Starch Gel Electrophoresis Interpretation

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Reference Manual

Techniques and Scoring Procedures for Starch Gel Electrophoresis of Enzymes from Maize (*Zea mays* L.)

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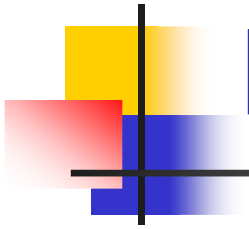
Technical Bulletin 286
March 1988
North Carolina Agricultural Research Service
North Carolina State University
Raleigh, North Carolina

Selection of Enzyme Stains

- Determined by Lab's requirements of data, cost and time
- Application:
  - Hybrid purity: selfs, off-types and variants
  - Inbred purity: off-types and variants
  - Breeder's seed purification
  - Export requirements

Evaluating Isozyme Gels

- Requires familiarity
- Bands observed represent multiple forms of an enzyme
- Knowledge of corn production basics and genetics very beneficial
- Can learn names of bands quickly
- To become proficient in problem solving takes years of experience



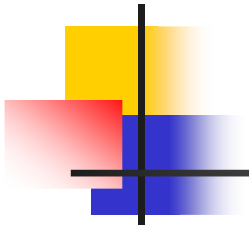
Definitions

- Genotype: Genetic constitution of an organism
- Phenotype: Observable physical properties of an organism
- Locus: The position that a gene occupies on a chromosome
- Allele: A possible form of a given gene occupying a given locus on a chromosome
- Allozyme: A single visible band indicates a homozygote for the particular enzyme assayed

Definitons

- Zymogram: Visual display of enzymes on an electrophoretic gel
- Homozygous: The presence of two identical alleles in an individual for a single genetic locus: A/A or 2/2
- Heterozygous: Having two different alleles of a gene at one locus, one inherited from each parent: A/B or 2/4

Terminology/Nomenclature



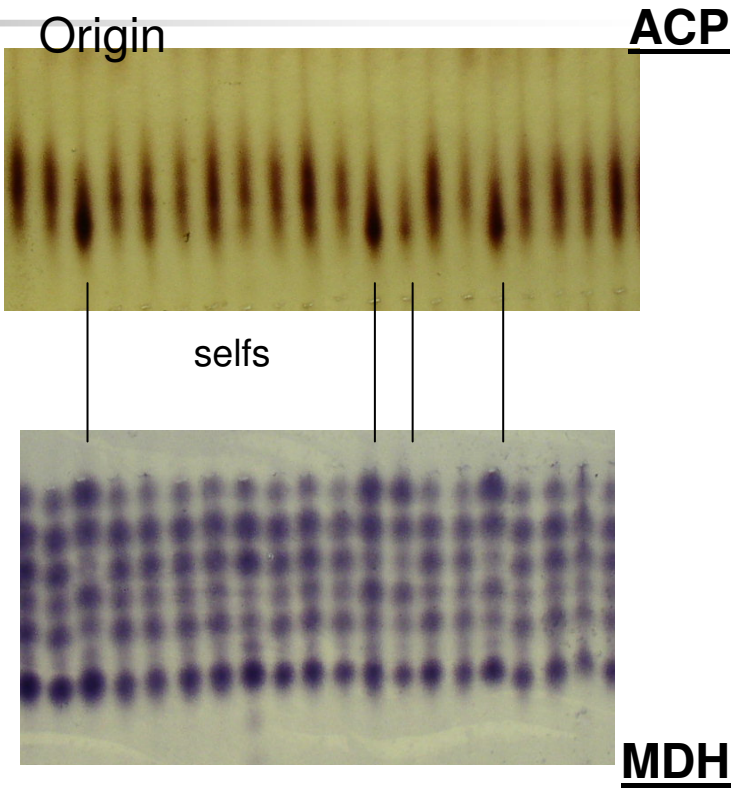
- Inbred: Single allele for all loci: A/A
- Single cross parent inbred: A/A or A/B
- Hybrid: Female and pollen parent combined genotypes : A/B
- 3-way cross: A/A and A/B

