Starch Gel Electrophoresis

Carol Betzel, RGT BioDiagnostics, Inc.
Pam Morasko, RGT Syngenta Seeds, Inc.

What is Starch Gel Isozyme Electrophoresis?

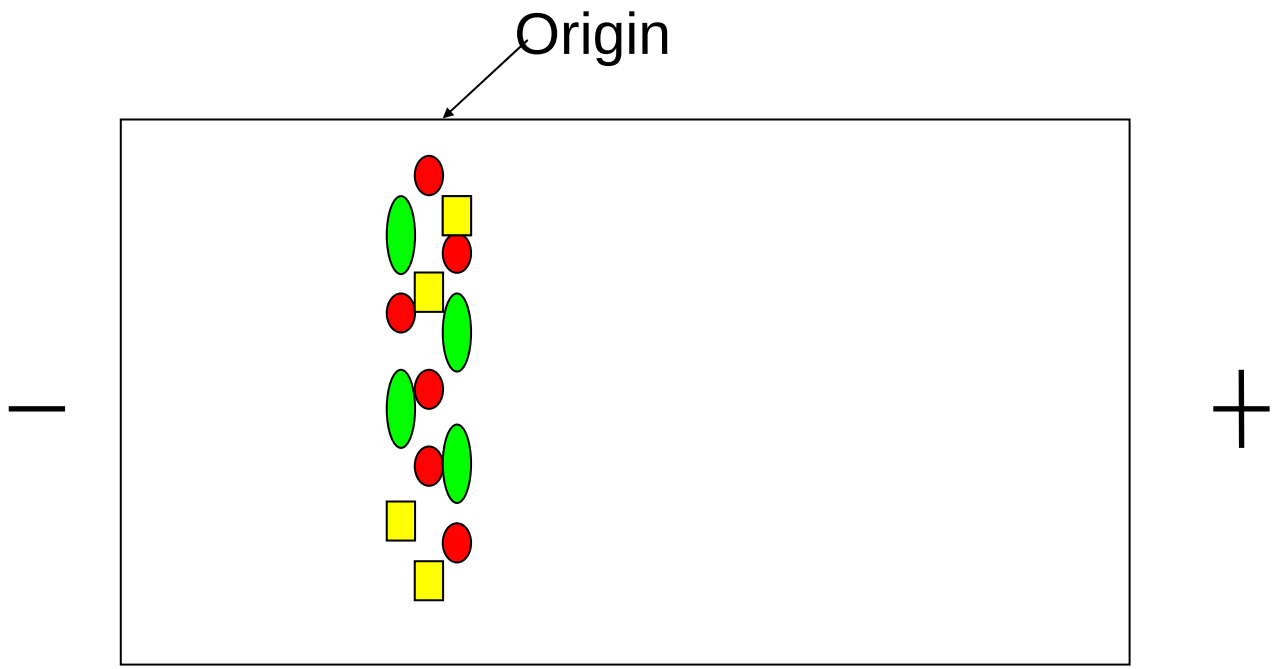
- **Isozyme Electrophoresis is a technique of separating multiple forms of an enzyme (isozymes) from each other.**

What are Isozymes?

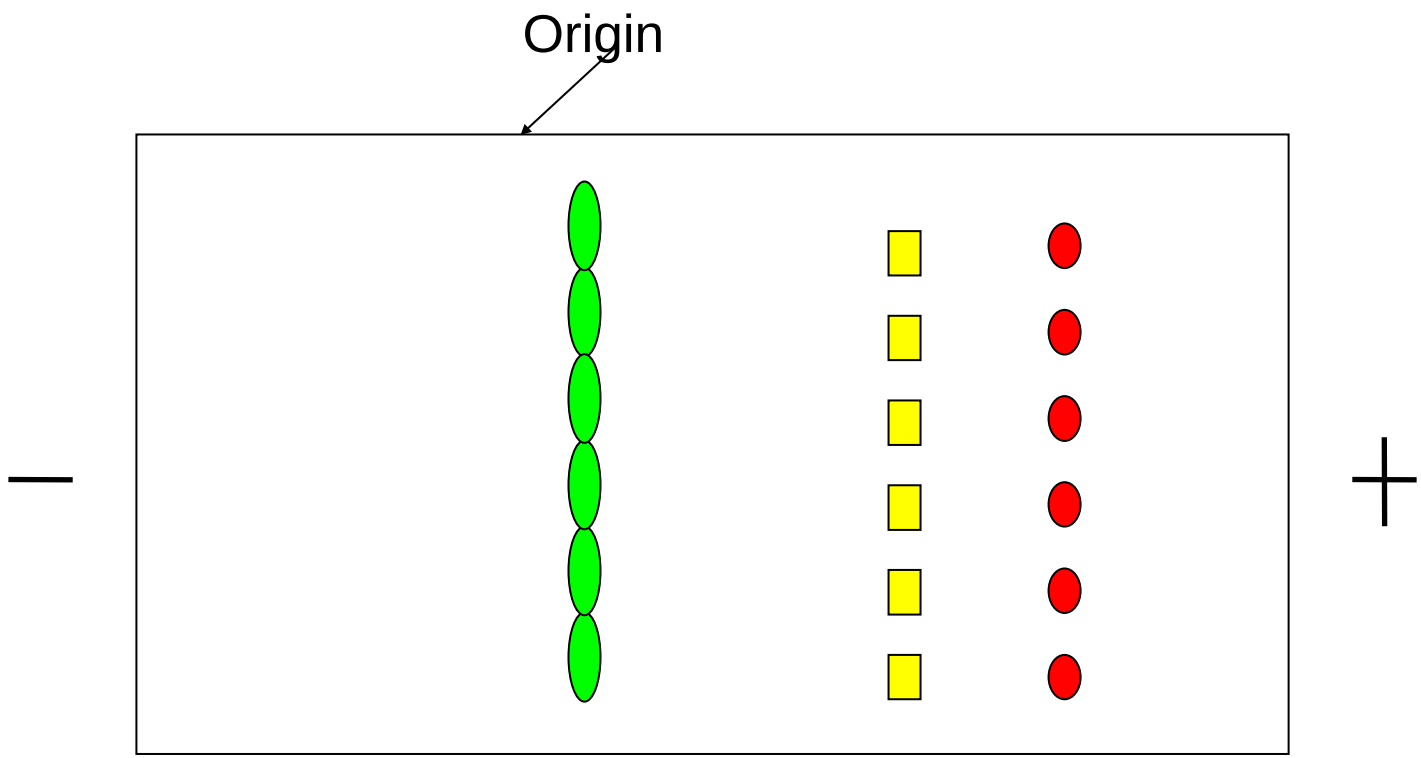
- **Isozymes are multiple molecular forms of specific enzymes.**
- **A single gene determines each molecular form of an isozyme.**
- **The occurrence of different forms of an isozyme is governed by simple Mendelian Genetics.**

Before Electrophoresis

Proteins are polarized molecules



After Electrophoresis



Pros

- Gives strong data genotypically of corn, etc
- Quick turn around time for results
- Starch matrix is suitable for isozymes to travel through and can get more than one slice from a gel
- Non-toxic
- Fairly inexpensive equipment

Cons

- What may be an offtype on the gel may or may not be expressed phenotypically
- Integrity of isozymes compromised by heat
- Must be live tissue to extract isozymes
- Not all tissues of corn band out the same i.e. Leaf tissue IDH run higher and less distinct than coleoptile tissue
- ACP not expressed in leaf tissue
- Not suitable for scanning or long term storing gels
- Lack of translucence and not suitable for quantitative measurement
- Starch suppliers
- Chemical supply/demand issues
- Some bands co- migrate and appear to be absent in a heterozygous combination PGM1&2 allele 9/9 4/4

Field vs Lab test

- Variant or offtype may be an insignificant agronomic characteristic
- Environmental factors can affect plant appearance
- Generally a correlation between field growouts and electrophoresis data

