# STARCH GEL ELECTROPHORESIS

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# Terminology:

- Allele: One of several alternate forms of a gene occupying a given locus on the chromosome.
- Allozyme: A single visible band indicates a homozygote for the particular enzyme assayed.
- Female Self: Isozyme patterns that are identical to the seed parent of a hybrid.
- Genotype: Total genetic makeup of an individual from their parent.

# Terminology:

- Heterozygous: When there are two different alleles of a gene at one locus.
- Homozygous: When there are two identical alleles of a gene at one locus.
- Locus: The chromosomal location of a gene on a chromosome in comparison to other genes on the chromosome.
- Male Self: Isozyme patterns that are identical to the pollen parent of a hybrid.
- Off-type: Isozyme patterns that do not match the hybrid or inbred in two or more loci.

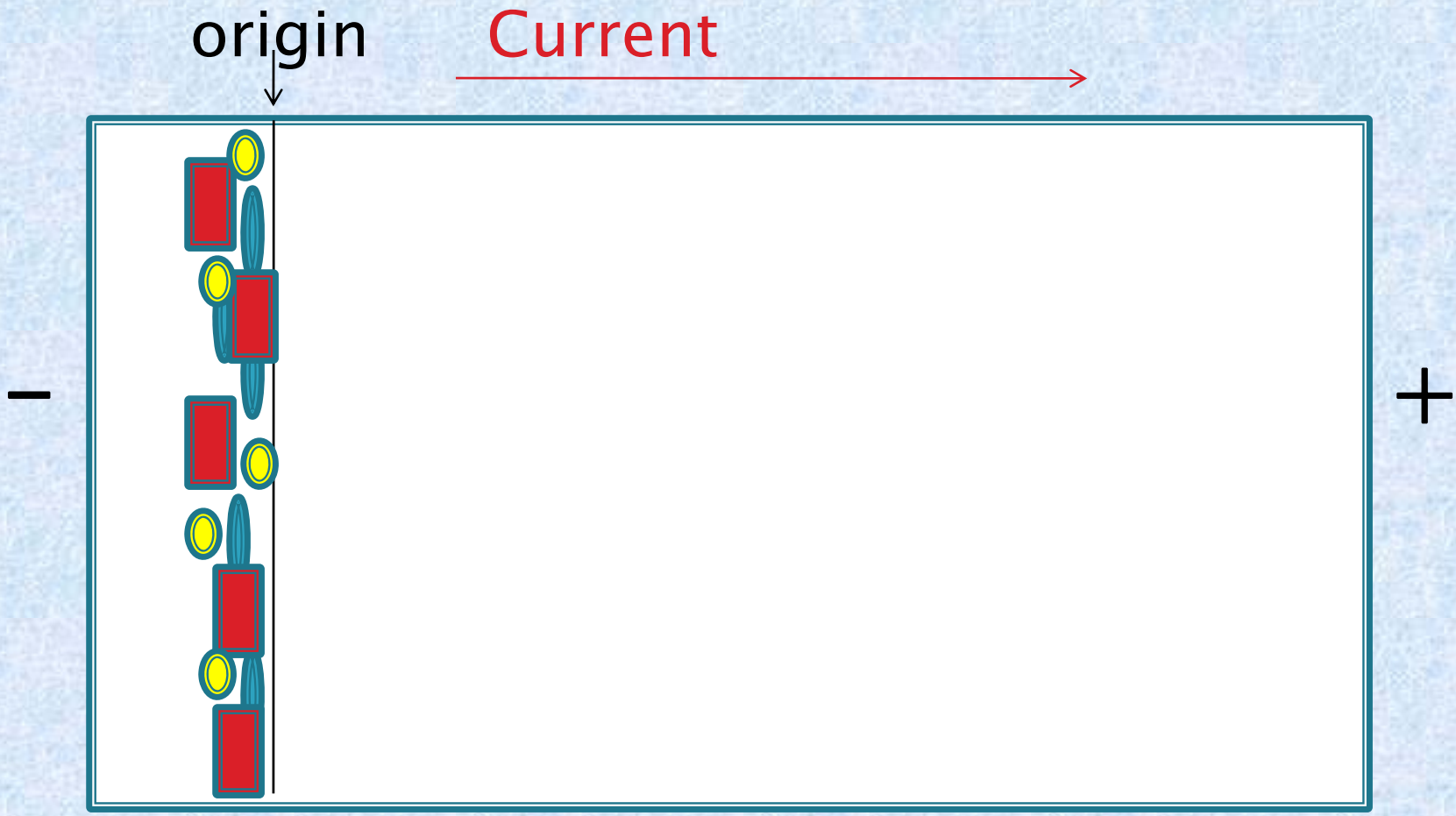
# Terminology:

- Phenotype: The observable physical characteristics of an individual.
- Seed mix: Observed isozyme patterns do not match the genetics from either parent of the hybrid.
- Variant: An observed difference in the expected isozyme pattern at only one locus.
- Zymogram: Display of enzymes on an gel (isozyme pattern).

