Genetic Varietal Purity Using Electrophoresis Methods

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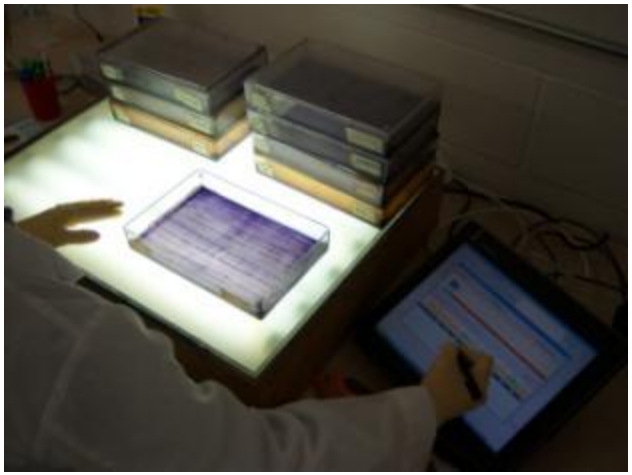

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Our Presenter

Jennifer Smetana

Electrophoresis

Genetic purity of a seed lot is important to know in the quality seed production. Genetic purity looks for differences in proteins (total protein profile or variety of enzymes) to create a "picture" for the variety or hybrid.

Hybrid purity or variety characterization may assist a seed producer in developing and marketing their seed.

Electrophoresis Techniques

Today we will cover two methods:

Isozyme testing = Corn

Isoelectric focusing = Other crops

Definitions

Female Selfs – Seed from the hybrid sample that has the same banding pattern as the female parent.

Male Selfs - Seed from the hybrid sample that has the same banding pattern as the male parent.

Offtypes – Seed from the hybrid sample that has a different banding pattern than the hybrid, female or male seeds.

Hybrid/Inbred Purity – the percent of the seeds that matches the major or expected pattern. *Females, Males and offtypes are subtracted from the total number of individuals run to determine the percentage.*

Variety Verification – Comparing a variety to a known/foundation seed sample.

Isozyme Electrophoresis

Method for Corn

Isozyme Electrophoresis

Method for Corn

Isozymes are multiple molecular forms of the same enzyme.

Corn has 10 pairs of chromosomes with genes at different locations (loci) on separate chromosomes that can code for different forms of each enzyme.

Some isozymic loci are polymorphic. *They have more than one allele that can code for the same enzyme at that location.*

Various types of corn may have a different allele at that particular location.

Isozyme Electrophoresis continued

Different alleles will results in various patterns when using starch gel electrophoresis for isozyme testing.

Typically 100 individuals are tested. Male and female lines are run to determine the hybrid pattern.

This test determines:

Hybrid Purity- Percentages of female selfs, variants, off-types and total purity.

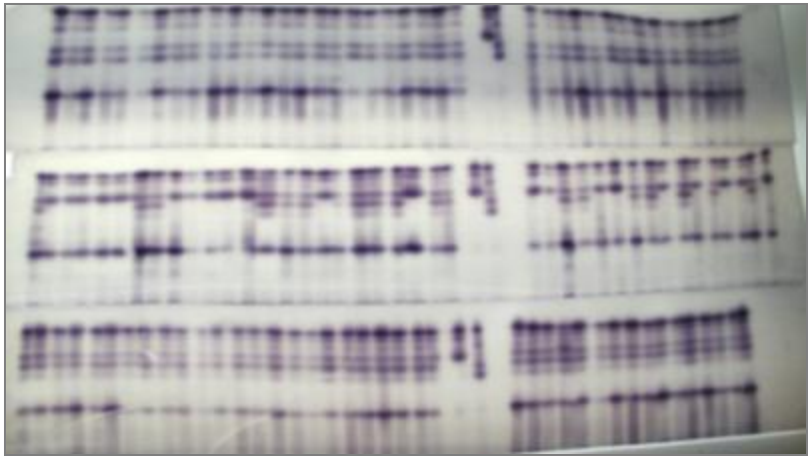
Inbred Purity – Percentages of variants, off-types and total purity.

Overview of Starch Gels

Multiple forms of each enzyme protein are separated on the gel because they travel at different rates due to their differences in net charge, size, and shape.

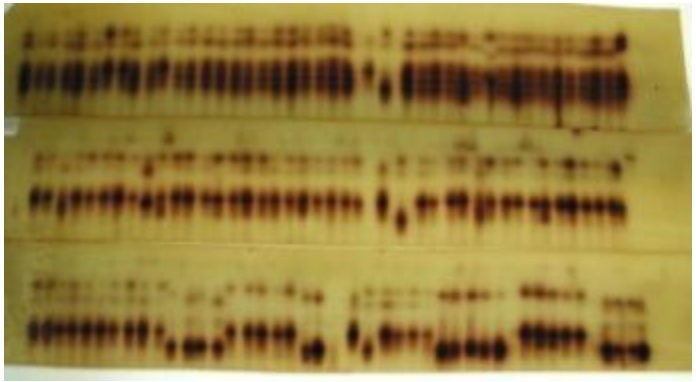
After gels are made they are cut into several thin slices.

