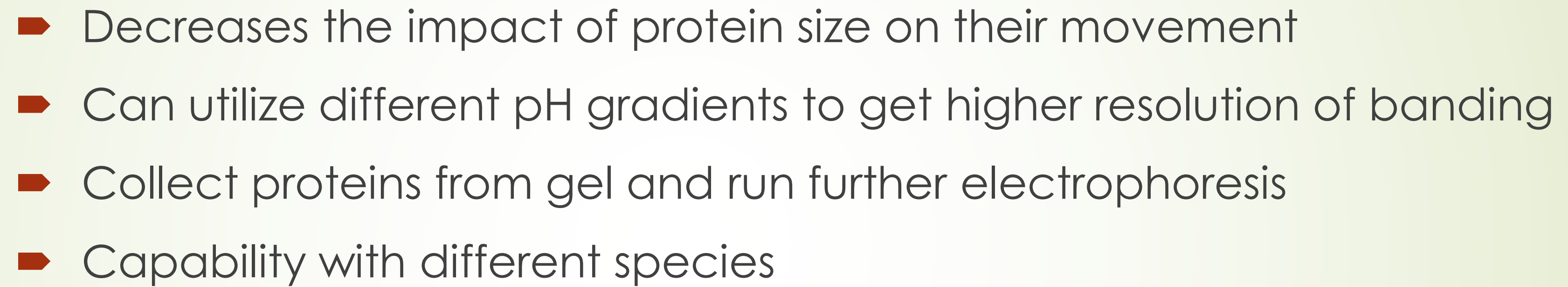


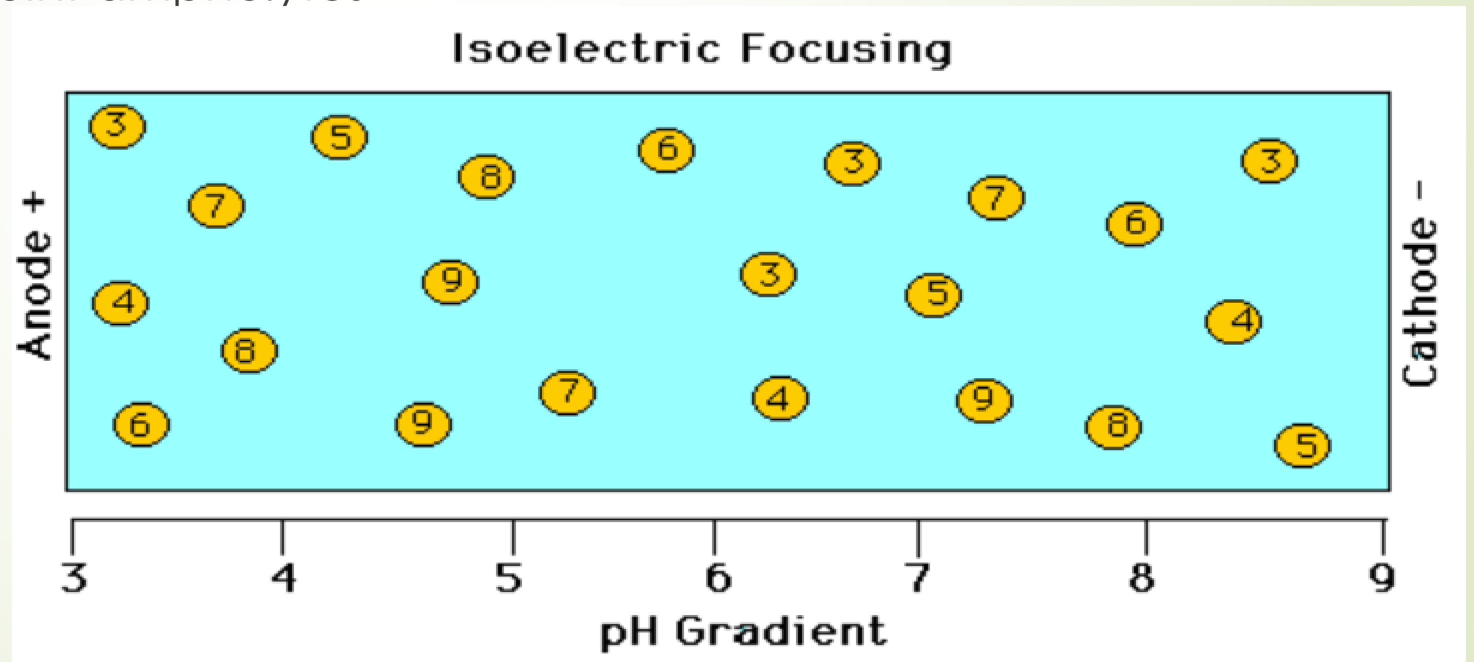
Image Christina Baker

Why IEF?