Female: A/B
Male: A/A

Hybrid: A/A A/B
Hybrid cross contains two genotypes

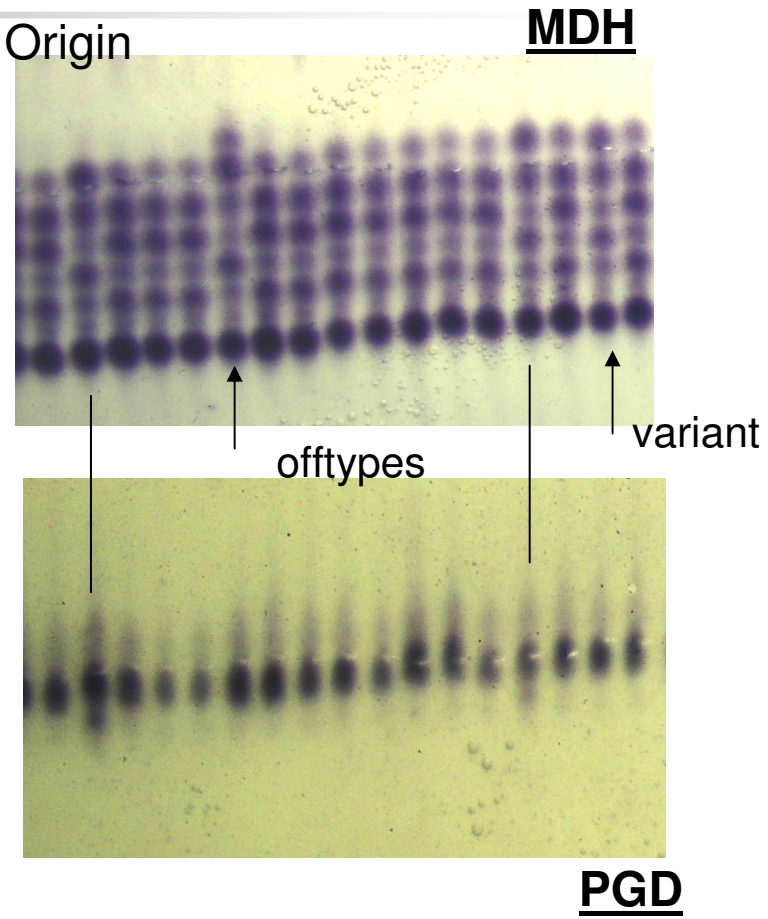
Terminology/Nomenclature

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



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

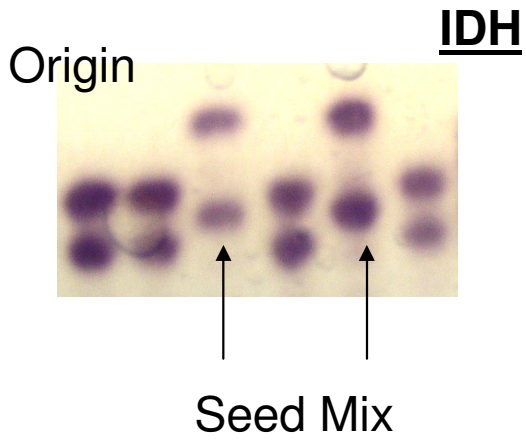
Contamination such as Seed Mix or Errors:

observed genotype does not have genetics from either parent.

Hybrid: A/B

Seed Mix: C/C

Inbred Seed Mix:



Terminology/Nomenclature

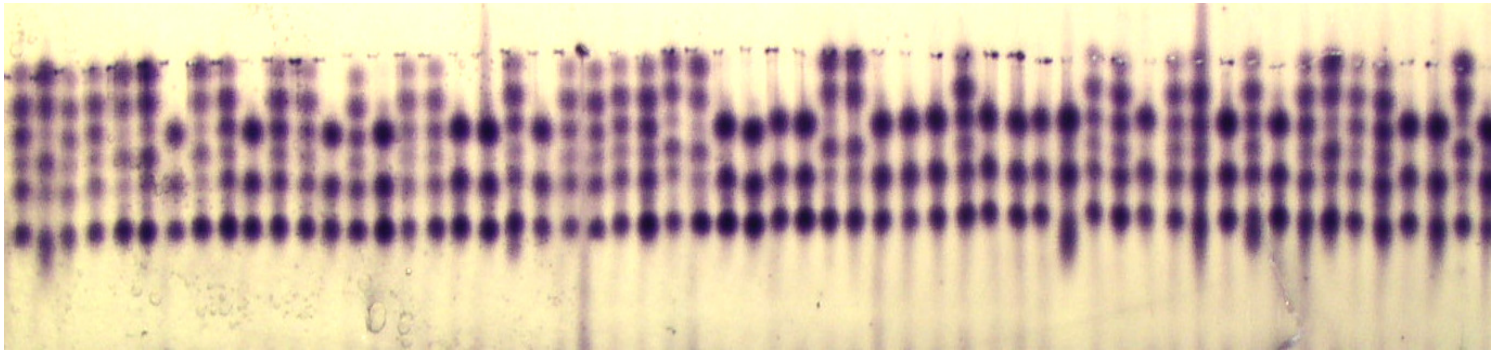
Segregation: Genetic variation in a population at one or more locus.

Follows Mendel's law

True segregation genetic ratio is 1:2:1

Origin

MDH



Segregating Inbred

Effect of Segregating Inbred

Single Locus Segregation

Female: A/A B/B A/B

Male: A/A

Hybrid: A/A A/B

Hybrid cross contains two genotypes

Effect of Segregating Inbreds

Segregation on two inbreds

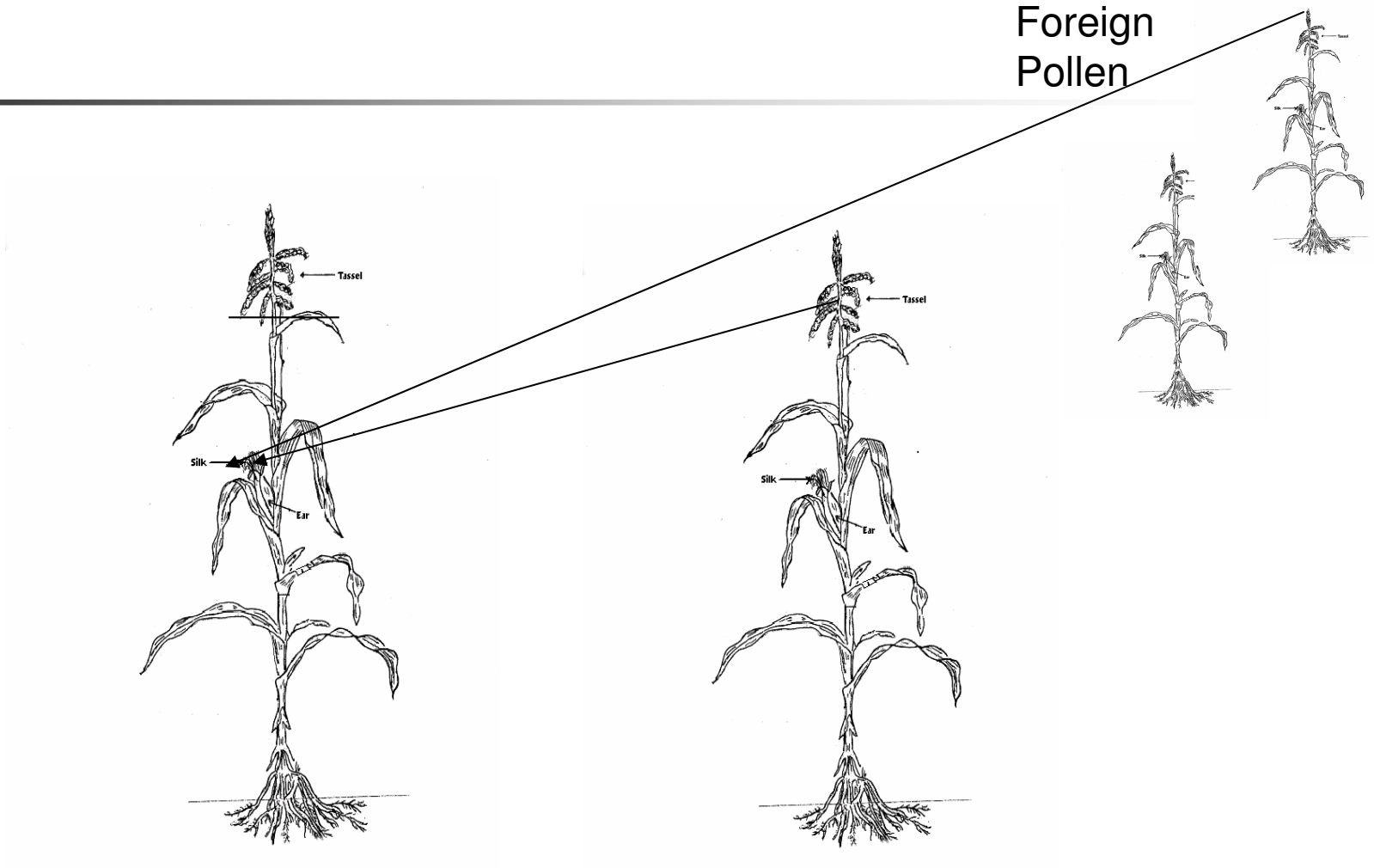
Inbred population contains three genotypes