Each slice can be stained for different enzyme activity.



Overview of Running Starch Gels continued

For some enzymes, one locus is studied while for other enzymes up to five loci are read, each of which could show a different pattern. Reading the patterns at several loci gives a fingerprint of the corn being tested.

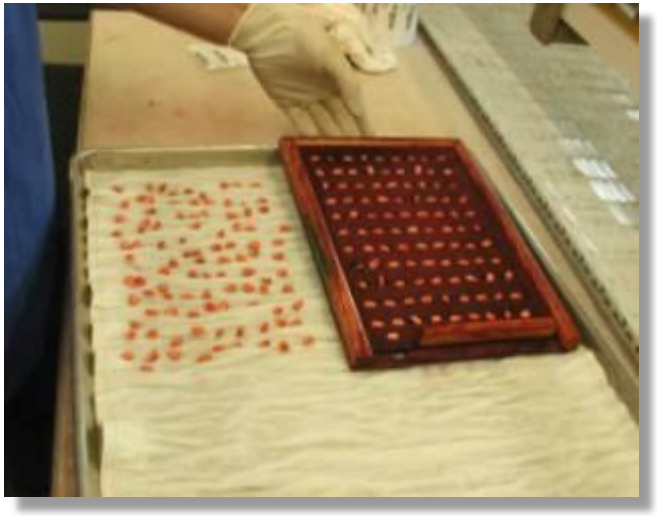


Information obtained is useful in analyzing hybrids. The percentage of selfs, offtypes, and variants can be determined for each seed lot.

1. Plant Isozymes

- Plant 4 100 seed samples on a tray.

- Grow for five days.

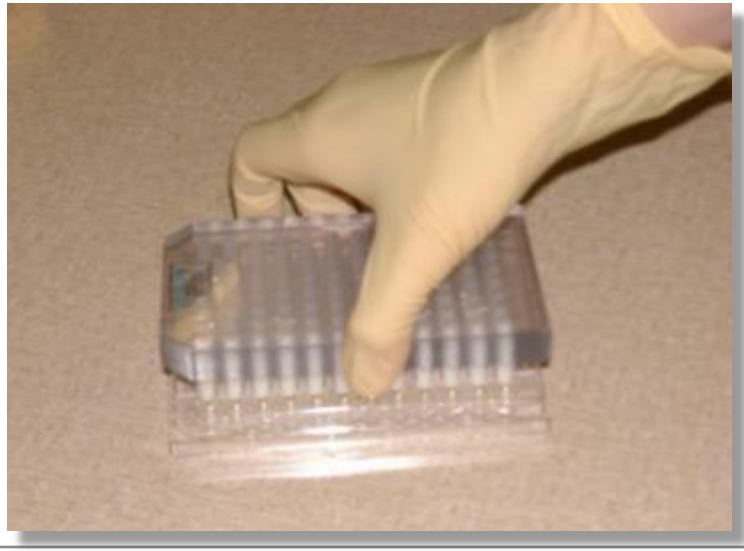


2. Harvest Samples and Extract the Proteins

- Harvest samples and place into 96 well plate.

  Plate contains an extraction buffer.

- Crush samples.

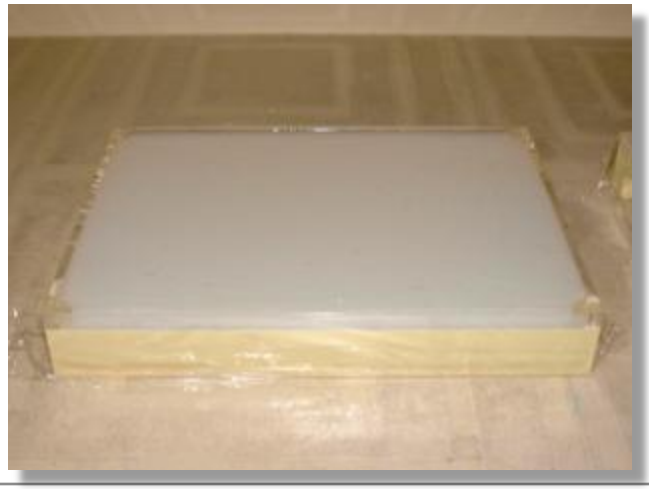


- Place in freezer overnight.

3. Make Isozyme Gel

- Heat a starch based solution.

- Pour hot gel mixture into a mold and allow to solidify.



- After solidification, wrap gel with clear plastic wrap for use the next day.