Estimation of Purity to determine error margin using ISTA SeedCalc 7

See ISTA website:

http://www.seedtest.org/en/home.html

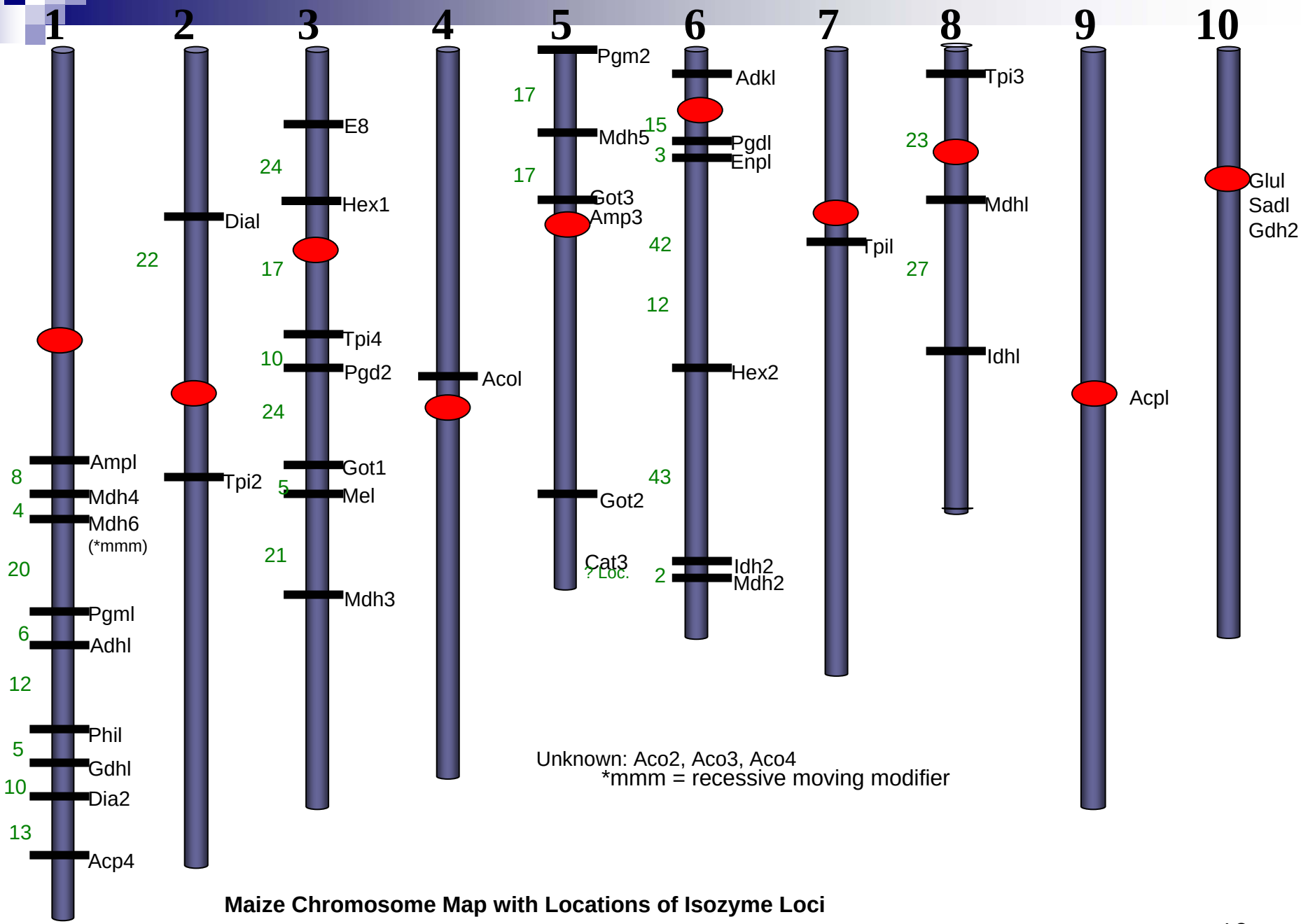
The Importance of Representative Sample

- Test results are reflective of the sample tested
- The test is only as good as the sample used
- The sample must reflect the entire seedlot to be valid