Female:	A/A	B/B	A/B
Male:	Y/Y	Y/Z	Z/Z

Hybrid:	A/Y	A/Z	B/Y	B/Z

Hybrid cross contains four genotypes

Corn Production Basics

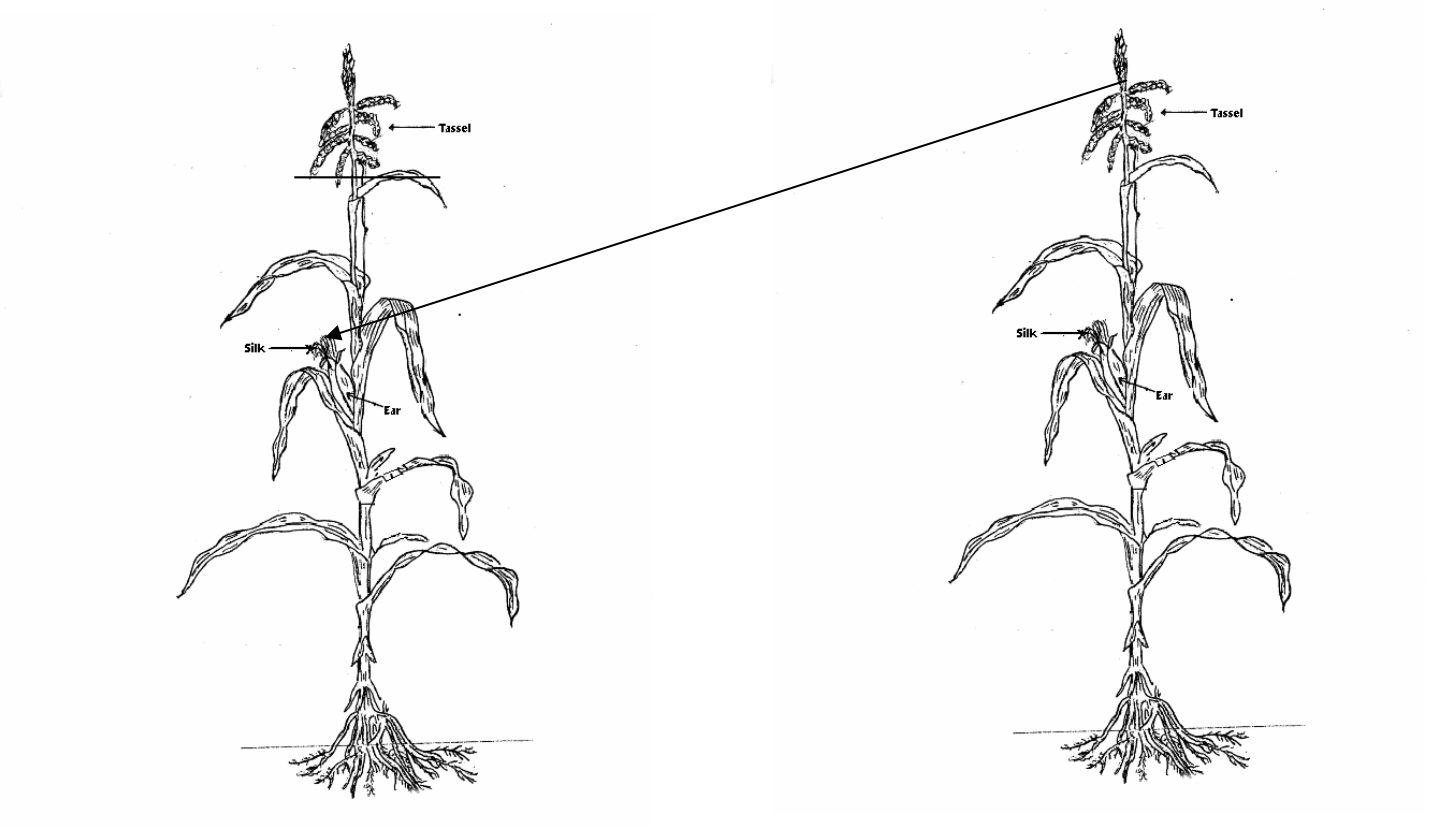


Seed Parent

Pollen Parent

Hybrid Seed Corn Production

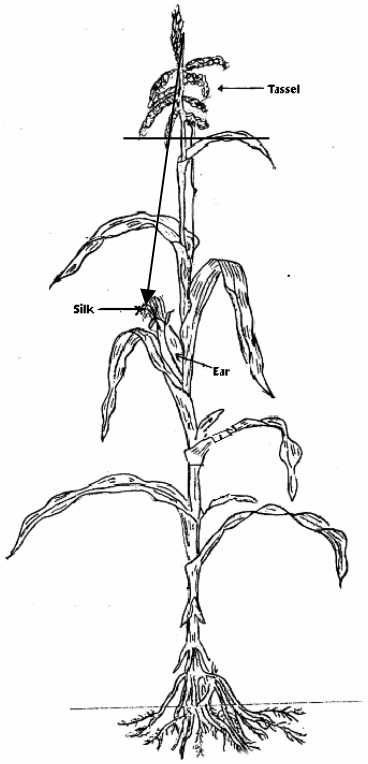