4. Prepare the Sample and Gel

A. Place paper wicks into the 96 well plate to soak up the extracted proteins.

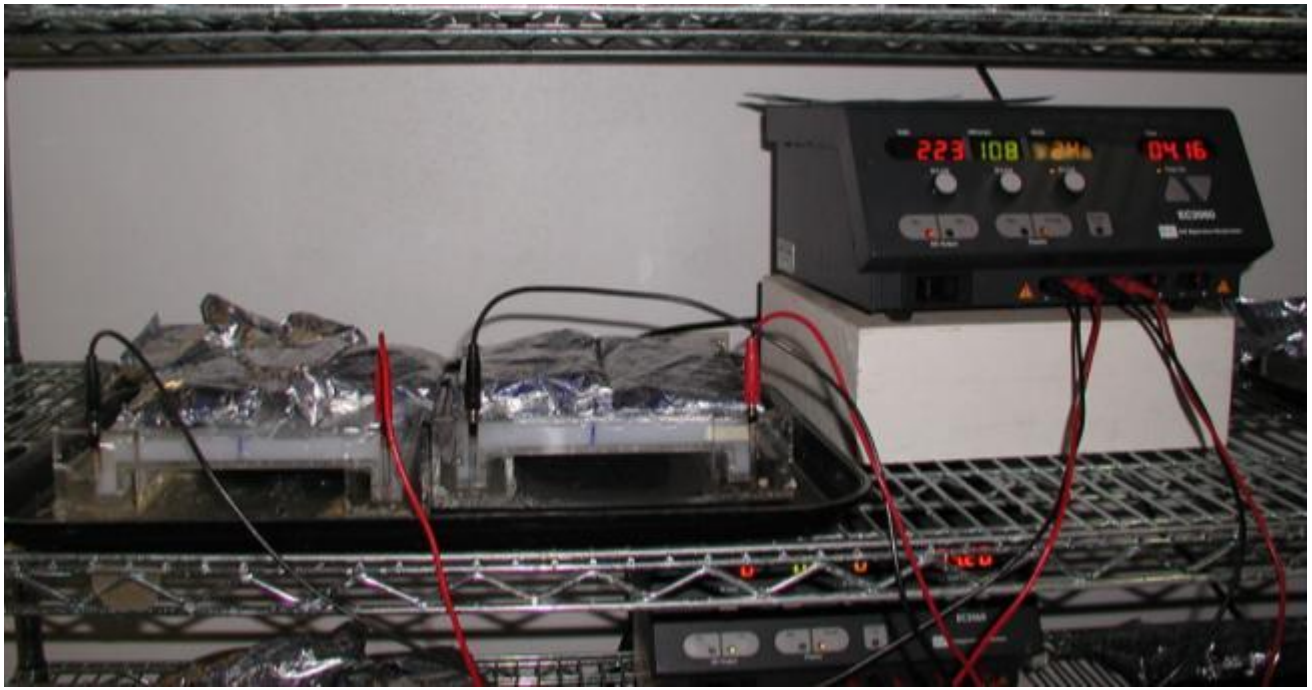
B. Cut/slice the gel.

C. Remove slice to allow the sample wicks to be placed into the gel.

5. Load Sample onto Gel

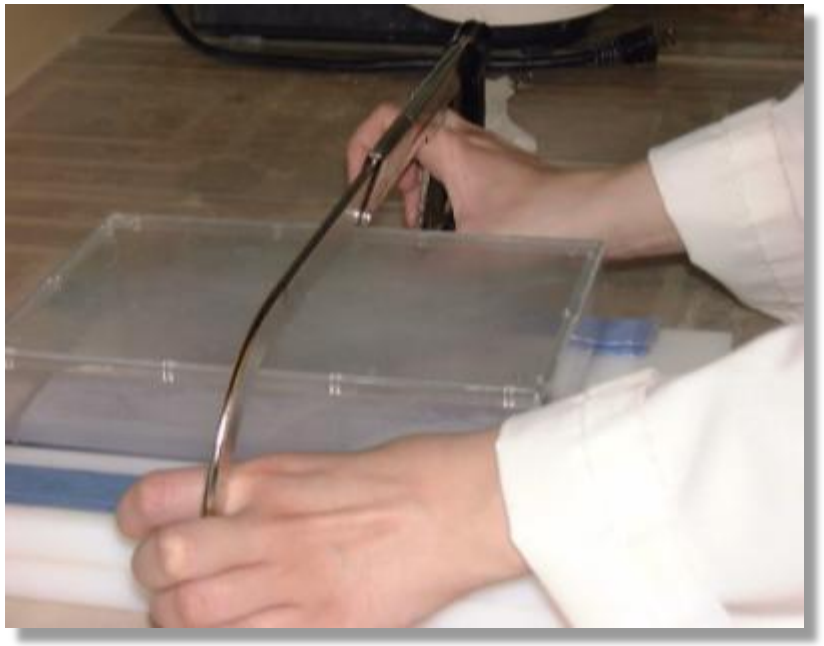
- Load samples onto the of gel.
- Place controls in the middle of the gel.
- Piece the gel back together.