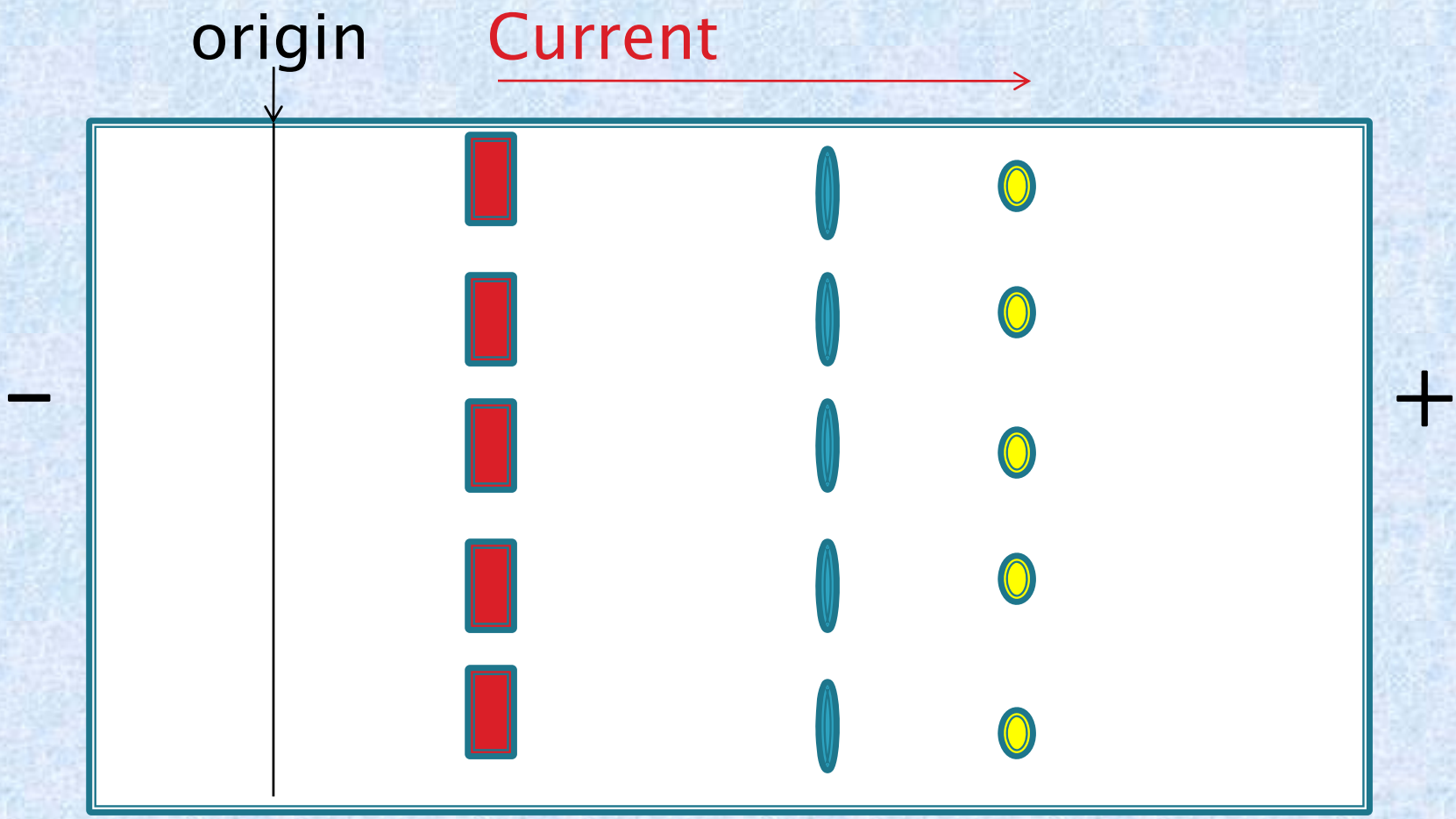
# What is Starch Electrophoresis?

- The practice of running an electrical current through a starch medium to separate isozymes, which are multiple forms of specific enzymes (proteins), based on charge and molecular size.