Decreases the impact of protein size on their movement
Can utilize different pH gradients to get higher resolution of banding
Collect proteins from gel and run further electrophoresis
Capability with different species

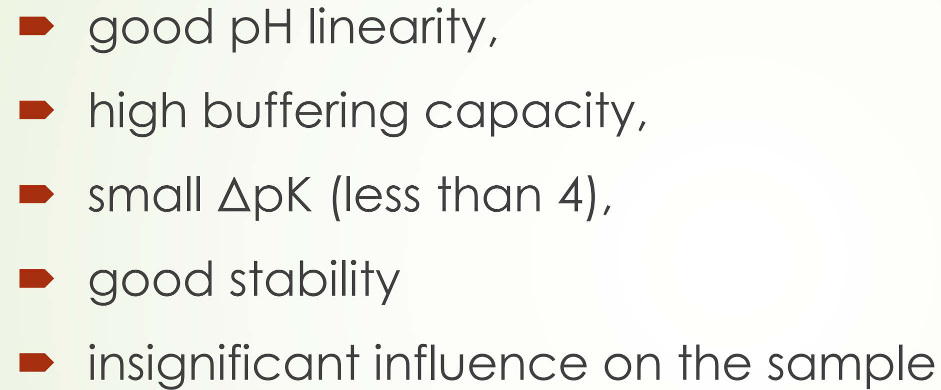
How does it work?

- Carrier ampholytes are small soluble molecules with both positive and negative charge properties
- When electricity is applied to the gel, it moves the ampholytes to their pI point creating a pH gradient in the gel
- Scientist can select the pH gradient in the gel by using different percentages of pI point ampholytes

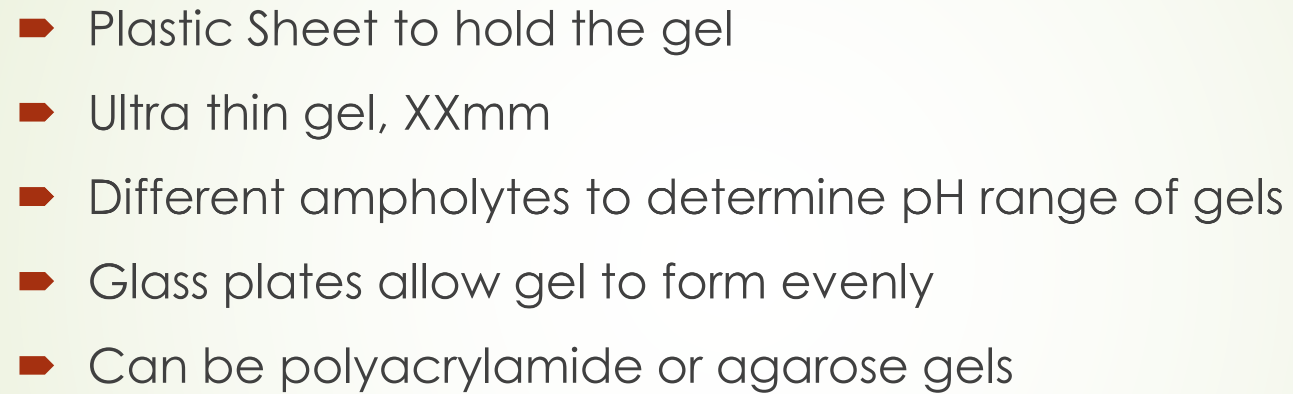


http://www.bioon.com/experiment/elec6/62193.shtml

What makes a good amphyolyte?