Hybrid A/B Production Field



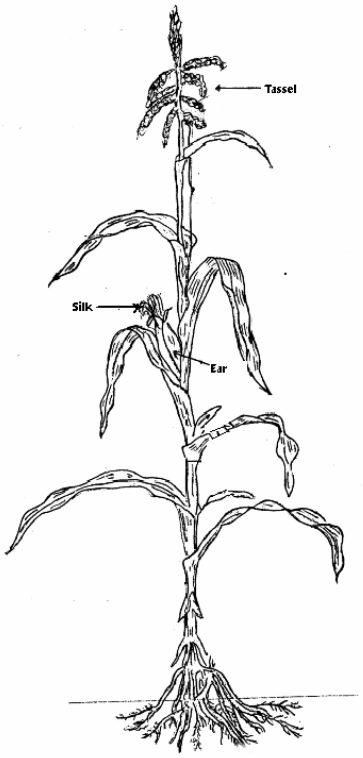
Seed Parent A/A

Pollen Parent B/B

Where do Selfs Come From?



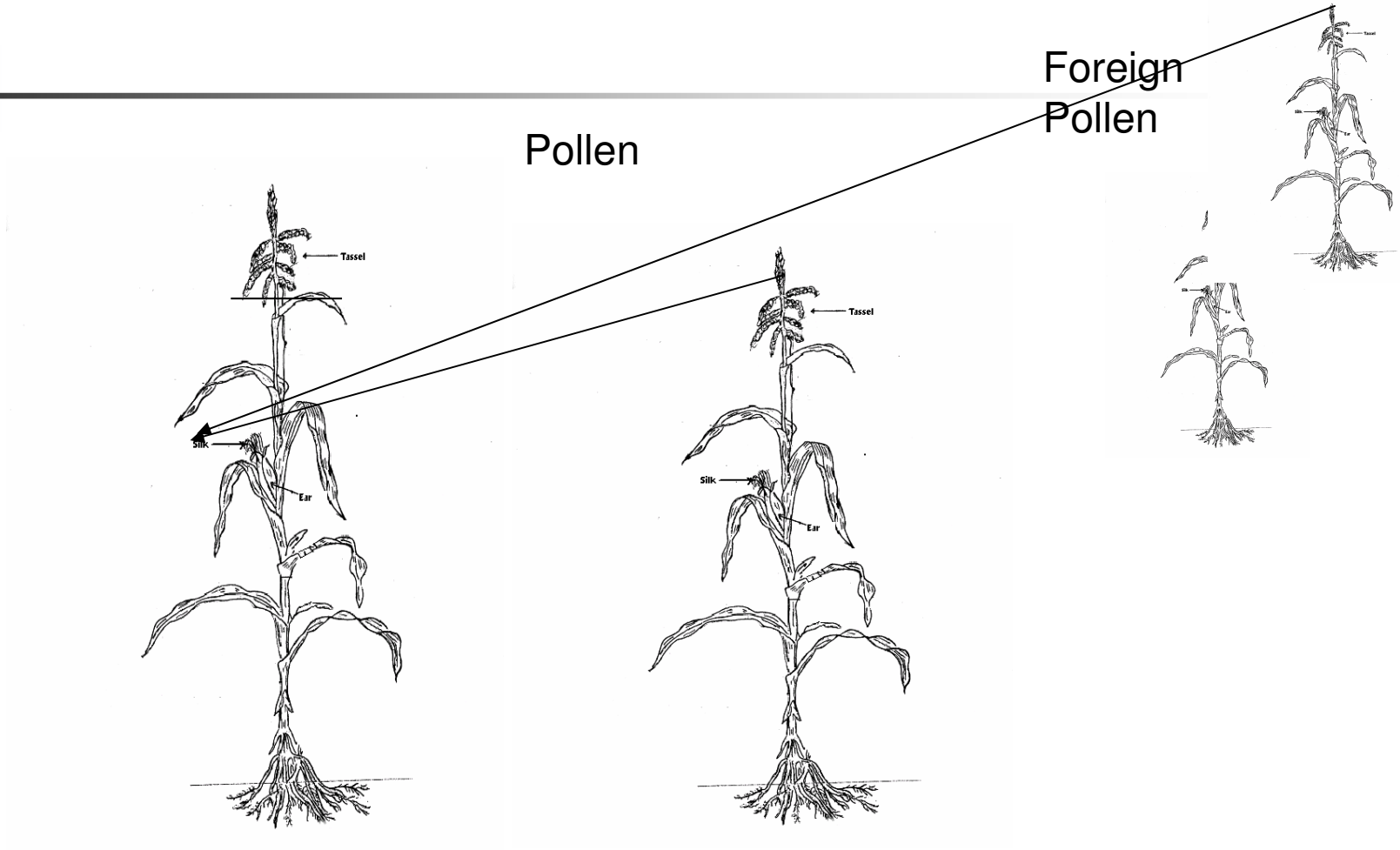
Pollen



Seed Parent

Pollen Parent

Where do Offypes Come From?