# Before Electrophoresis



# After Electrophoresis:



# Steps of Electrophoresis

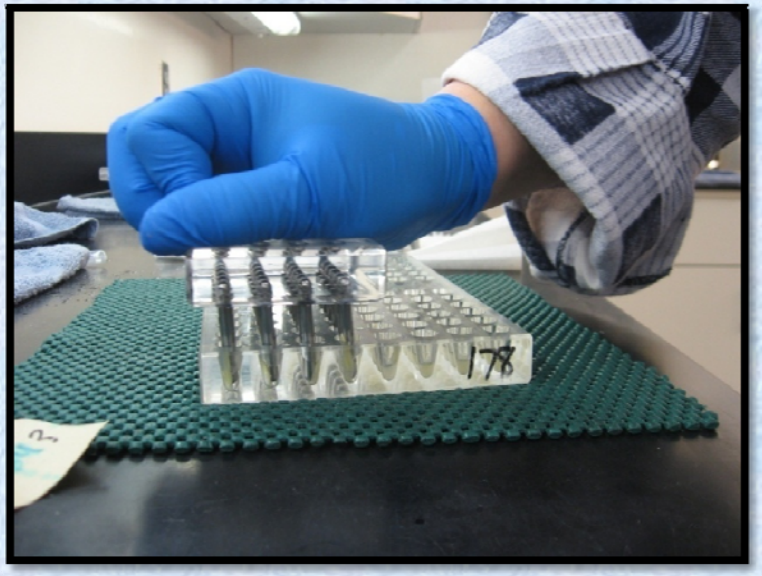
- Sample Preparation
- Gel Making
- Loading, running and slicing gels
- Preparation of buffers and stains
- Score and interpretations of gels

# Sample Preparation

- Seeds are planted on moist germination paper.
- Left in dark germinator for 4-7 days.

# Sample Preparation

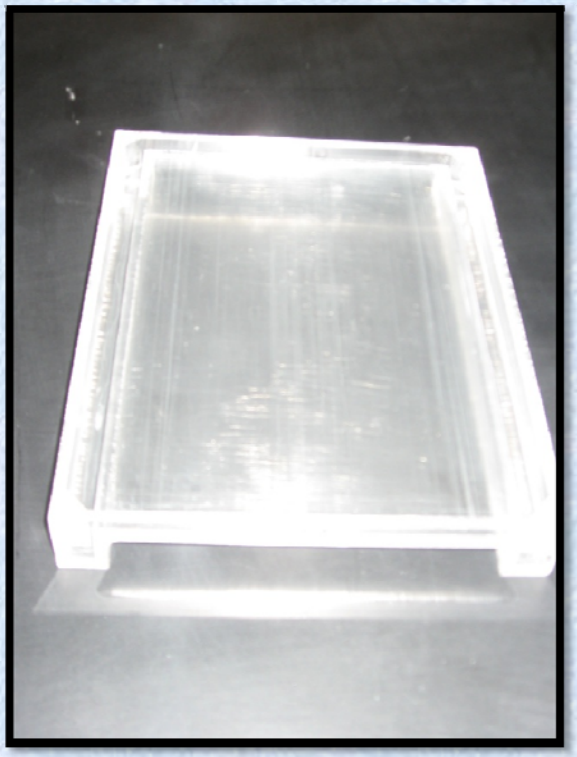
- Coleoptiles, leaf, root, or pollen are extracted and placed in wells containing extraction buffer.
- Samples are crushed in the buffer in order to release the proteins.
- Samples must be kept cool so not to denature the proteins.



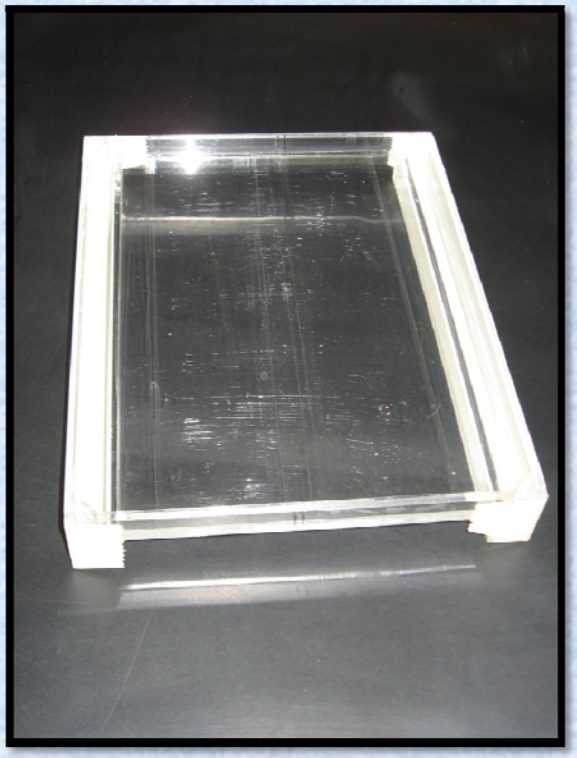
# Making Gels:

- Determine what gel system.
- Need consistent starch and sucrose product.
- Need consistent gel making practices.