6. Gel Electrophoresis



- Place gel in a gel tray containing electrode buffer.
- Run for 3½ to 4 hours.

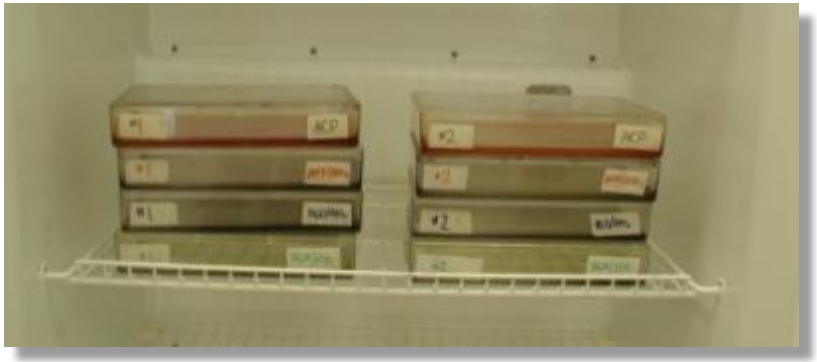
7. Slice the Gel



- Slice gel sample into thin slices for staining

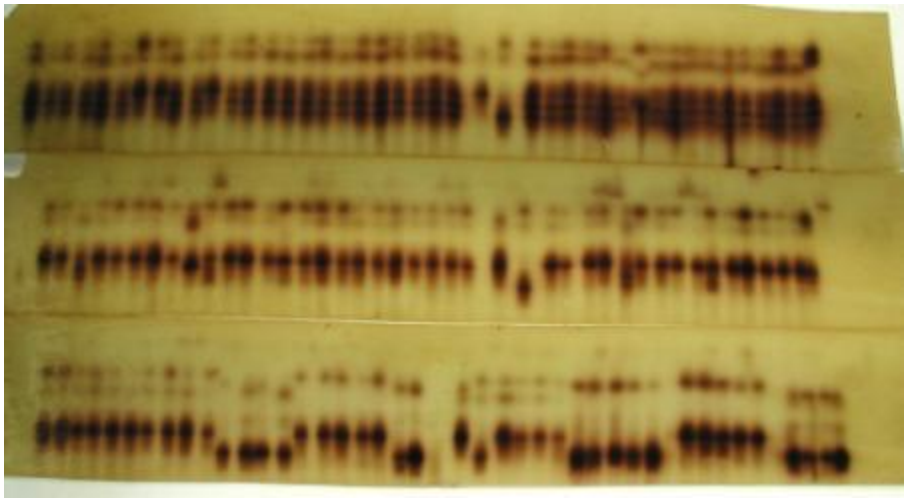
8. Stain the Gel

- Place thin slices into boxes.
- Stain gels for ten different isozyme loci.
- Incubate in incubator for approximately one hour.