Basic Technique

- Stuber’s Technique
- Other Lab Technique

Chromosome Map

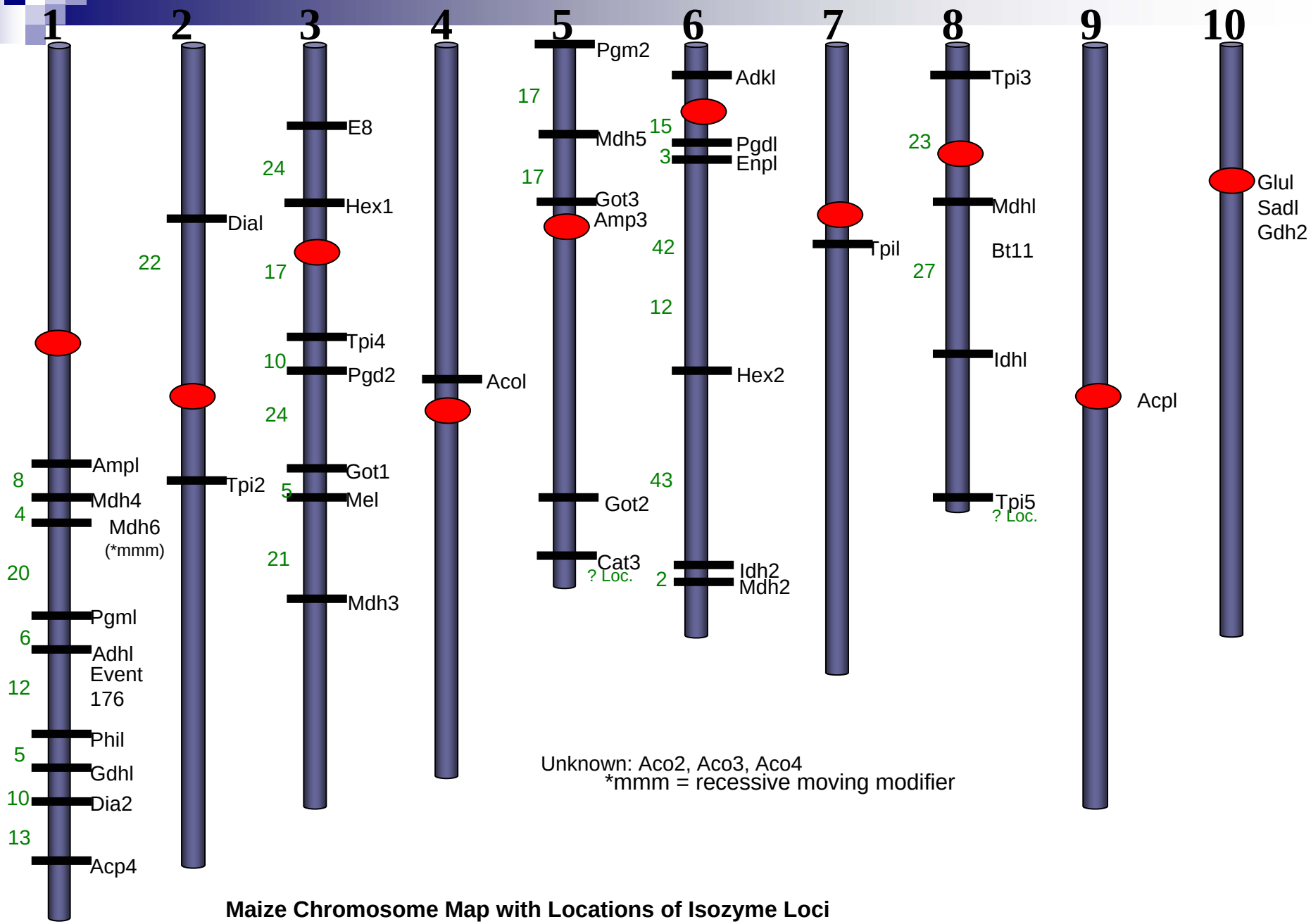


Unknown: Aco2, Aco3, Aco4

*mmm = recessive moving modifier

Maize Chromosome Map with Locations of Isozyme Loci

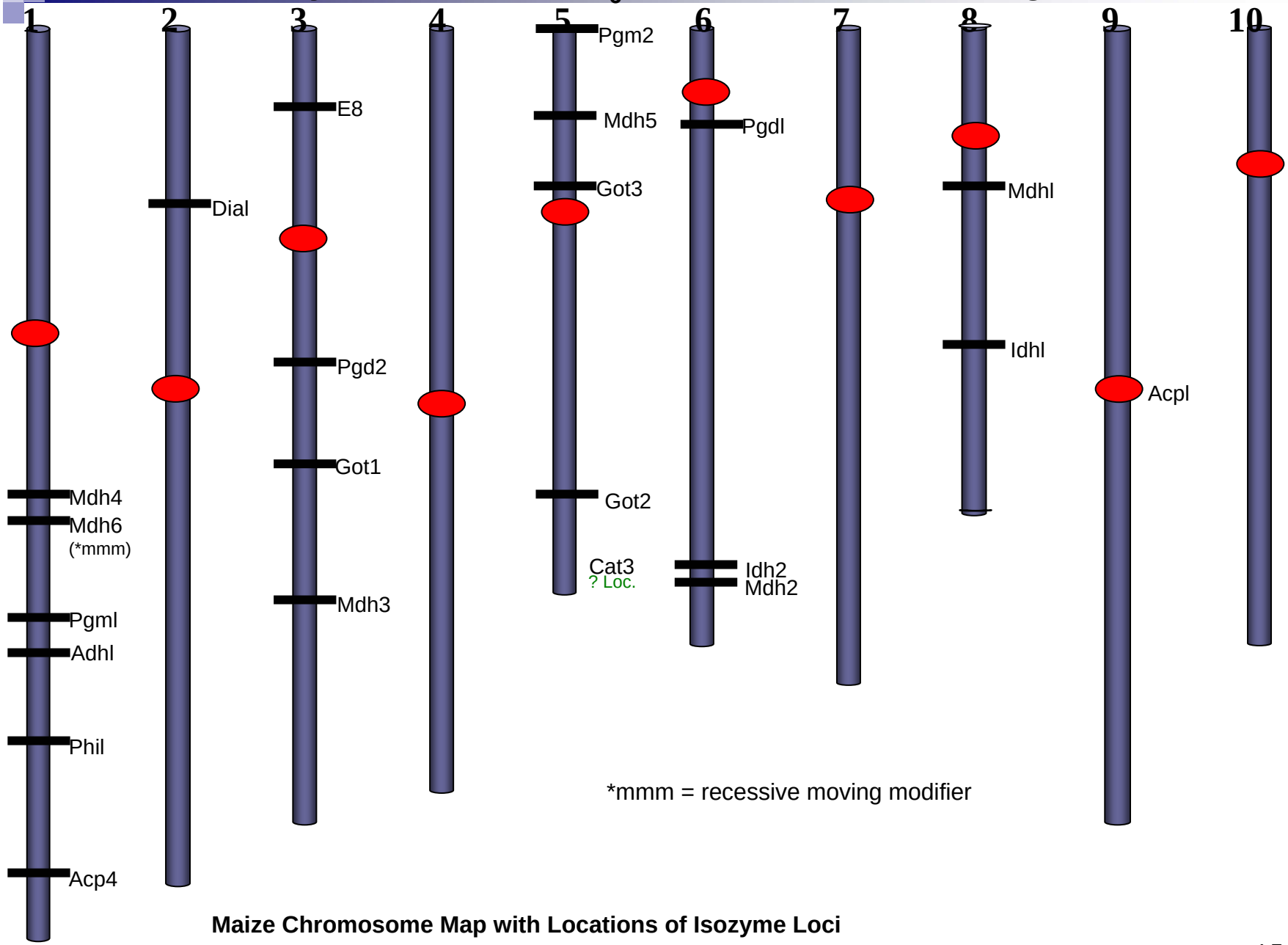
Reference: *Techniques and Scoring Procedures for Starch Gel*



Maize Chromosome Map with Locations of Isozyme Loci

Reference: *Techniques and Scoring Procedures for Starch Gel*

Isozymes Tested on S_6 Derived Sublines at Stage 3

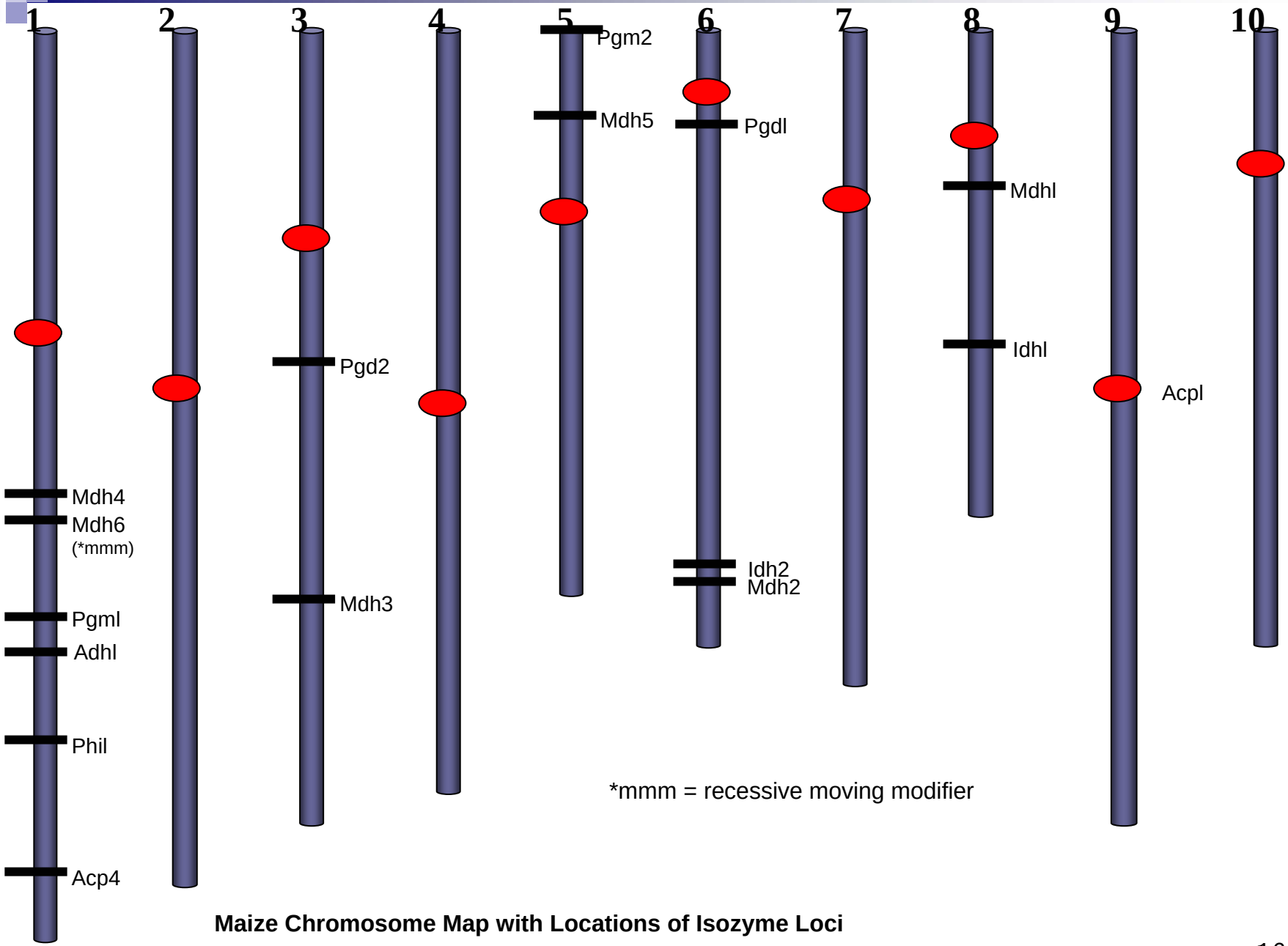


*mmm = recessive moving modifier

Maize Chromosome Map with Locations of Isozyme Loci

Reference: *Techniques and Scoring Procedures for Starch Gel*

Isozymes Tested on BSI-Derived Sublines at Stage 4



*mmm = recessive moving modifier

Maize Chromosome Map with Locations of Isozyme Loci

Reference: *Techniques and Scoring Procedures for Starch Gel*

Sample Receiving/Planting

- Chain of custody and tracking of sample
- Size of sample
- Planting and conditions of crop

Gel Making

- Determine gel system needed
- Multiple or single gel
- Consistent starch product suppliers
- Consistent taring and gel making procedure

Trouble shooting for gel quality

- Water quality
- pH
- Use of PGD
- Starch and sucrose amounts

Electrophoresis Process

Settings for Constant Power

- Temperature
- High temp increases migration and evaporation of buffer
- Noted by burn marks on gel and long separation of patterns

Proper gel and electrode buffer concentrations and pH

Adjusting the pH or increasing ionic strength lowers the resistance through the gel. Constant current allows isozymes to migrate slower.