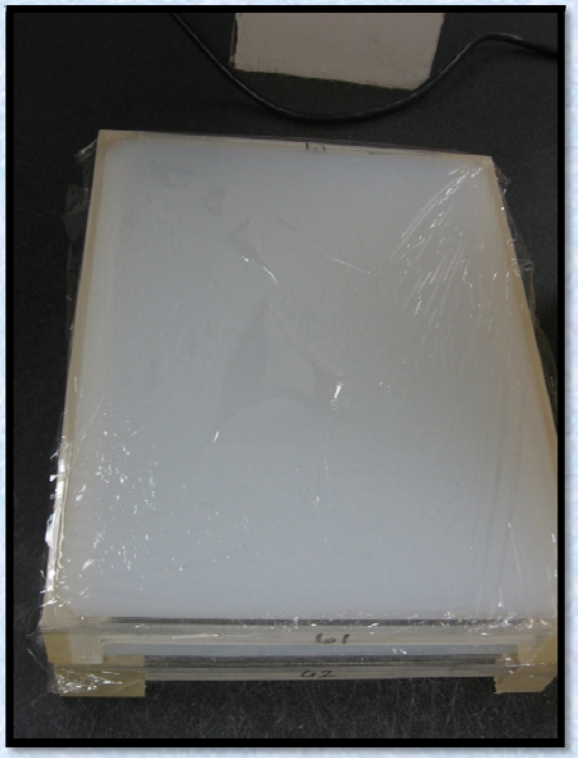
# Gel Tray



Gel Tray

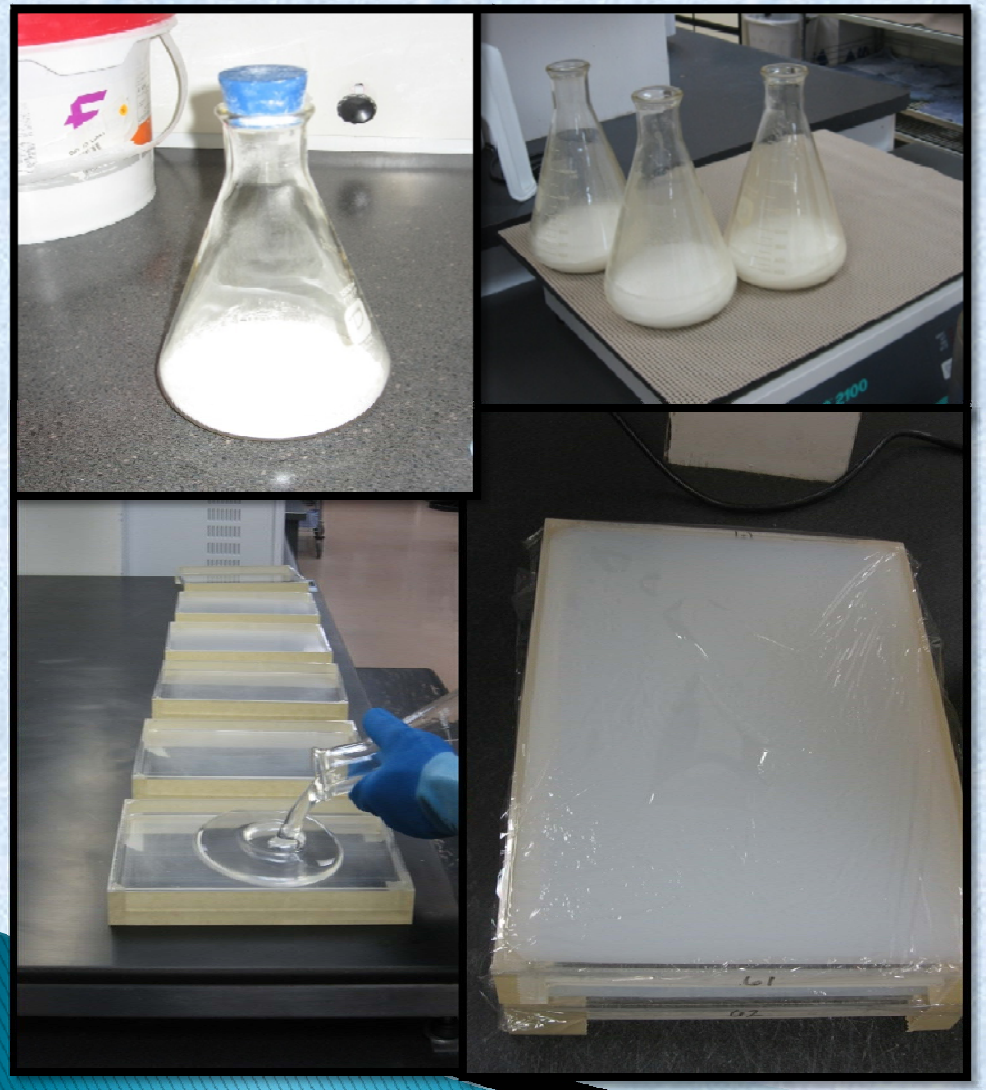


Taped Tray



Poured Gel

# Gel Pouring



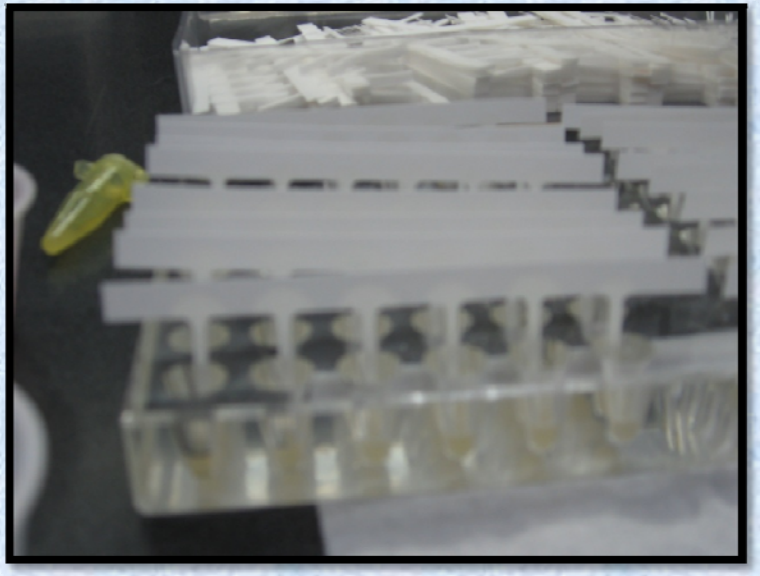
- Starch and sucrose, predetermined amount, are added to specific amount of buffer and heated.
- The solution is poured in a tray and is allowed to sit and cool. Then the gels are wrapped with plastic to avoid further drying.

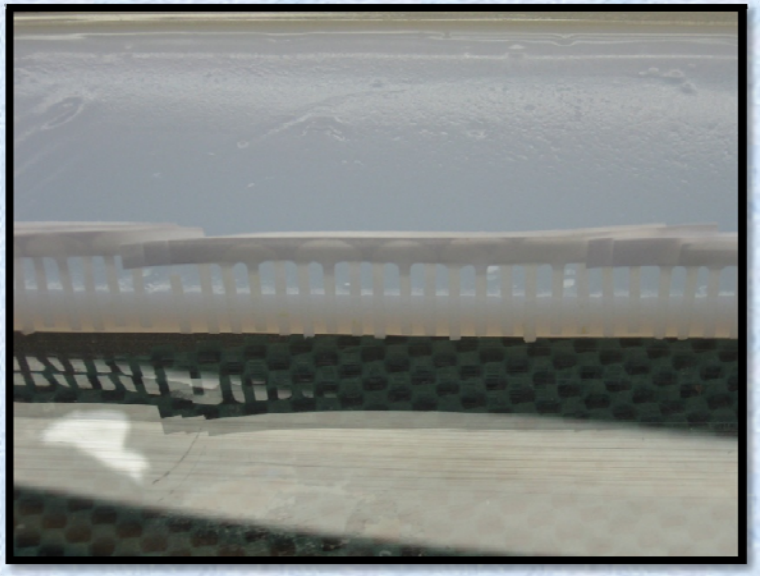
# Gel Quality

- Quality of Water
- Starch source
- Starch and sucrose amounts used in making gels
- Proper gel preparation procedures
- Ionic Strength of Buffer
  - Increase in strength: sharper separation and decreases migration speed
  - Decrease in strength: poorer separation and increases migration speed, less heat
- pH
  - Higher pH less anodal migration: tighter bands
  - Lower pH more anodal migration: more spacing between bands

# Gel Loading

- Paper wicks are used to transfer samples to gel.
- Keep the lanes in order!