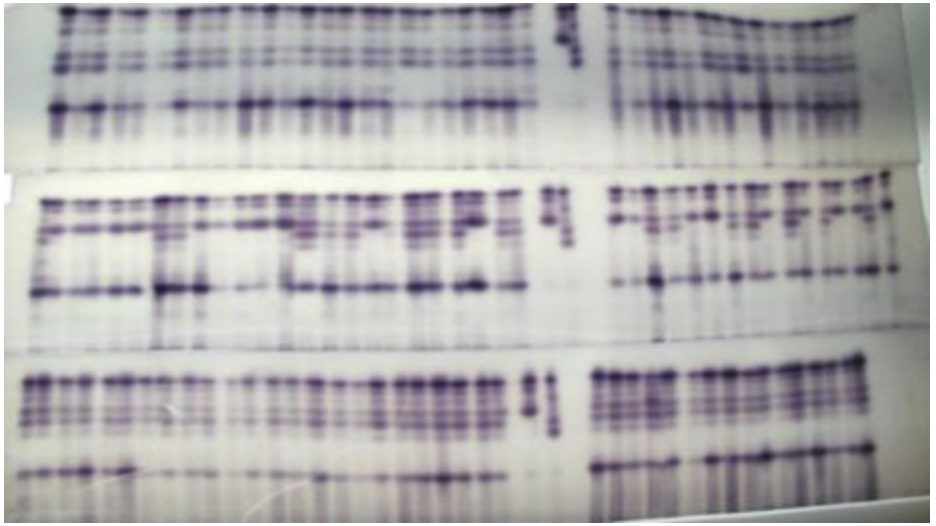


Stained Isozyme Gel

ACP Stain

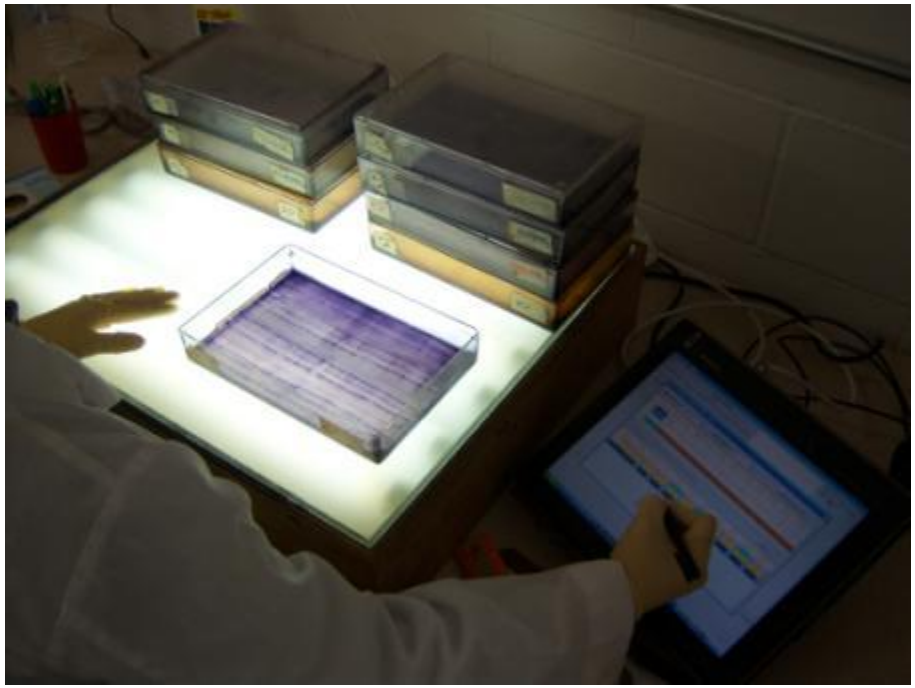


MDH/ADH Stain

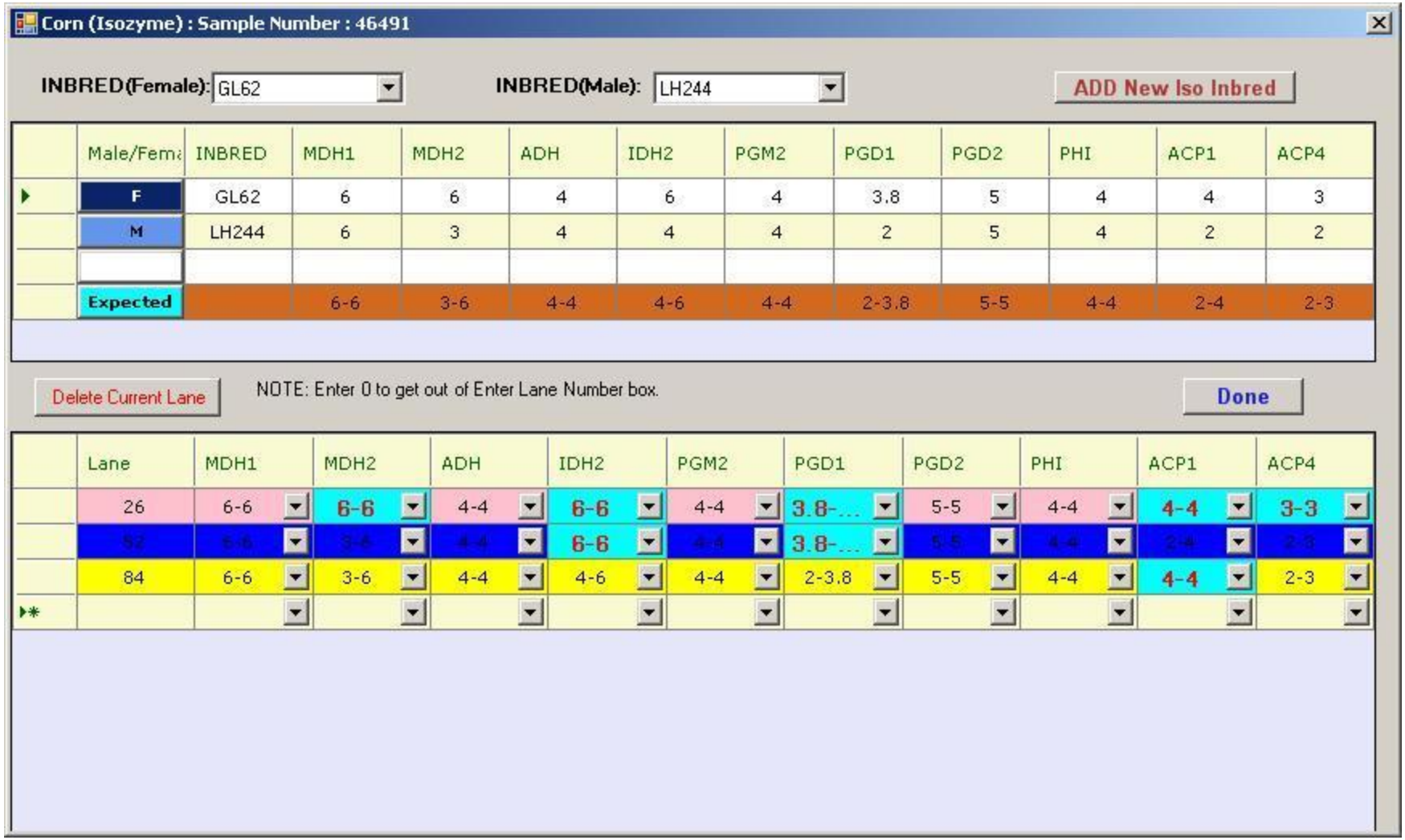


Reading Gels and Reporting Results

- Read gels using a light table.
- Determine the 10 loci's banding patterns and give a numeric score.
- Scores recorded into SGS computer system. *Test results immediately available on website.*



Example of a Isozyme Score Sheet



	Male/Fema	INBRED	MDH1	MDH2	ADH	IDH2	PGM2	PGD1	PGD2	PHI	ACP1	ACP4
▶	F	GL62	6	6	4	6	4	3.8	5	4	4	3
	M	LH244	6	3	4	4	4	2	5	4	2	2
	Expected		6-6	3-6	4-4	4-6	4-4	2-3,8	5-5	4-4	2-4	2-3

	Lane	MDH1	MDH2	ADH	IDH2	PGM2	PGD1	PGD2	PHI	ACP1	ACP4
	26	6-6	6-6	4-4	6-6	4-4	3,8-...	5-5	4-4	4-4	3-3
	52	6-6	3-6	4-4	6-6	4-4	3,8-...	5-5	4-4	2-4	2-3
	84	6-6	3-6	4-4	4-6	4-4	2-3,8	5-5	4-4	4-4	2-3
▶*											

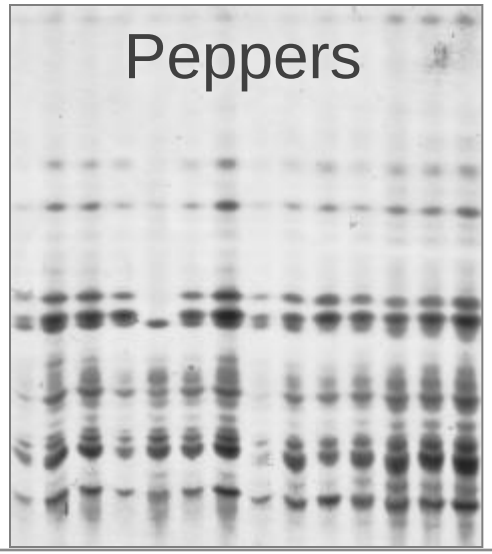
ISOELECTRIC FOCUSING

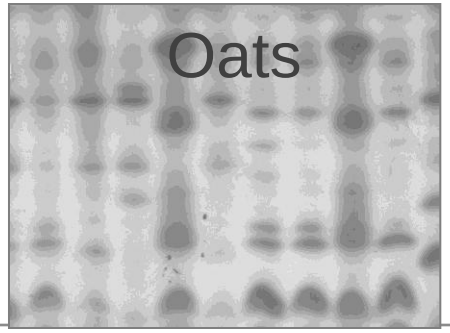
Method For Other Crops




ISOELECTRIC FOCUSING
IEF

- Electrophoresis in a pH gradient.
- A separation method that resolves proteins on the basis of their isoelectric points.
- Different molecular components of the protein are detected according to their position in the gel.





ISOELECTRIC FOCUSING

IEF

- Inbred and hybrid purity testing is a quality assurance test to evaluate true-types, off-types and self levels.
- Test 96 individuals typically.
- Run male and female lines as controls to enable female and male self detection.
- Hybrids – Determine percentages of female selfs, male selfs, off-types and total purity.
- Inbreds – Determine percentages of off-types and total purity.

Overview of IEF

(Isoelectric Focusing)

- Crush seeds or plant material and extract proteins.
- Use 1 of 5 extraction buffers depending on seed/tissue type.
- Pipette extracted proteins onto an agarose gel.
- Run for 80 minutes at an average of 50 watts.
- Stain gel with either a total protein stain or an enzyme stain.

Banding patterns can differ greatly and prove to be an excellent tool for variety verification and hybrid purity.