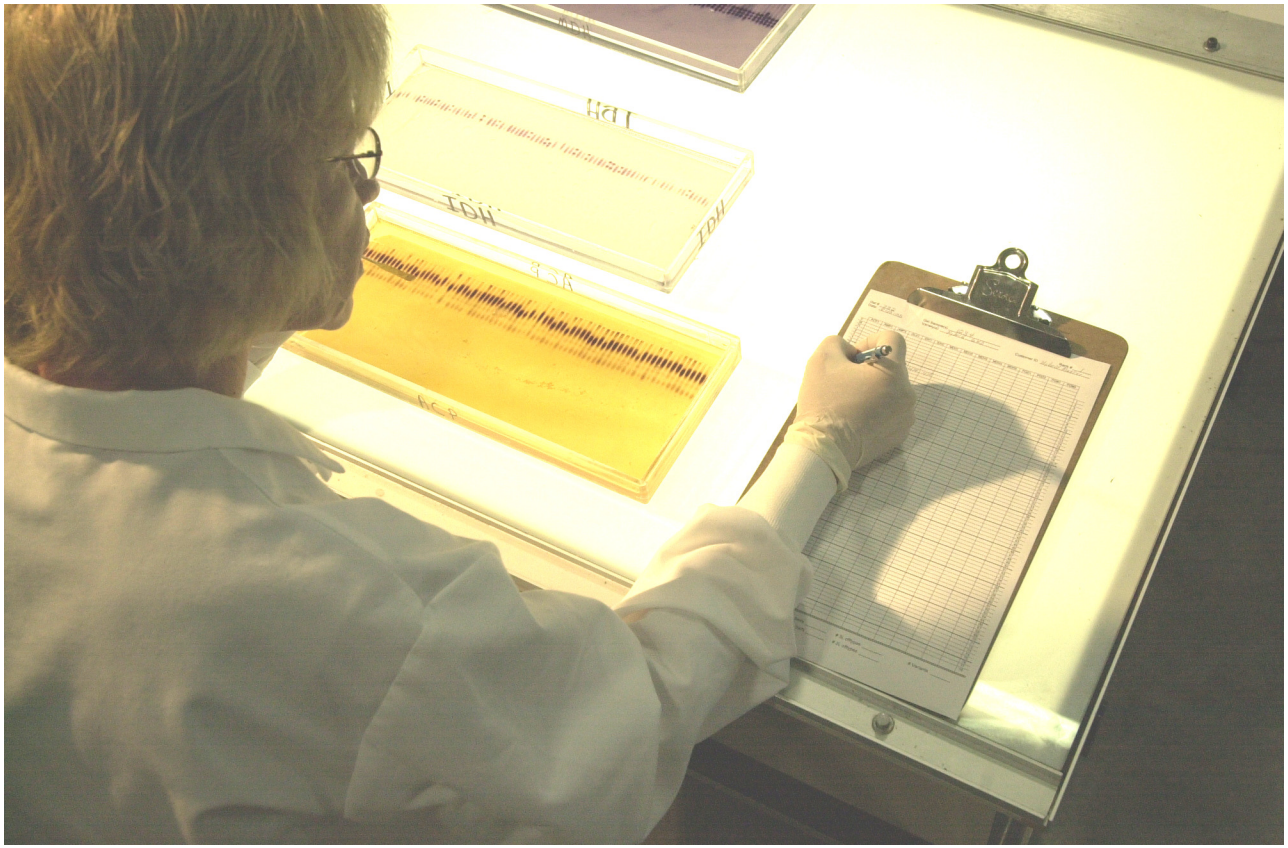
Seed Parent

Pollen Parent

Off-type Sources

- Outcross--foreign pollen
- Self—female self or male self
  - Male self—from contaminated female, or lodged male plant
- Rogue (volunteer) plant
- Genetically impure parent seed