Increasing ionic strength of gel buffer gives sharper separations and decreased mobilities

Ohm′s law E=IR

- Voltage=Current x Resistance

Decreasing ionic strength gives faster but poorer separation, less heat and increased mobility through the gel

- Higher the pH the less anodal migration giving tighter bands that doesn't run very far on gel
- Lower the pH the greater the anodal migration giving more spacing between bands
- Molecular sieving effect varies with gel concentration and diffusion is limited with big molecules in gel matrix of starch

Loading Gels

- Paper wicking of sample , pipetting technique, others
- Keeping lanes in correct order when loaded

Prep and Staining Gels

- Know your chemical MSDS for safety
- Different pH values for loci stain systems
- Proper chemical and concentrations prepared
- Incubation times and conditions
- Slice sequences and thickness

Evaluation of Gels

- Use of nomenclature of alleles
- No standard allele translation
- Need for standard control on each gel slice i.e. parents, known pattern control

Scoring of Gels

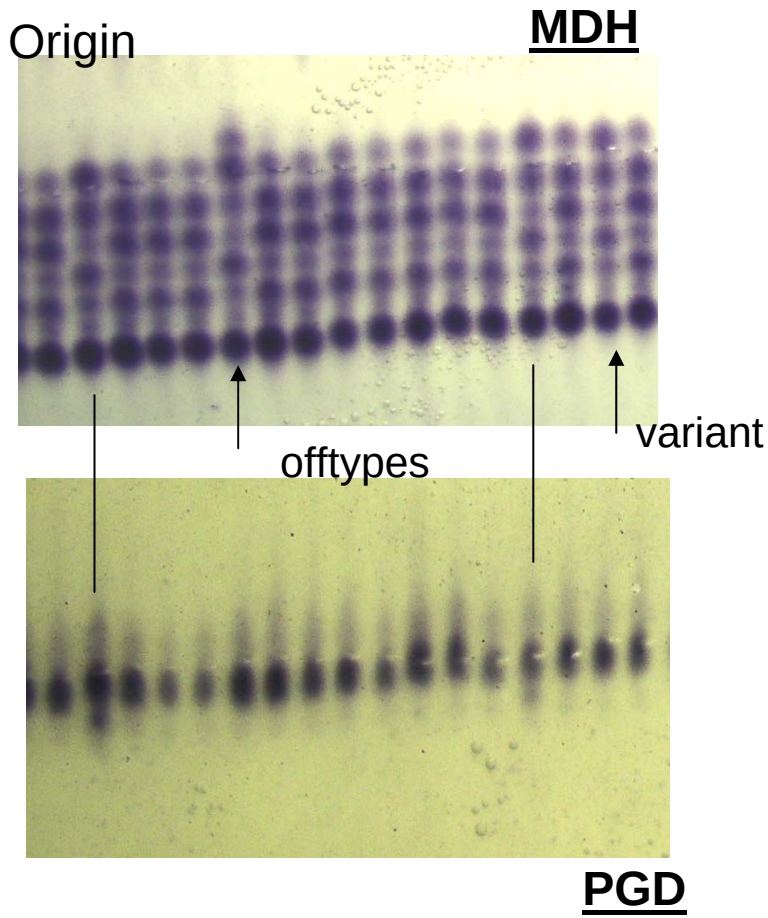
- Documentation on paper and/or computer
- Use of Stuber's manual for identifying patterns
- Keeping a history of inbreds and hybrid patterns
- Photography
- Fixing gels
- Storage of gels
- Mold/artifacts or new band? Are protein bands in response to mold?

Terminology/Nomenclature

- Inbred: Single allele for all loci: A/A
- Single parent cross inbred: A/A or A/B
- Hybrid: Female and pollen parent combined genotypes : A/B
- 3-way cross: A/A and A/B

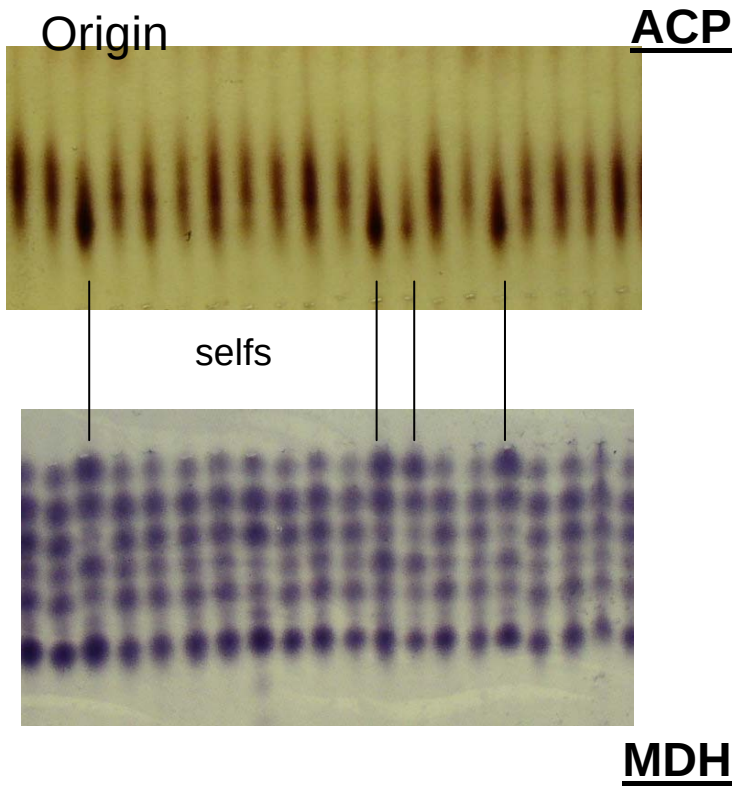
Terminology/Nomenclature

- Variant: Differs genotypically from hybrid or inbred at 1 locus
- Offtype: Differs genotypically from hybrid or inbred at 2 or more loci



Terminology/Nomenclature

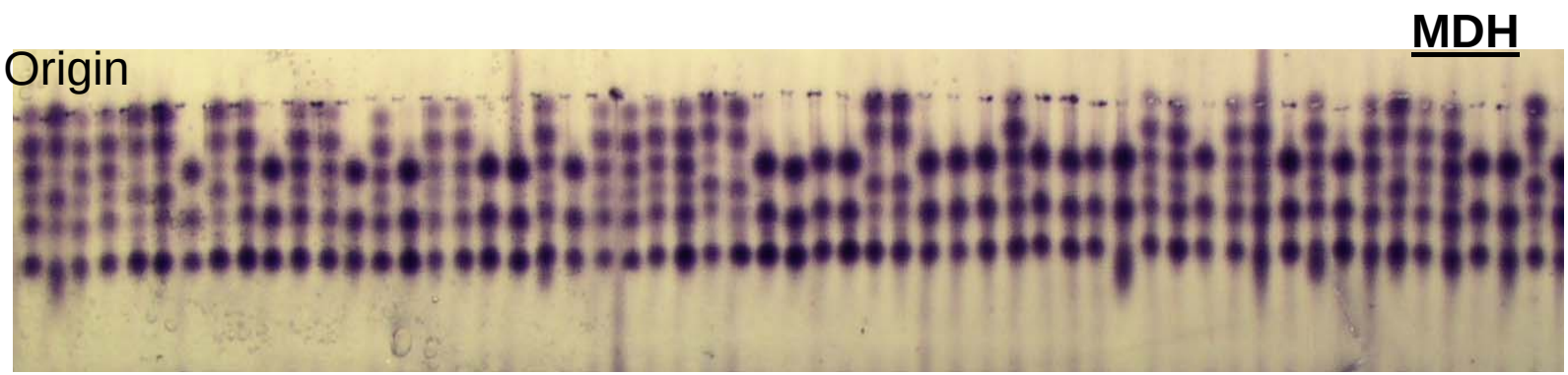
- Female Self: Genotype identical at all loci of seed parent
- Male Self: Genotype identical at all loci of pollen parent



Terminology/Nomenclature

Segregation: Heterogeneity and heterozygosity results at one or more locus and or any other loci.

Follows Mendel's law



Segregating Inbred

Effect of Segregating Inbred

Single Locus Segregation

Inbred population contains three genotypes

A/A B/B A/B

Hybrid cross contains two genotypes

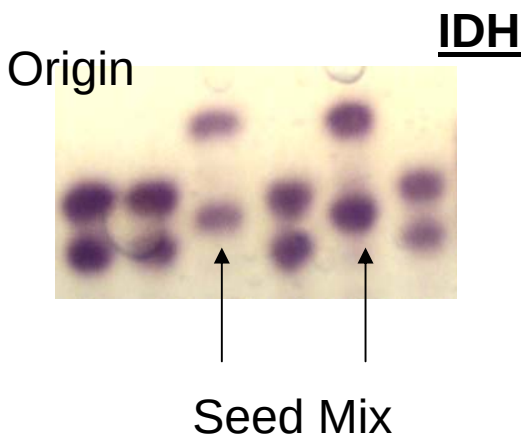
Contamination such as Seed Mix or Errors

Observed genotype does not have genetics from either parent.