Extract Proteins from Seed

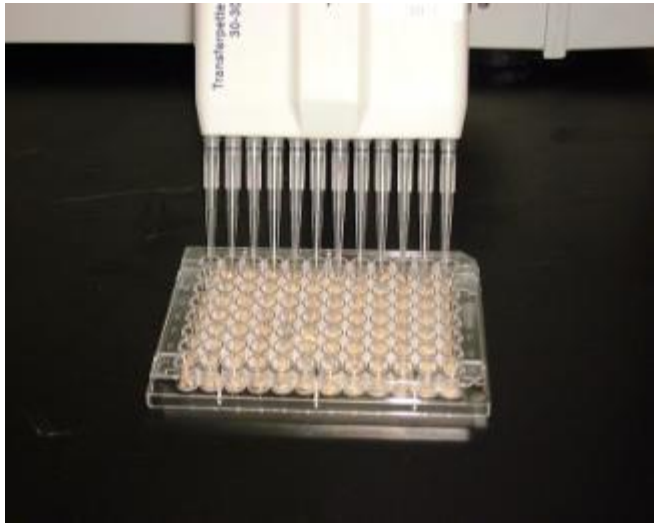
Two methods

Individuals

- Place individual seeds into wells on a 96 well plate.
- Crush seeds.
- Add extraction buffer.

Population Sample

- Grind 2 grams of seeds into fine powder when running a population sample.
- Weigh a subsample of powder into a tube for extraction.



Prepare the Gel

Use pre-cast agarose gels for IEF.

1. Saturate wicks with electrode solutions at the anode and cathode areas of the gel.

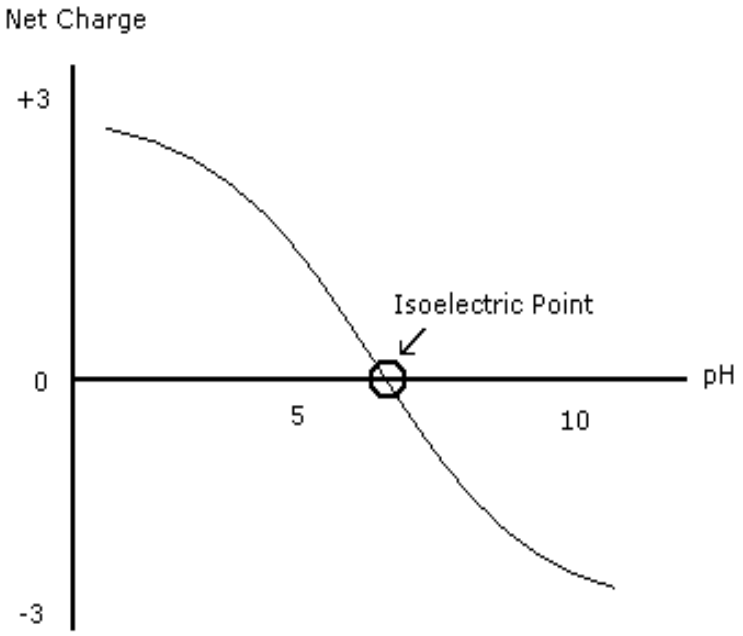
   (these wicks help to create a pH gradient)

2. Load sample onto template.

3. Run gel for approximately 1 ½ hours.

How Proteins Move

- Proteins are either positively or negatively charged.
- When proteins are at a pH where their charge is zero, they are at their isoelectric point (pI).
- When an electric current is applied to the gel, a pH gradient is formed and this allows the proteins to migrate to their pI.