- Use controls, a known banding pattern, for helping interpreting results.
- Use female and male controls for hybrids, unless the parents have been previously recorded.
- Place a marker dye to measure migration distance.





# Running Gels

- Place the loaded gel in the buffer tray.
- Cover the gels with a cold pack.
- Gels are run in a cooler or refrigerate room to control heat.
  - Helps stabilize gels
  - Keeps the lanes consistent
  - Helps to keep the proteins from denaturing.

# Running Gels


- Connect gel tray to an electrical power source
- Power source has electrodes (leads), + and –
- Choose constant
  - Milliamps (Current)
  - Watts (Power)
  - Volts (Voltage)

# Running Gels

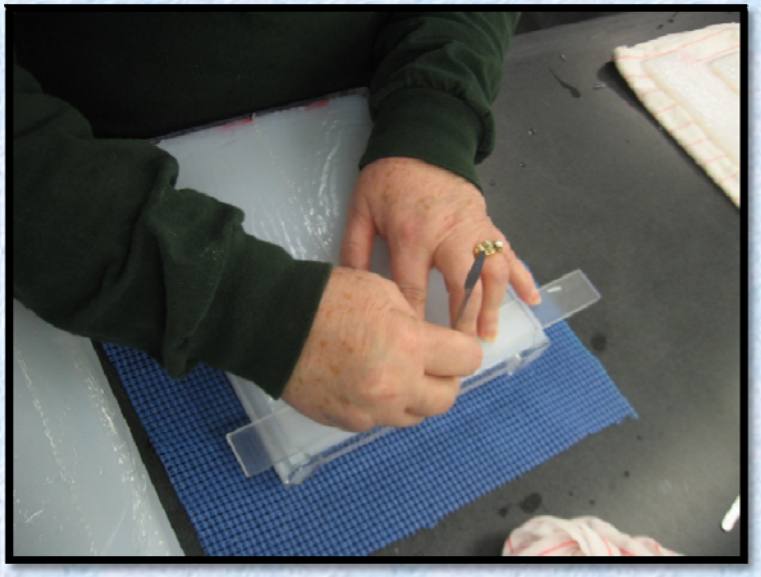
- Ohm's Law
  - I=E/R
    - Electric current is proportional to voltage and inversely proportional to resistance
  - Electrical Parameters
    - I = Current (amps)
    - E = Voltage (volts)
    - R = Resistance (ohms)
    - P = Power (watts)