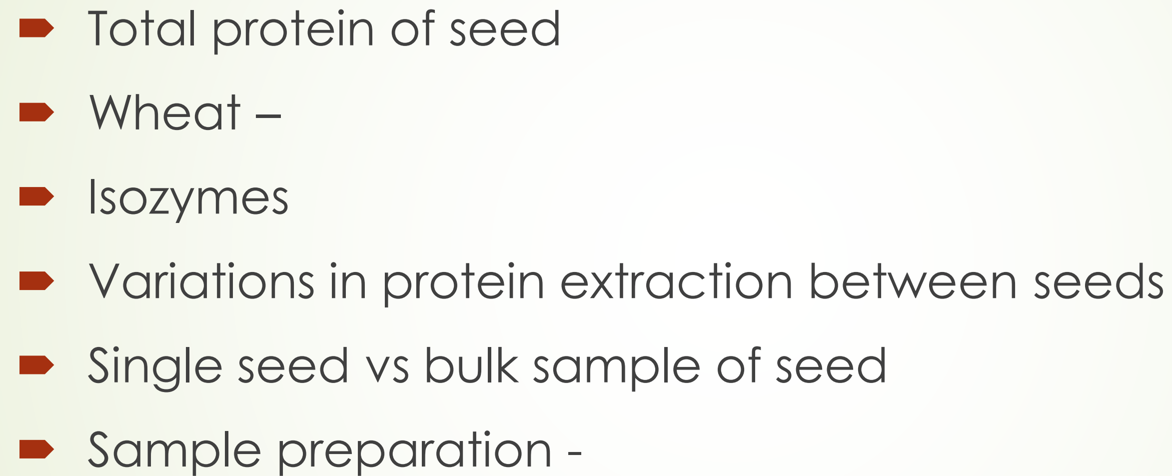

good pH linearity,
high buffering capacity,
small ΔpK (less than 4),
good stability
insignificant influence on the sample

Gels



- Plastic Sheet to hold the gel
- Ultra thin gel, XXmm
- Different ampholytes to determine pH range of gels
- Glass plates allow gel to form evenly
- Can be polyacrylamide or agarose gels

Extraction of Seed proteins


Total protein of seed
Wheat –
Isozymes
Variations in protein extraction between seeds
Single seed vs bulk sample of seed
Sample preparation -