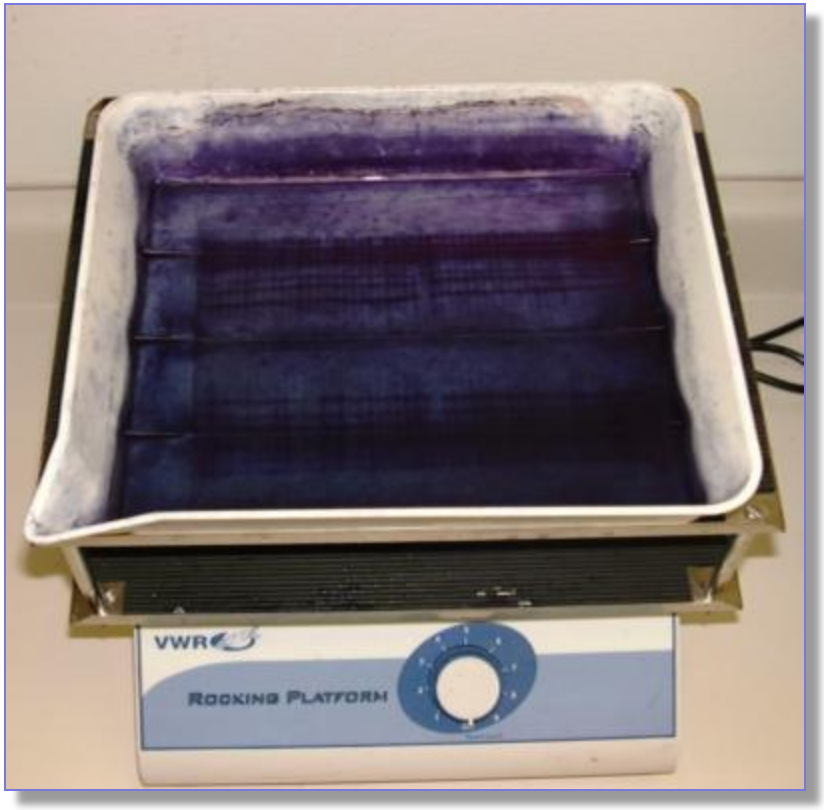


Stain the Sample

1. Place gel in staining solution. *(use either an enzyme stain or a total protein stain)*

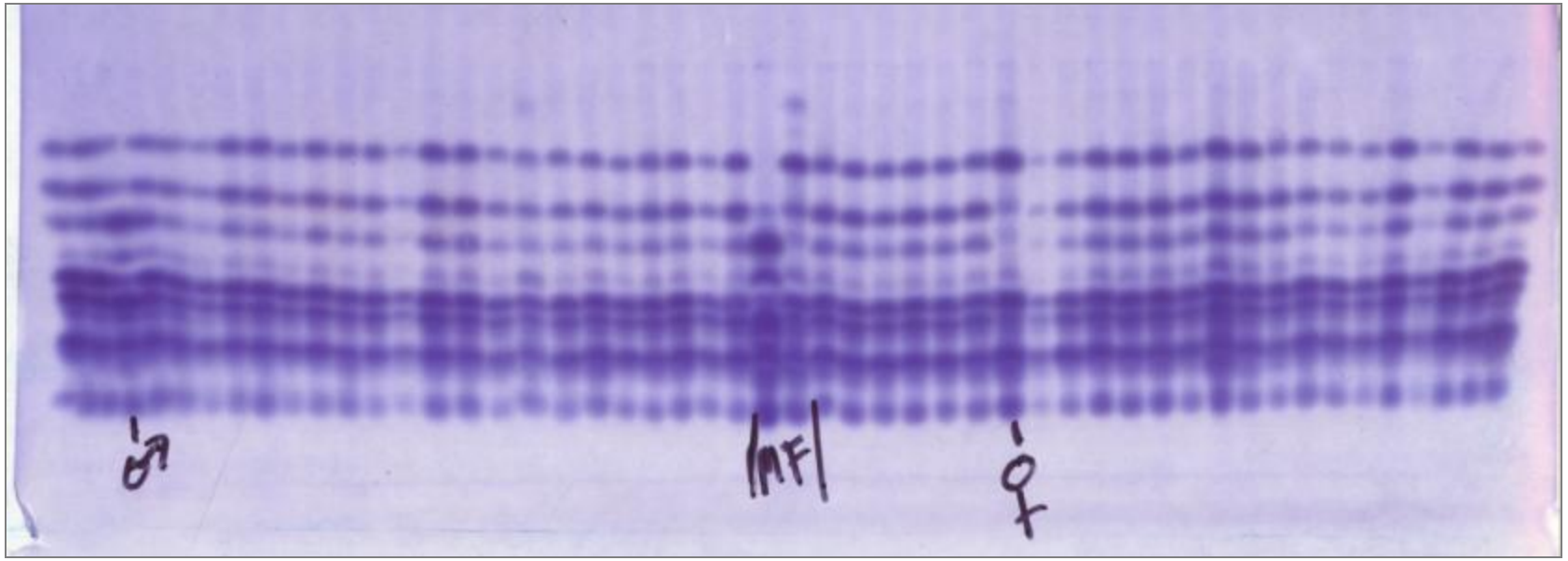
2. Stain for five minutes to one hour. *(type of stain determines the time)*

3. Dry gel before interpreting the sample.



Analysis of the Gel

1. Run a control. (female and male inbreds or a known sample)
2. Analyze banding pattern in comparison to the control.
3. Report any genetic differences as either female selfs, male selfs or offtypes.



Why Use IEF?

- Inexpensive
- Quick – results in as little as 24 hours
- Accurate
- Determines Genetic Purity
- Varietal Identification/Protection

Remember us for your testing needs- SGS Agricultural Services

Laboratory

- Seed Testing
- GMO Testing
- Soil Testing
- Feed and DDG Analysis
- Food Safety Testing
- Product Development Research
- Seed Treatment Analysis

Field

- Contract Research
- Field Mapping and Grid Sampling
- Field Audit Service

Thank you for attending our webinar.

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