# Running Gels

- Migration
  - Size of gel
  - Voltage amount
  - Temperature
  - Ionic Strength of the buffer
  - Net charge of the protein
  - Molecular size and shape of the protein
- Time
  - Run time 2-10 hours
  - Longer the run time the more separated the bands become.

# Slicing Gels

- Place a notch in the corner of the Gel.
  - Helps to keep orientation of the gel
- Slice the gel into several slices.
- Each slice is placed into separate stain trays.
- Each slice is stained with a different protein stain.

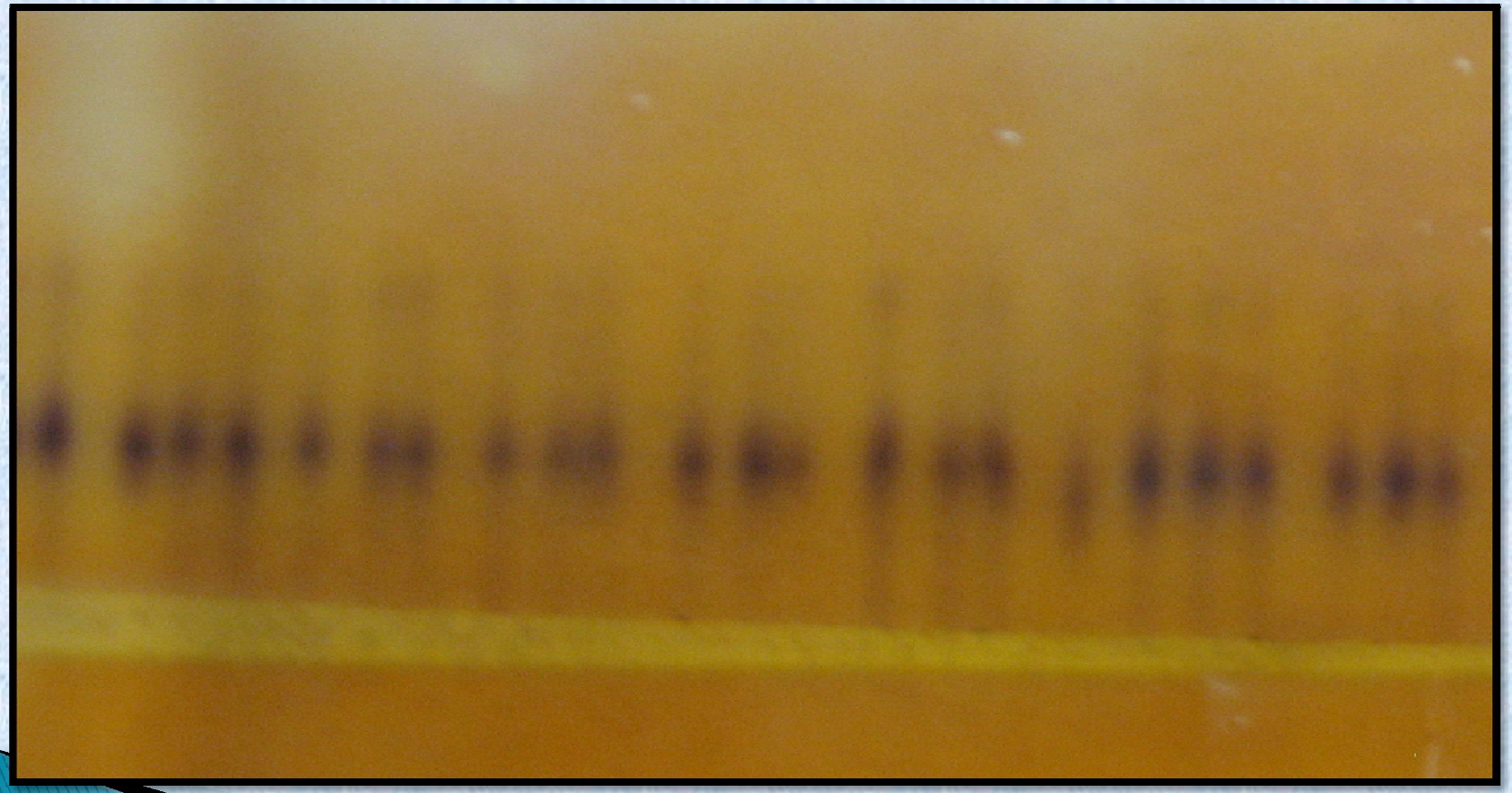


# Staining Gels

- Know MSDS of chemicals for safety.
- Immersing the gels in different stains will develop different enzymatic activity bands on the gel.
- Different stains use different pH to help develop different loci stain systems.
- Incubation times and conditions vary for stain systems.