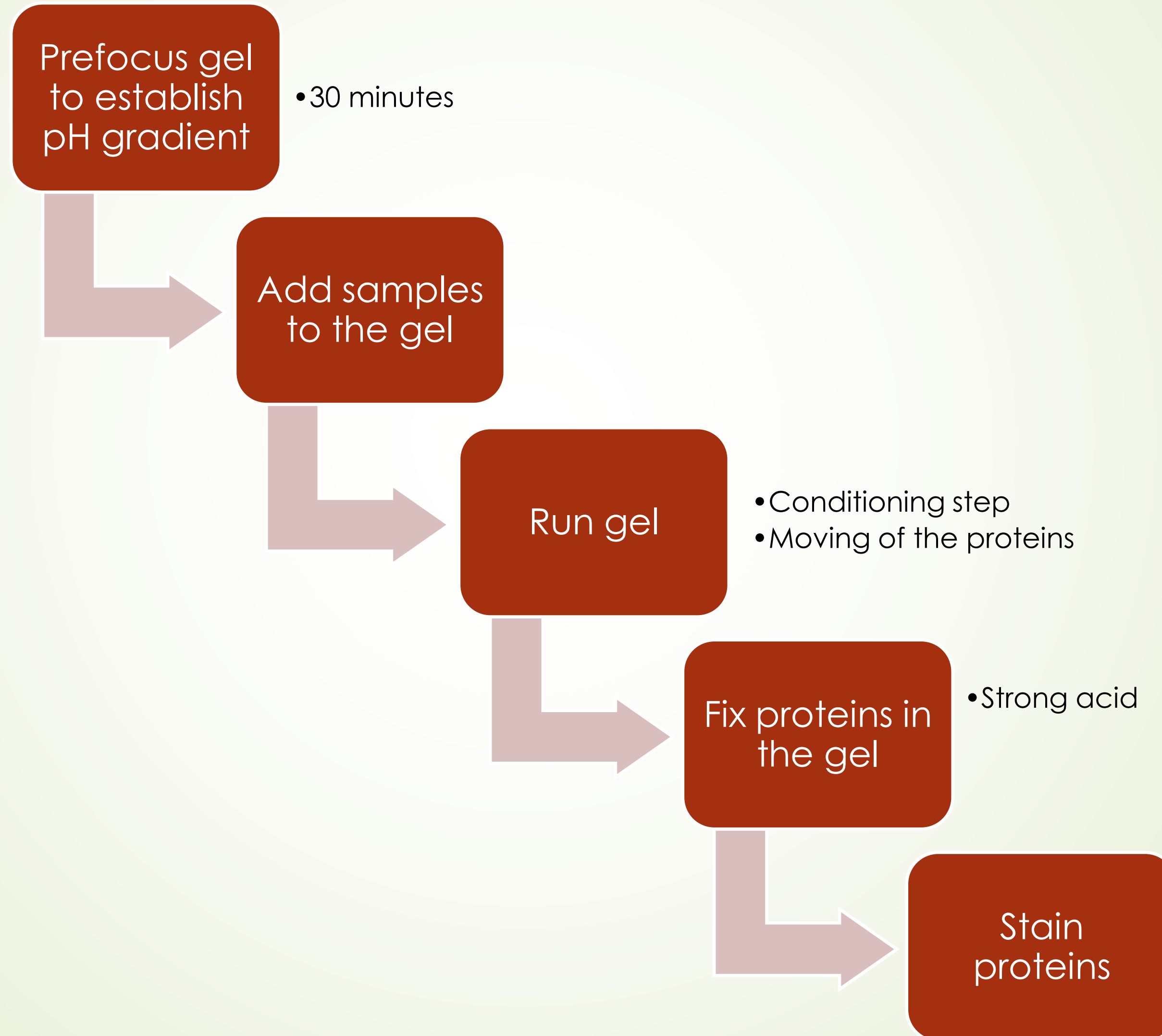
Equipment Needed

- Circulating Chiller
- Cooling plate to keep gel cool while electrophoresis is running
- Electrophoresis units, able to get to 2000V
- Set up to allow for application of electrical field (Anode/ Cathode wires)
- Sample application strips
- Staining containers
- Shakers
- Ability to handle chemicals safely

Controls

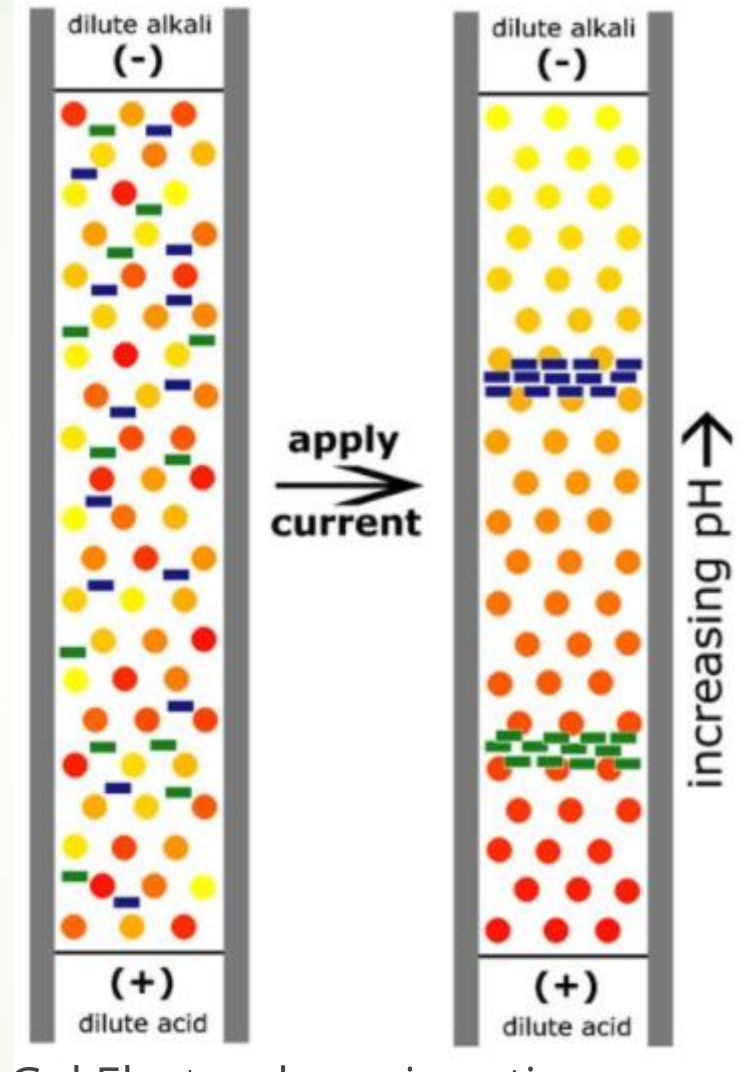
- Protein ladder
- Known parents or varieties to compare results