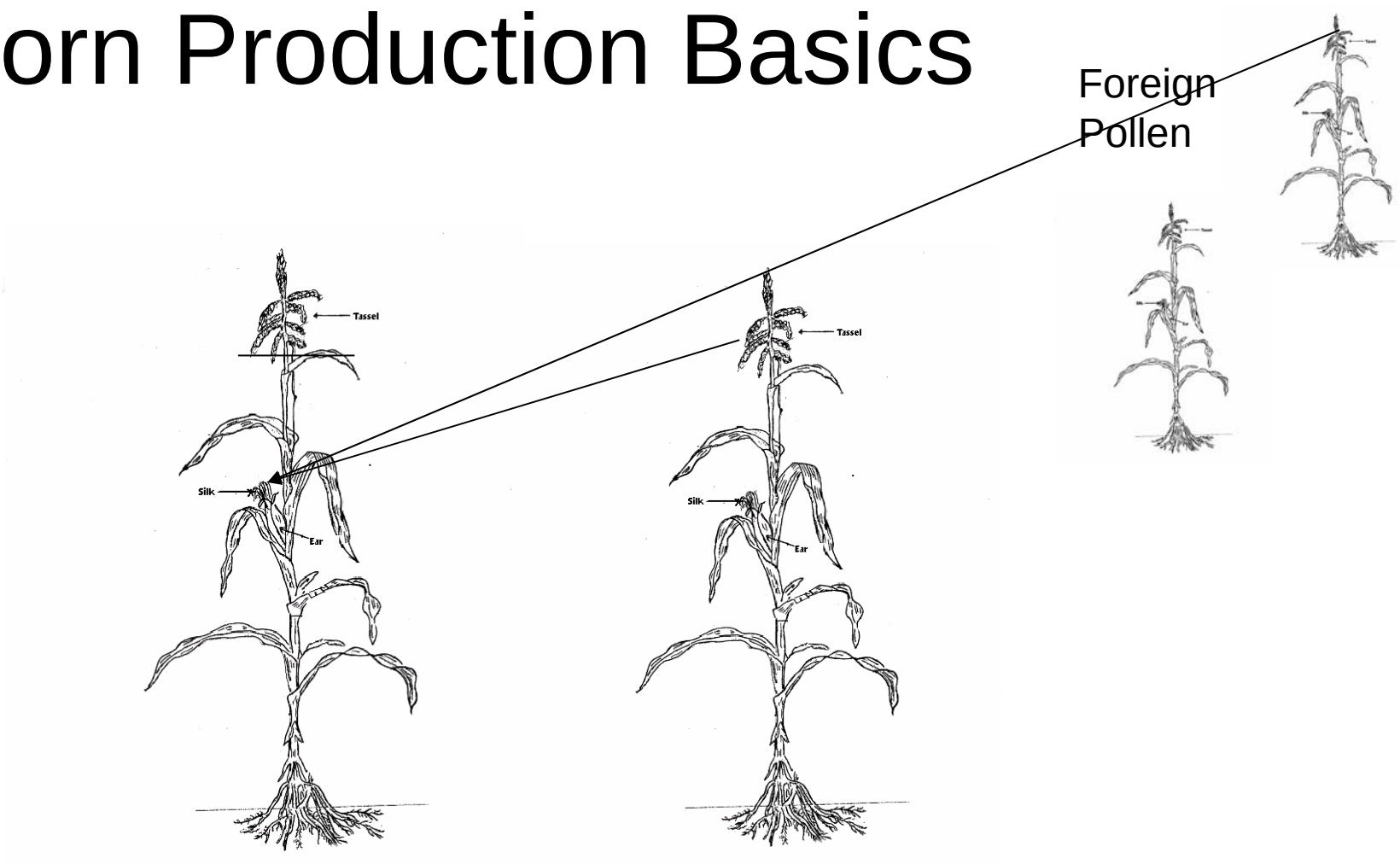
Hybrid: A/B

Seed Mix: C/C

Inbred Seed Mix:



Corn Production Basics

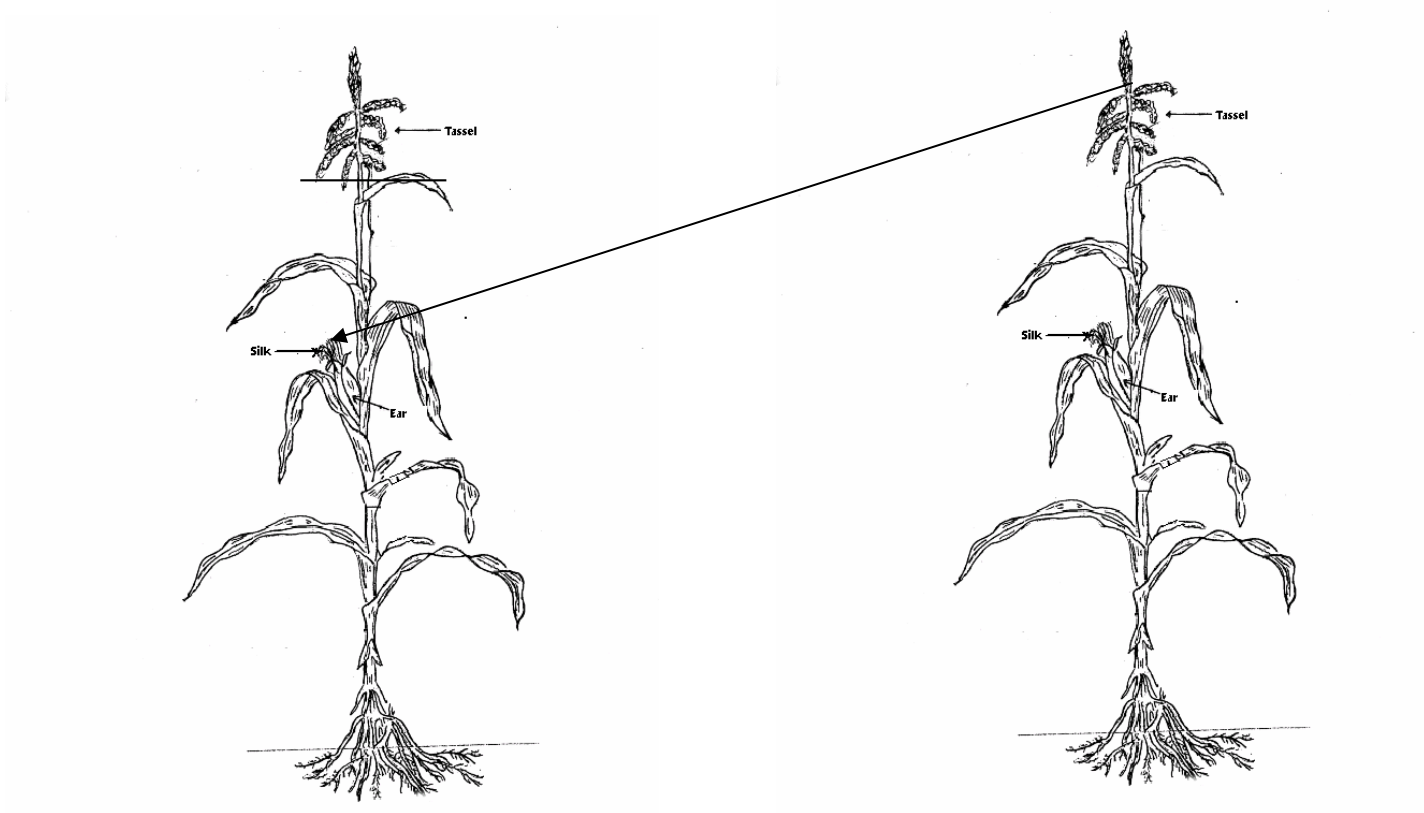


Seed Parent

Pollen Parent

Hybrid Seed Corn Production

Hybrid A/B Production Field



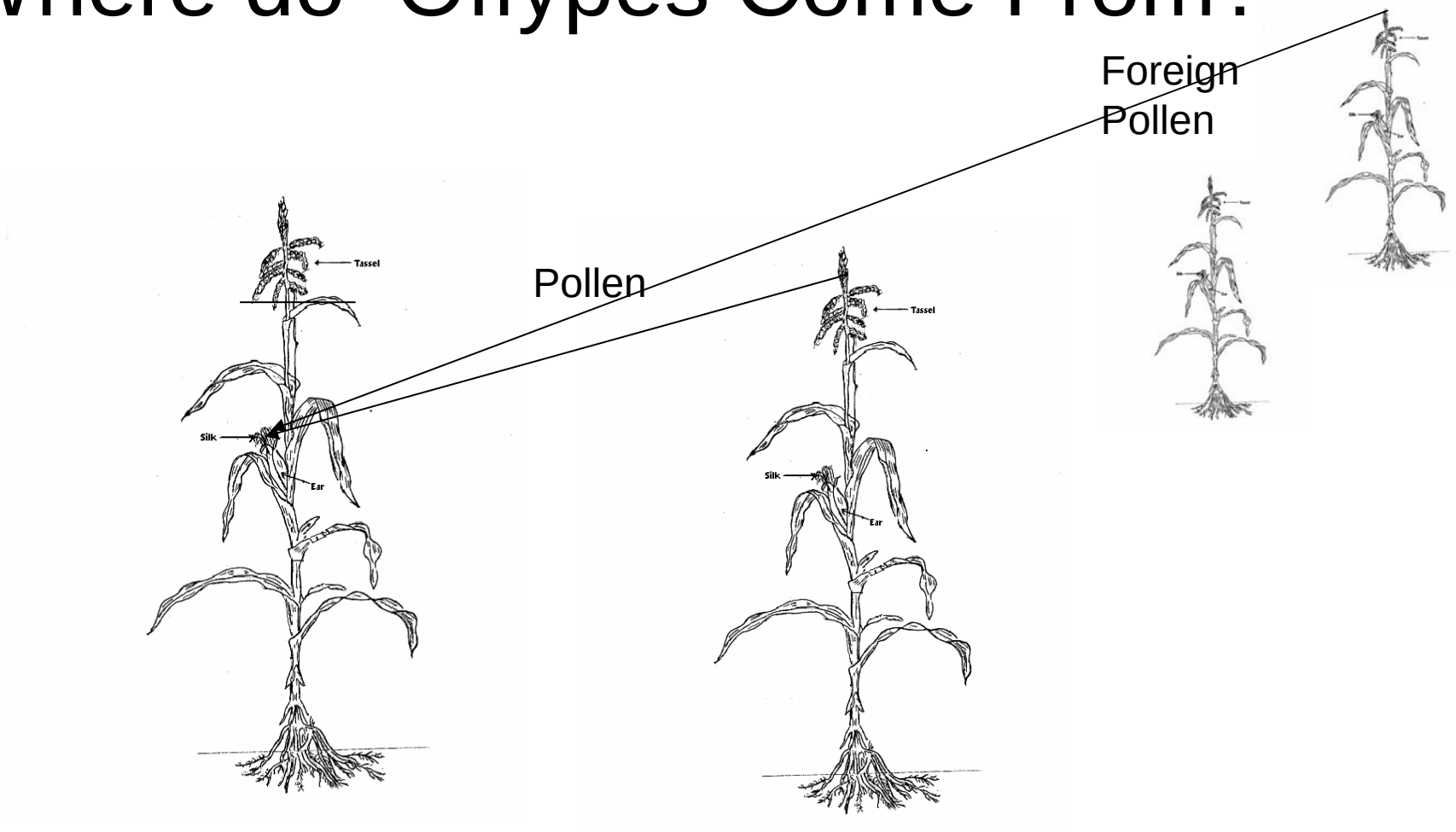
Seed Parent A/A

Pollen Parent B/B

Mendelian Inheritance

Female Banding Pattern	+	Male Banding Pattern	=	Hybrid Banding Pattern
2/2		4/4		2/4

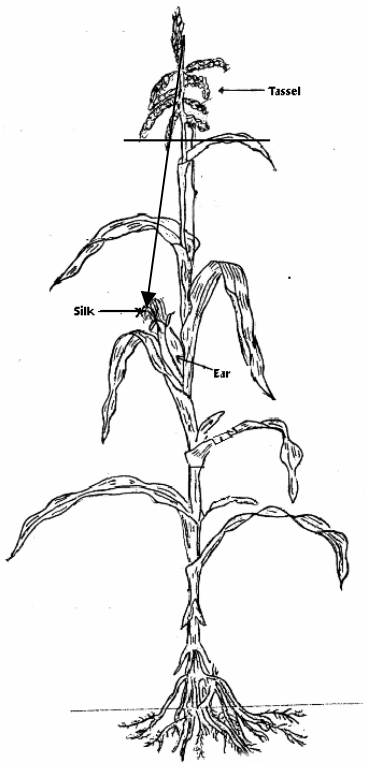
Where do Offypes Come From?



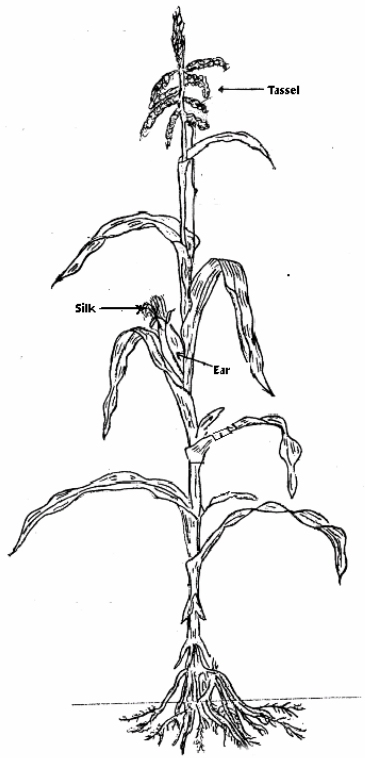
Seed Parent

Pollen Parent

Where do Selfs Come From?