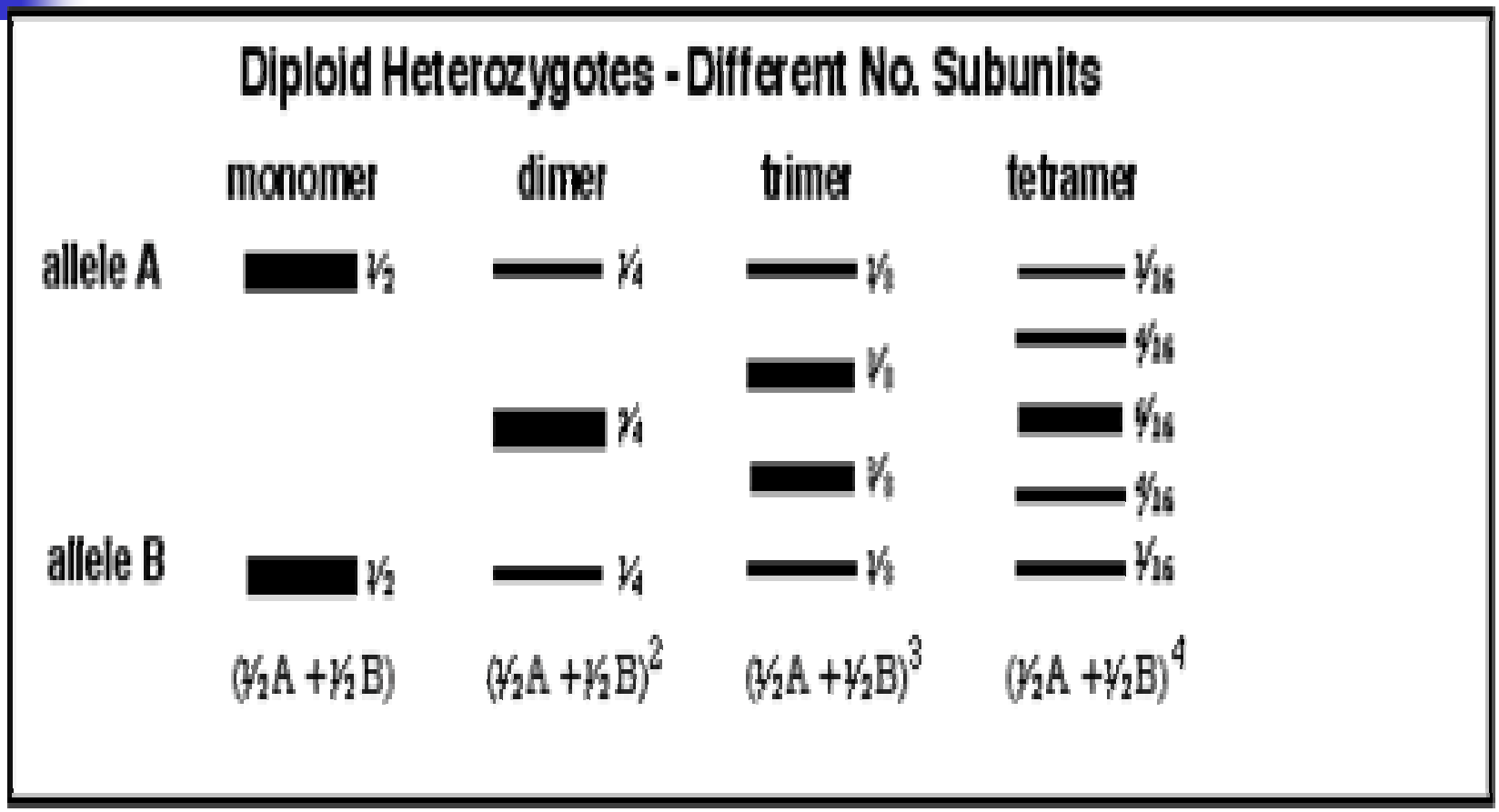
Evaluation of Gels



Evaluation of Gels

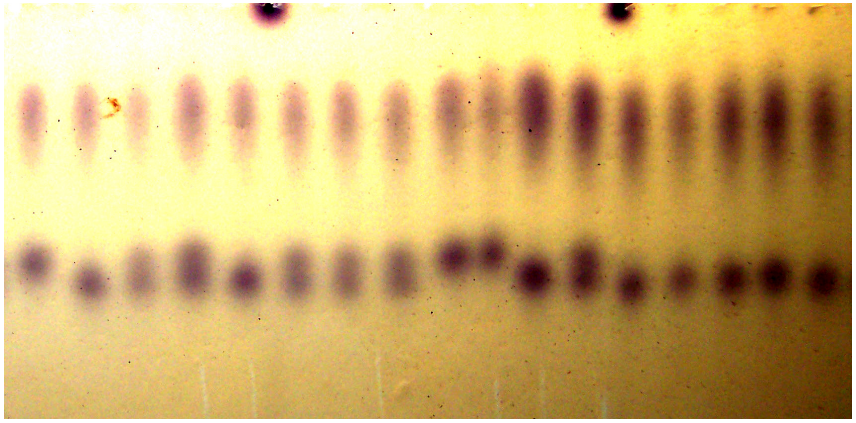
- Use of nomenclature of alleles
- Need for standard control on each gel slice i.e. parents, known pattern control
- Parent seed not needed if banding patterns are know

Evaluation of Gels



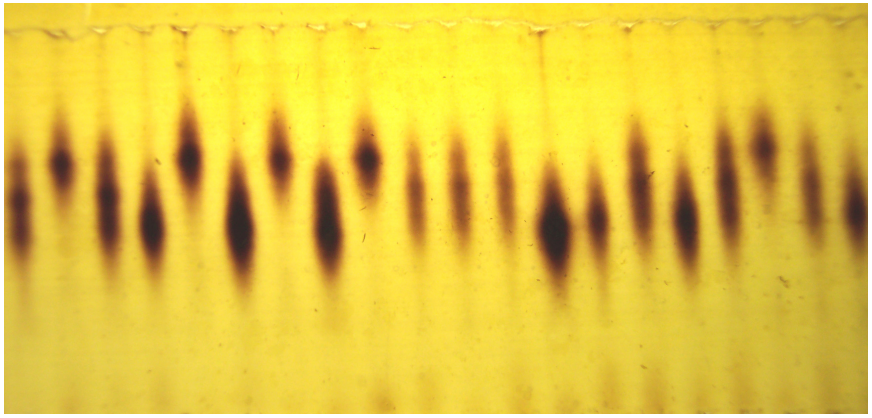
Evaluation of Gels

- Monomer



AMP

- Dimer



ACP

Mendelian Inheritance

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

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, drying gels

Problem Solving

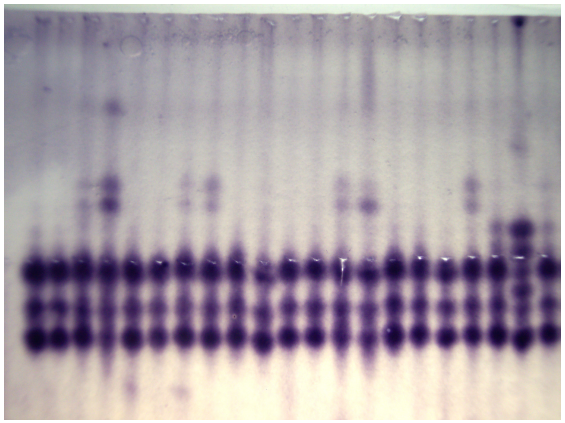
- Wrong bands
  - Gel slice in wrong tray
  - Data entry error—wrong variety name (check seed packet)
  - Wrong pedigree information
  - Trait on different parent (check actual hybrid production field records)
  - Labeled wrong (recheck source material)
  - WRONG