Process



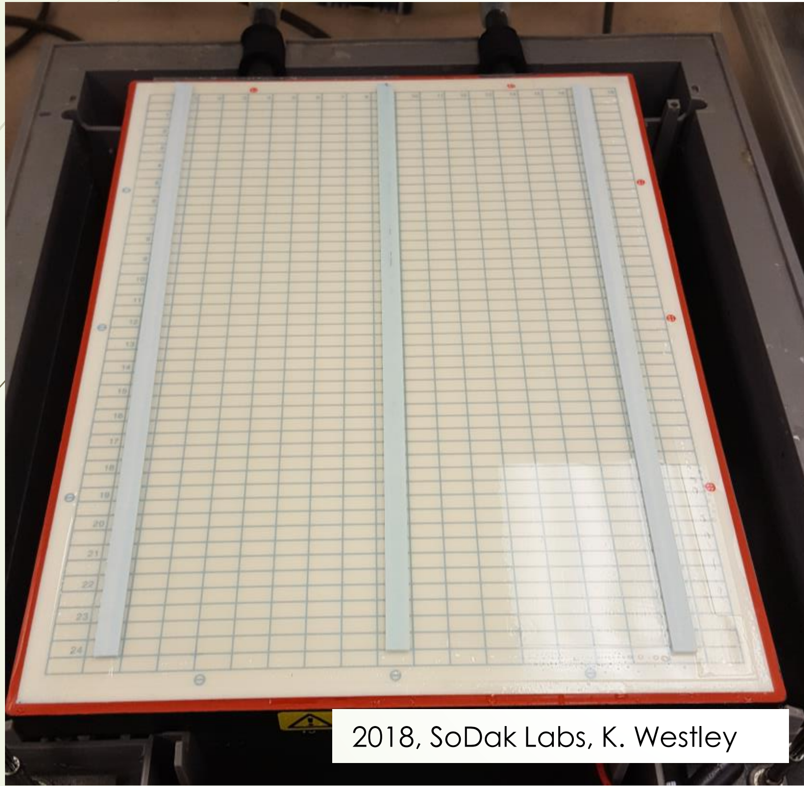
Prefocus Gels

- Create pH difference
- Anode and Cathode areas
- pH of anode strip – 4
- pH of cathode strip – 10
- Increasing voltage to move ampholytes



Gel Electrophoresis native: mobility = (voltage)(charge)/(mass)
Published byKyle Wilson