Pollen



Seed Parent

Pollen Parent

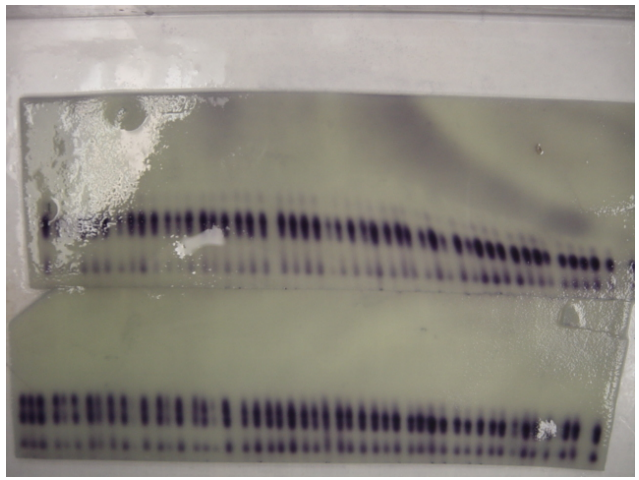
Mechanical Mixtures or Errors

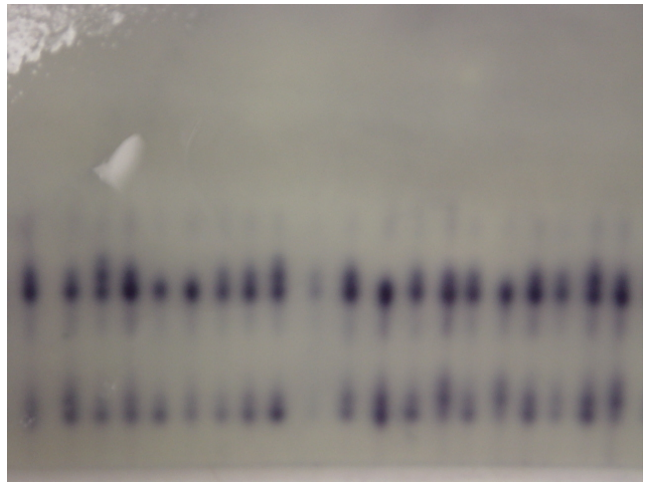
Contaminated Parent Seed Stock

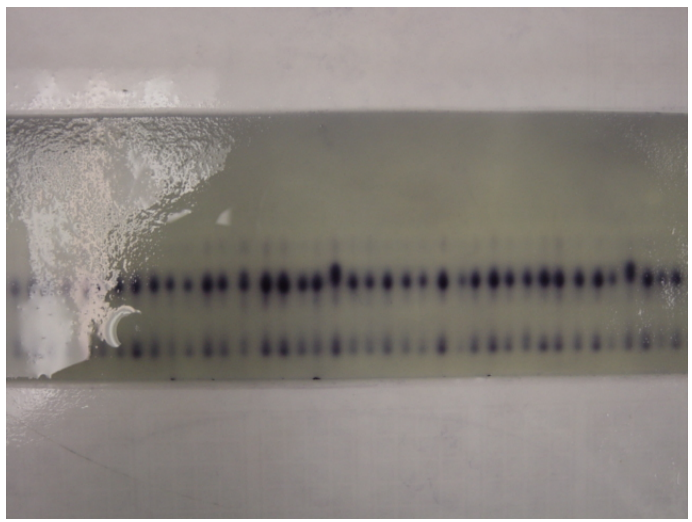
Field Growout

Evaluation and Scoring Handouts

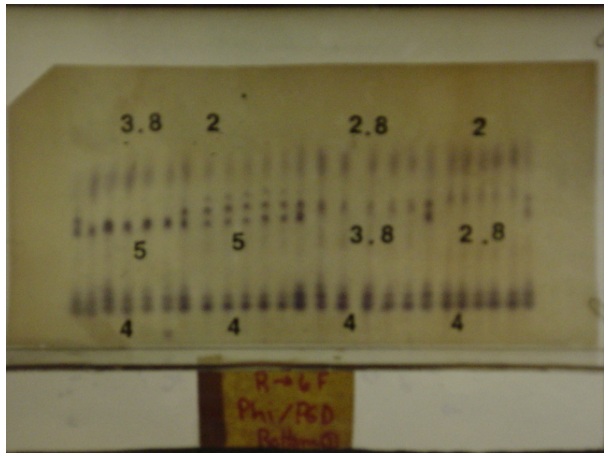
Gel Pictures 1

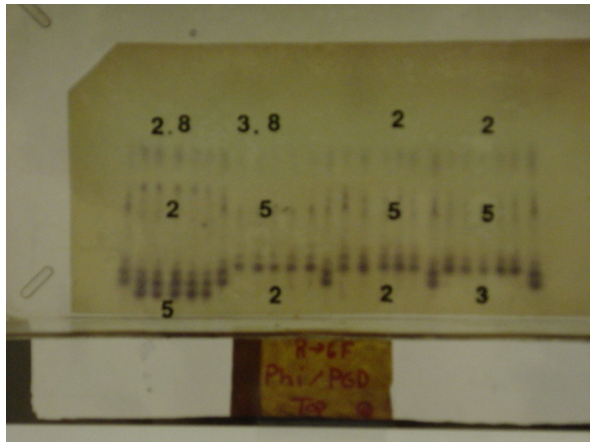


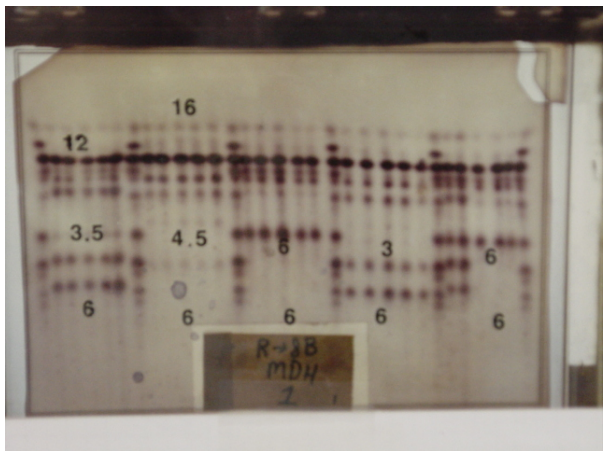


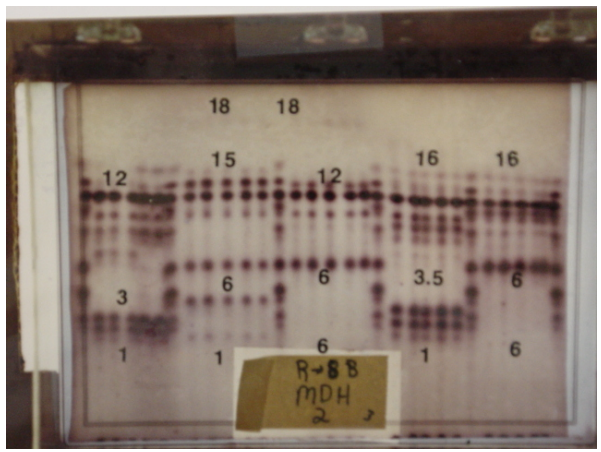


Gel Pictures 2

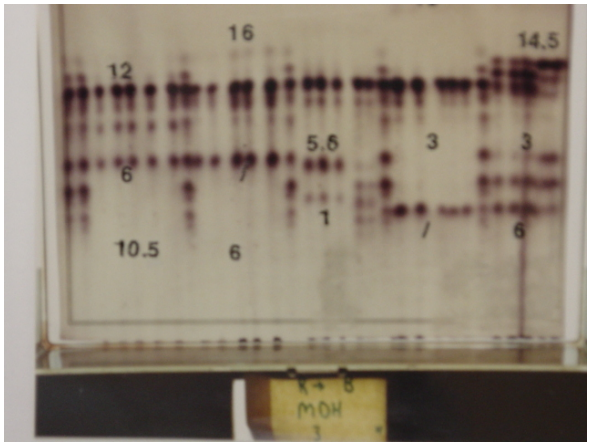


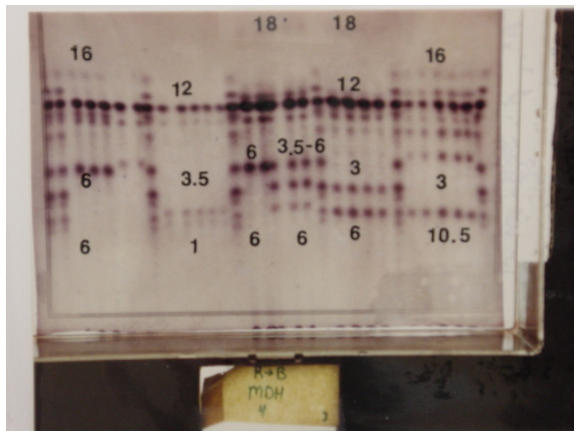


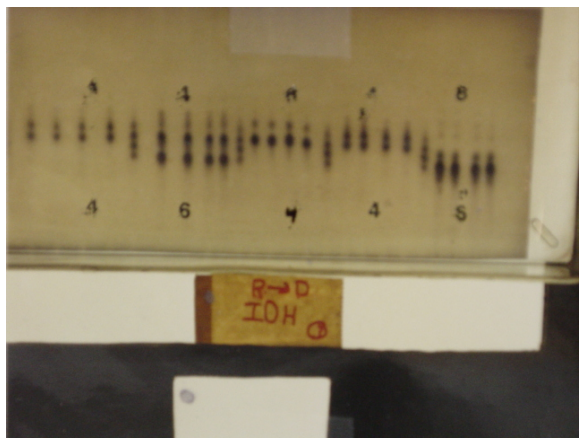


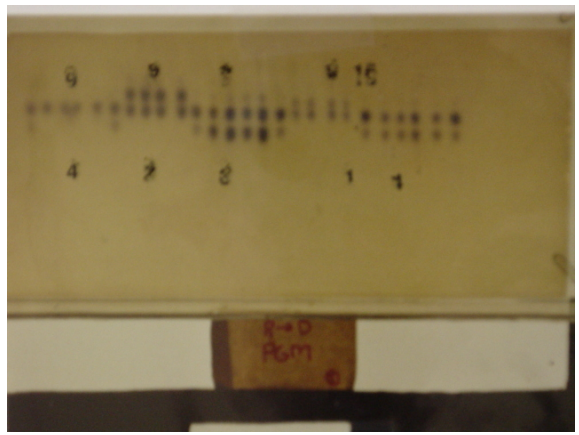


Gel Pictures 3