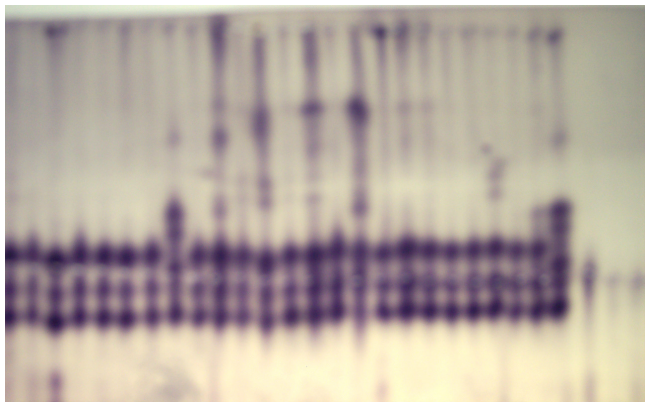
Analysis Challenges

- Traits: can change banding patterns in ACP, PGD, etc., or change to or from segregating locus
- Reversed Pedigree
- Traits on parents are reversed

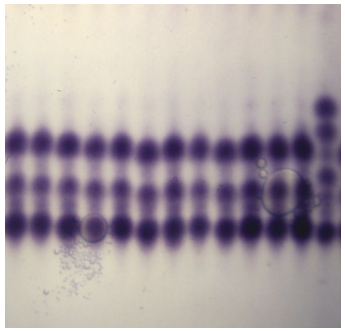
Analysis Challenges

- Mold/fungus/artifacts or new band?
  Are protein bands in response to mold?





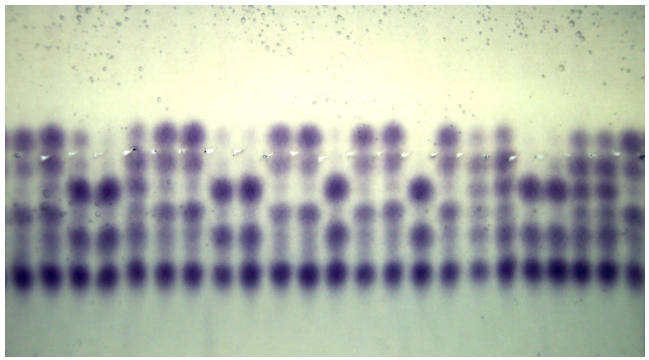
MDH



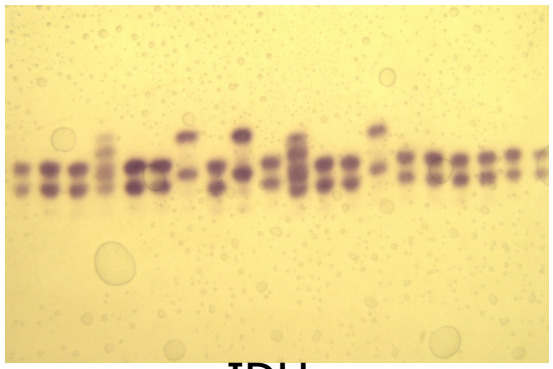
(no fungus bands)

Analysis Challenges

- Segregation
  - Line is not "fixed"
  - Is low level of variation segregation? (Not 1:2:1 ratio)
  - Report in comments that line is segregating at a specific locus or loci



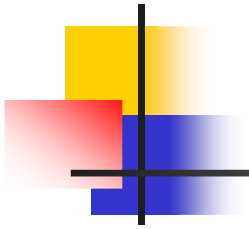
MDH



IDH

Analysis of other crops

- No Standardized Nomenclature:
- Canola
- Sunflower
- Sorghum
- Soybeans
- Other crops?