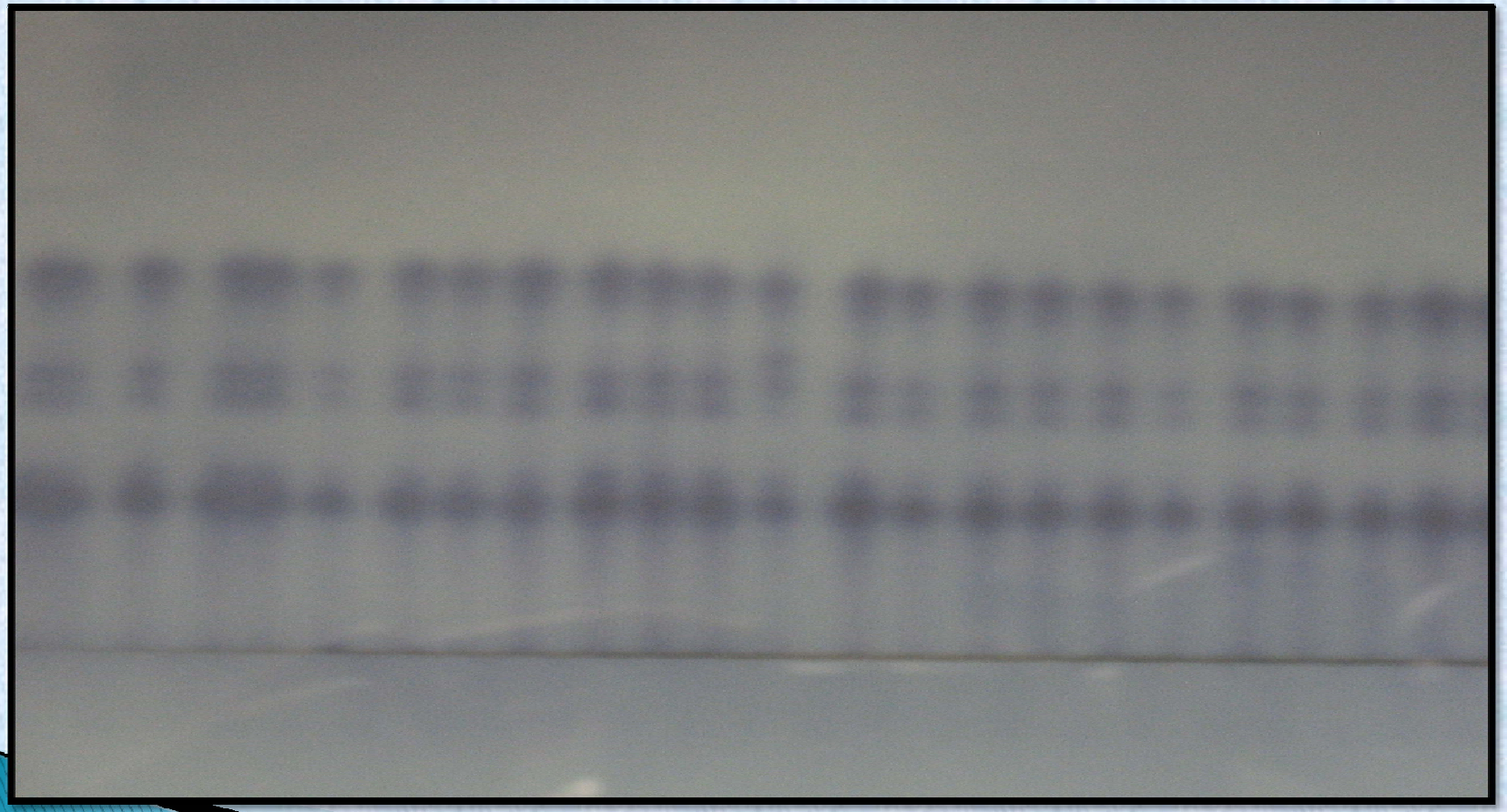
# Staining Gels

ACP – Acid Phosphatase



# Staining Gels

MDH – Malate dehydrogenase



# Gel Interpretation

- Selection of enzyme systems (stains) are determined by the lab's requirement for data.
- Familiarity with gel systems are needed for evaluation of gels. This helps in understanding expected banding patterns and detecting the differences.
  - Controls used are helpful for referencing patterns
    - Known pattern
    - Parents for hybrids
  - Keeping a history of inbred and hybrid patterns
- Understanding of basic Mendel's genetics.