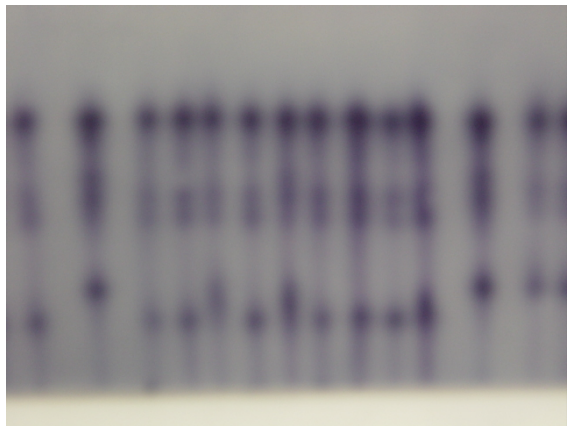


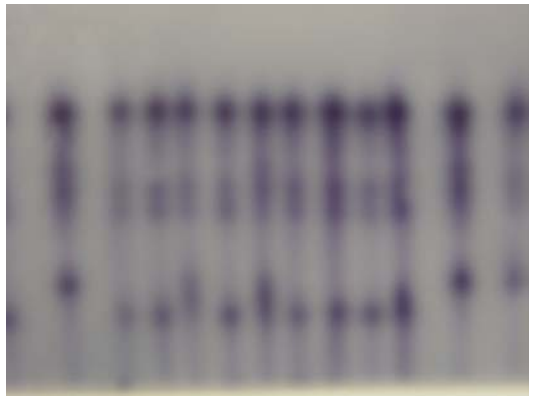


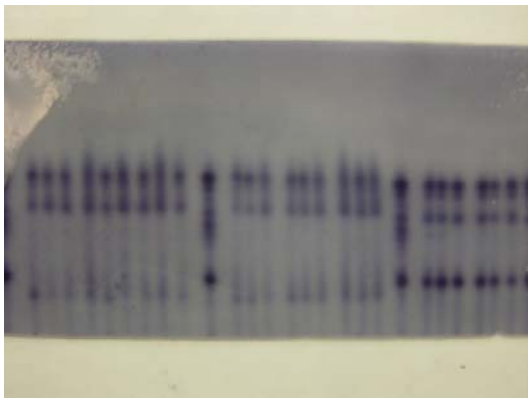


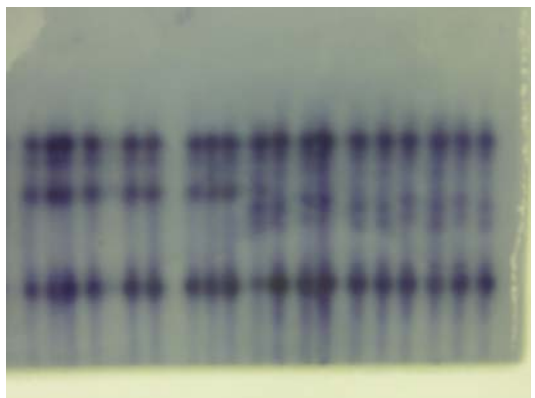


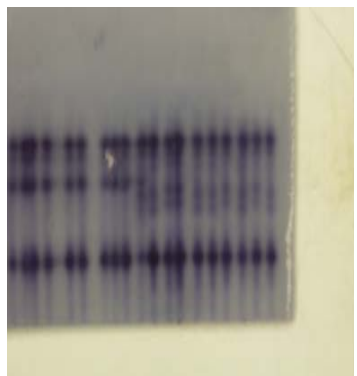
Gel Pictures 4









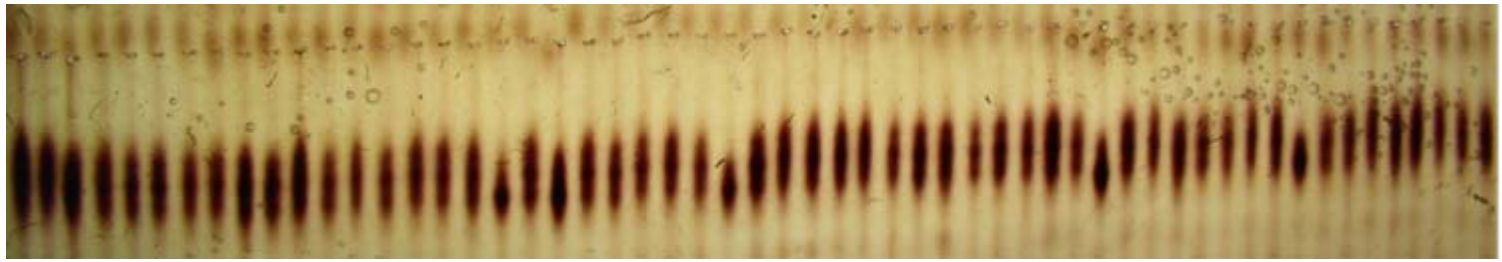


Gel Pictures 5

Gel 7488.4

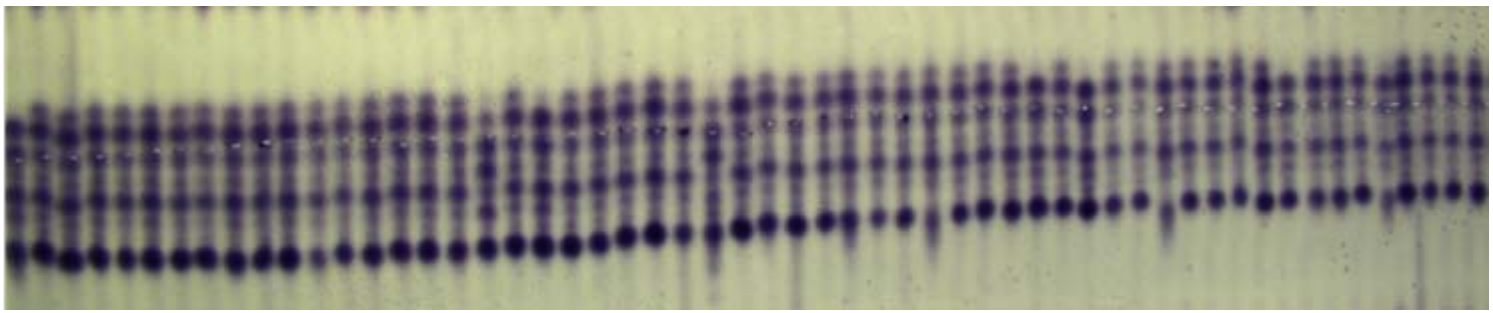
Control at positions 20 and 40.
Female genotype same as control
Origin

ACP



MDH

Origin



Control at positions 20 and 40.

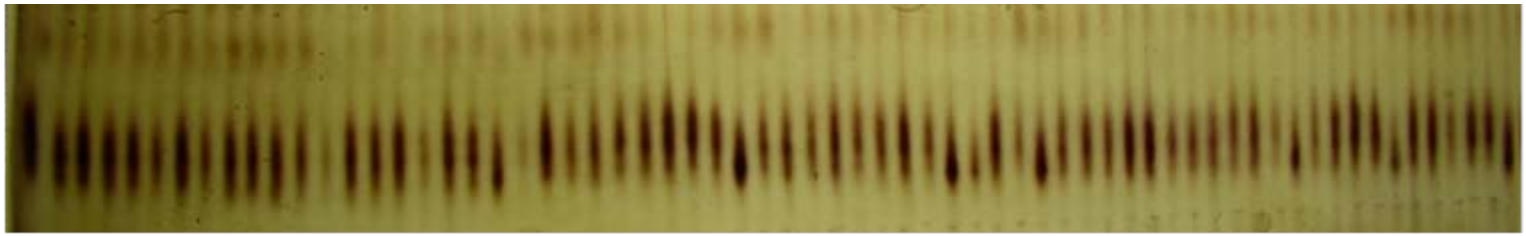
Gel 7493.13

Control at positions 20 and 40

Female genotype same as control

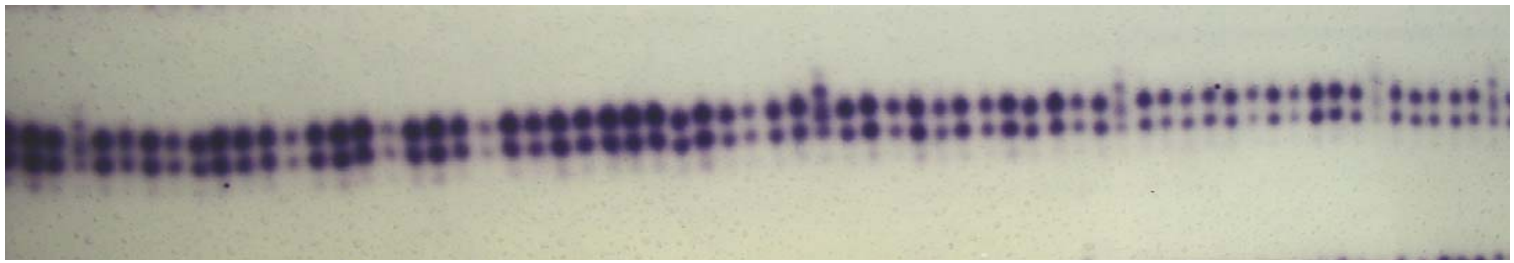
Origin

ACP



IDH

Origin



MDH

Origin