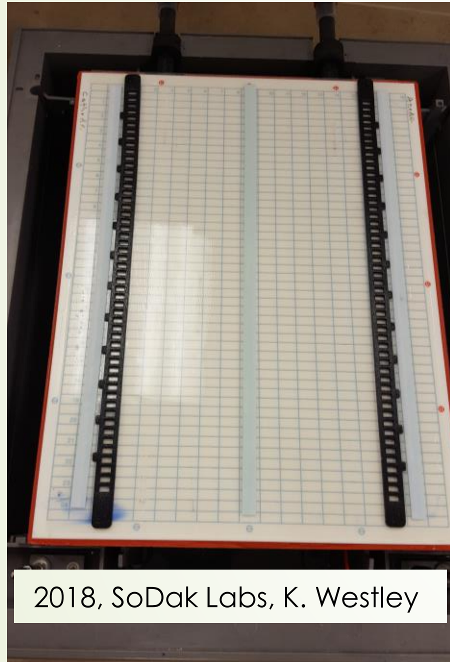
Prefocus gel



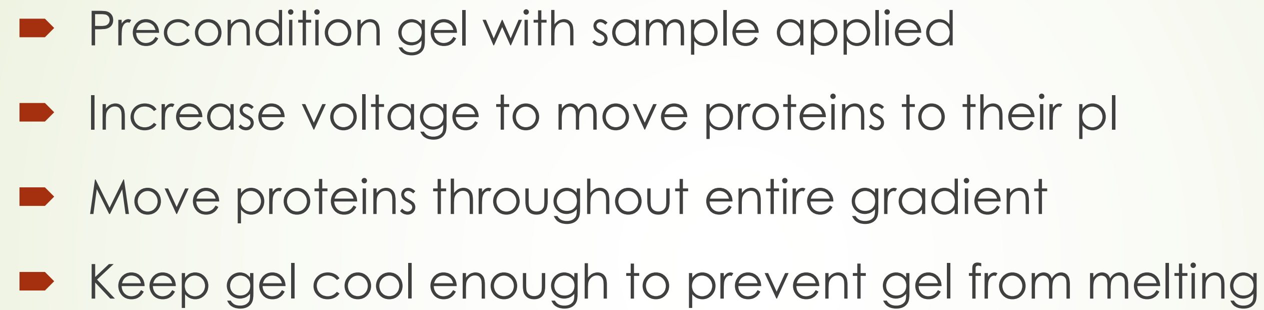
2018, SoDak Labs, K. Westley

2018, SoDak Labs, K. Westley

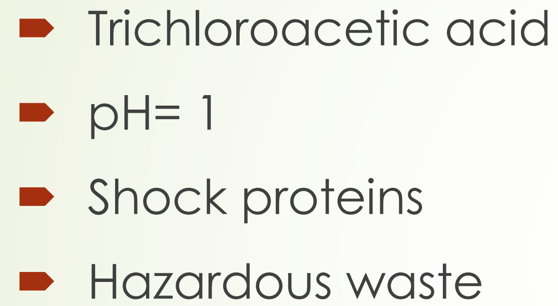
Sample Application


2018, SoDak Labs, K. Westley

Gel electrophoresis


Precondition gel with sample applied
Increase voltage to move proteins to their pI
Move proteins throughout entire gradient
Keep gel cool enough to prevent gel from melting

Fixing proteins in the gel



- Trichloroacetic acid
- pH= 1
- Shock proteins
- Hazardous waste

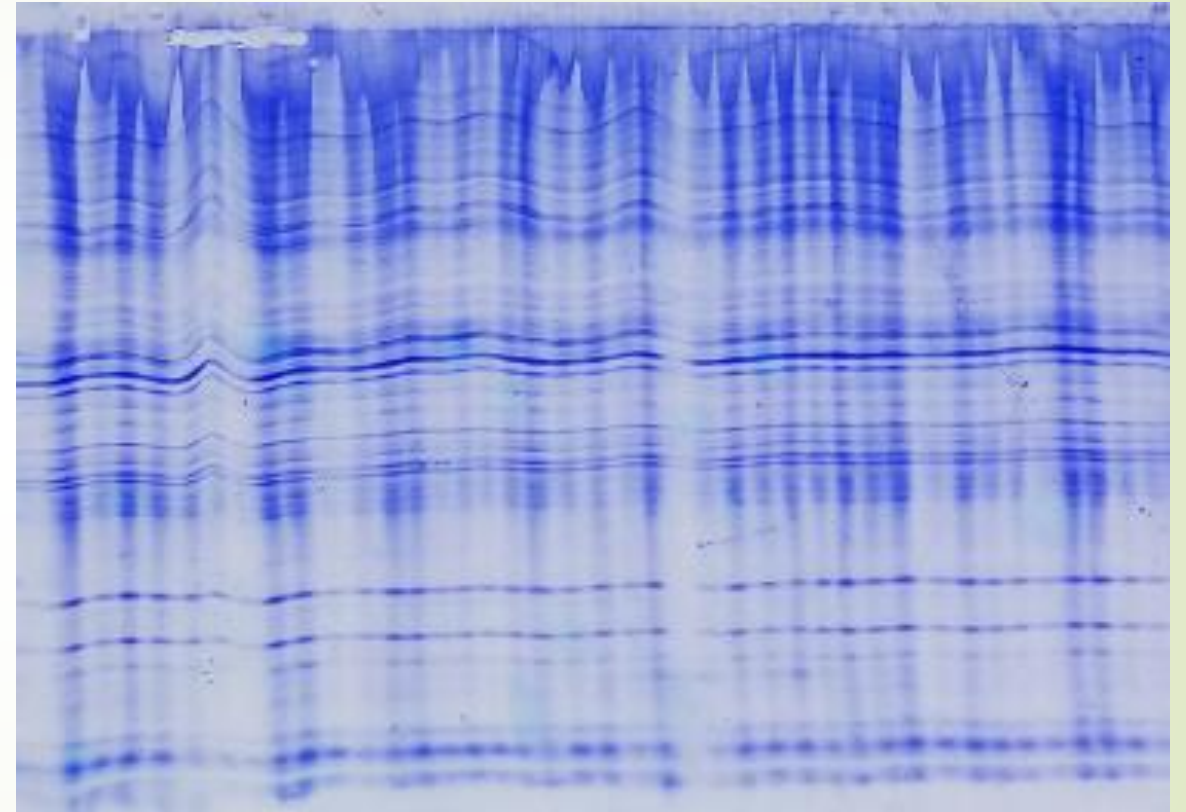
IEF – Steps of the Process

- Sample processing
  - Total Protein of seed
- Running of gels
  - Prefocus gel to establish pH gradient
  - Load gels
  - Run gels for 2.5 hrs
- Staining –
  - Prep staining solutions
  - Stain and dry gel
- Evaluation-
  - Read gels
  - Report results

Seed Academy

Stain Proteins

- Coomassie Blue stain
- Silver Stain



Seed Academy