# Gel Interpretation

- Documentation on paper and/or computer
- Photography
- Fixing gels
- Storage of gels

# Gel Interpretation

Inbreds:

Self crossing which results the same allele at all loci.

Cross:

A/A X A/A

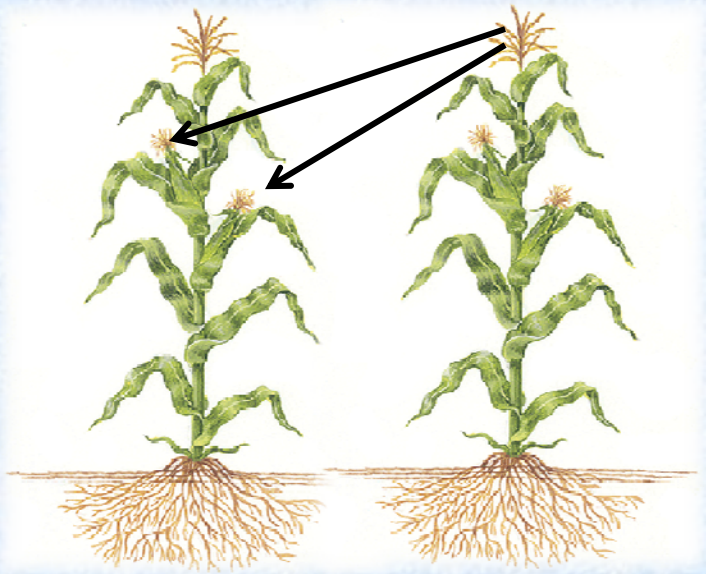
Inbred= A/A

- Identify
  - Off-types
  - Variants
  - Seed Mix

# Gel Interpretation

Hybrids:

Crossing where the female and male parents come from separate sources. Creating different allele possibilities at the loci.



Cross:

A/A X B/B

Hybrid = A/B

- Identify
  - Female-Self's
  - Male-Self's
  - Variants
  - Segregation
  - Off-types (outcrosses)

# Uses for Electrophoresis

- Seed Quality → customer
  - Shorter turn around than grow outs.
- Can help identify problems in the field from field samples.
- Breeder's seed
  - Helps to eliminate segregating lines.

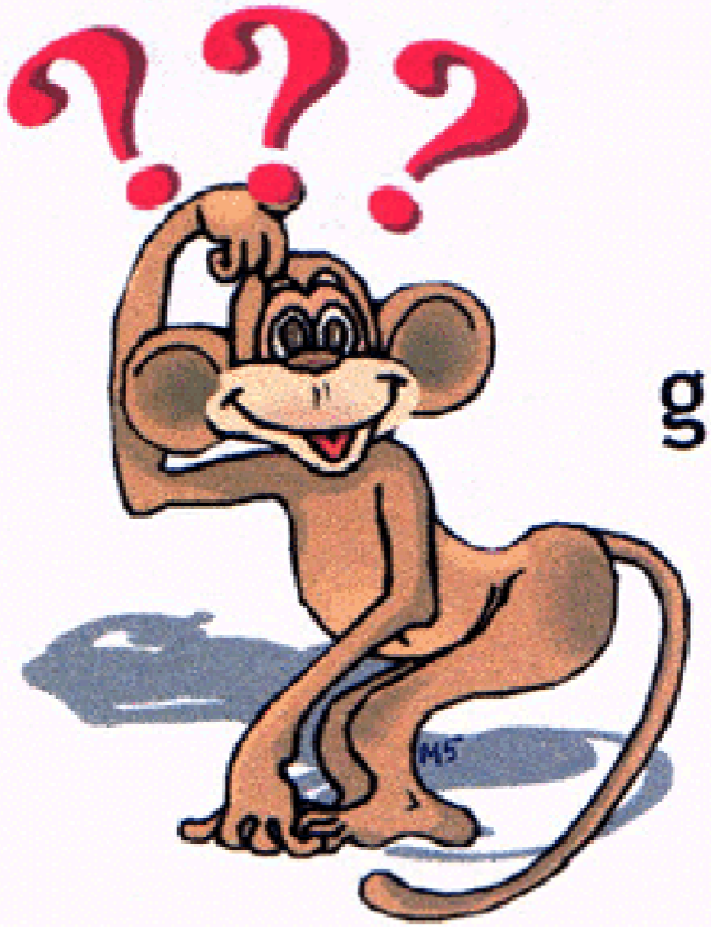
# Pros for Starch Electrophoresis

- Quick turn around time
- Gives strong data genotypically
- Can not be masked by environmental factors
- Isozymes can travel through the starch matrix
- Can get more than one slice from a gel
- Non-Toxic
- Inexpensive
- Can identify problems

# Cons for Starch Electrophoresis

- Off-type or variant may not be significant agronomic characteristic
- No long term storage of gels
- Limited number of loci tested
- Must have live tissue to extract enzymes from
- Not all tissue express the same enzymatic activity bands

# QUESTIONS?



Questions are guaranteed in life; Answers aren't.