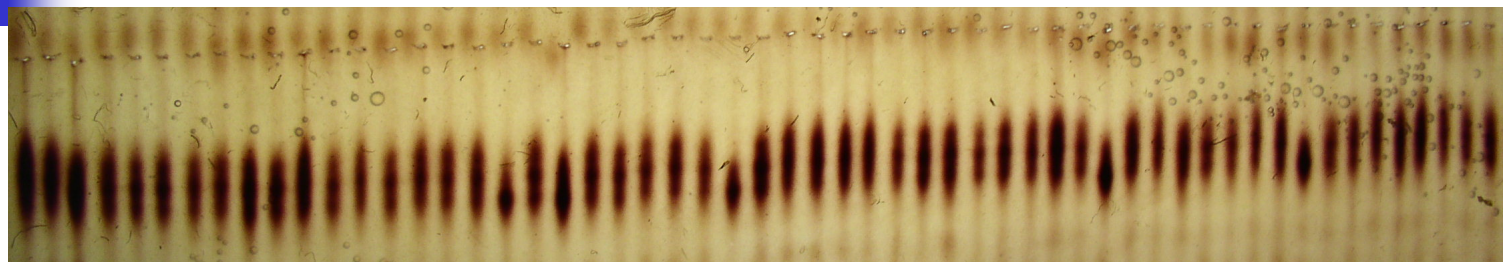


Evaluation and Scoring Handouts

Gel 7488.4

ACP

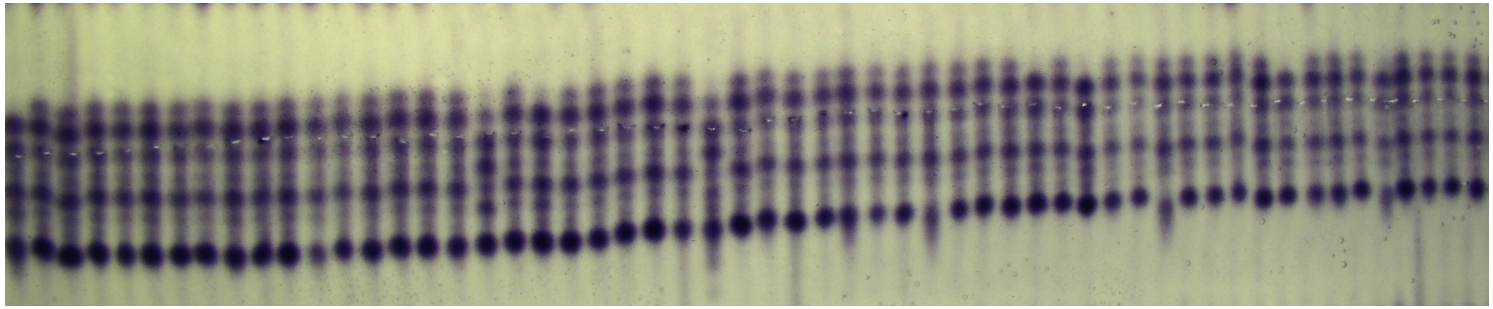
Origin



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

MDH

Origin

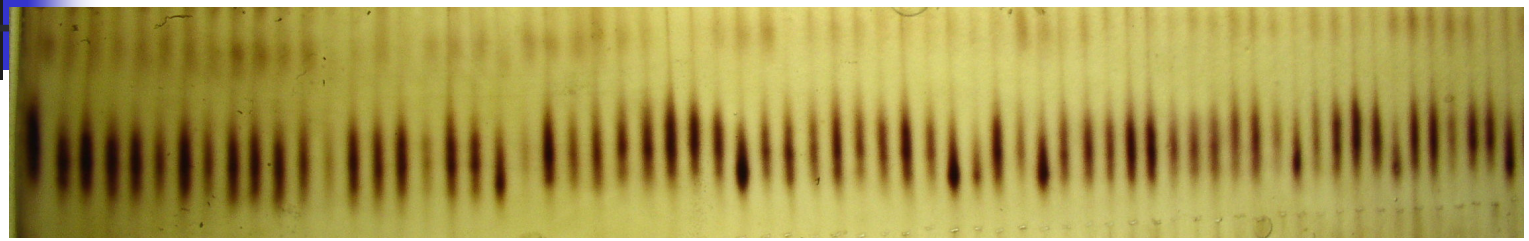


Control at positions 20 and 40.

Gel 7493.13

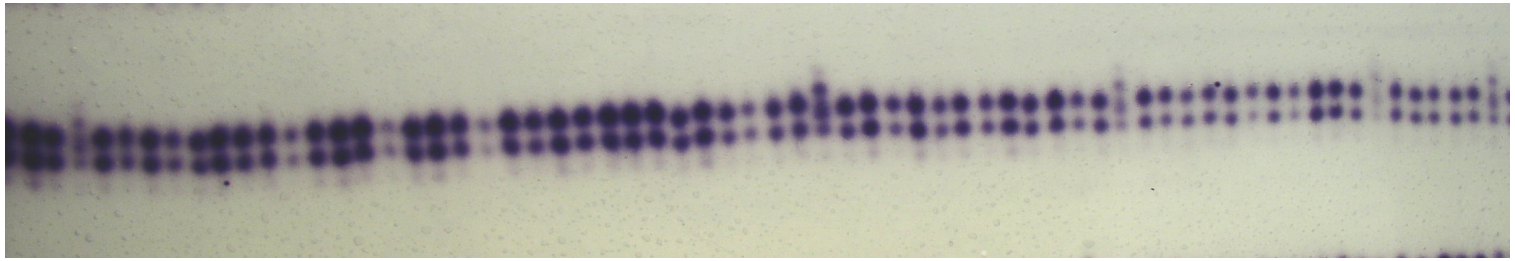
Control at positions 20 and 40
Female genotype same as control
Origin

ACP



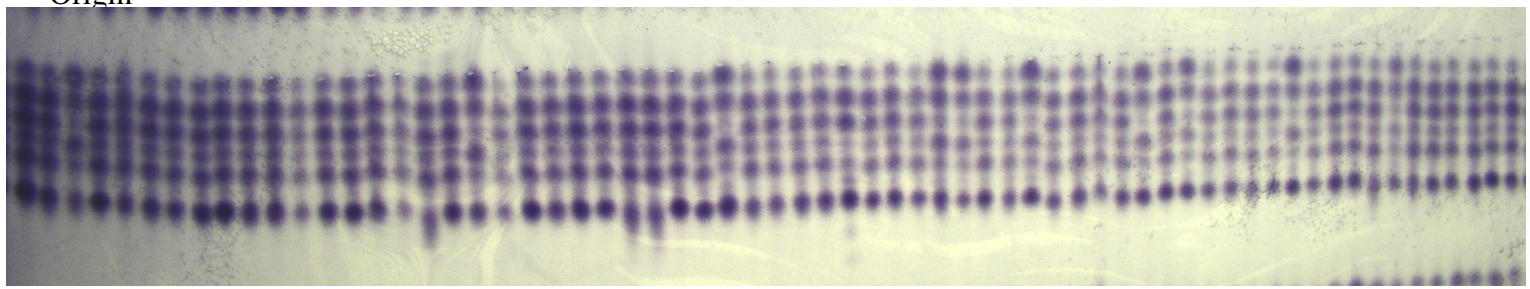
IDH

Origin



MDH

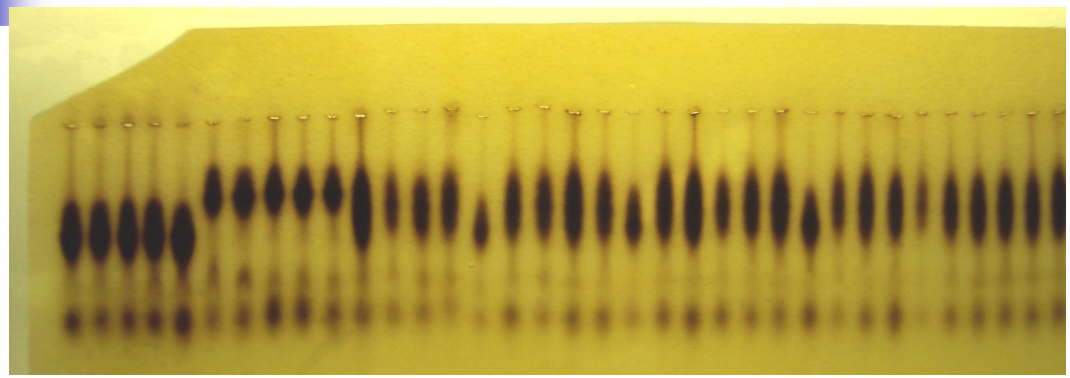
Origin



Sample 546864

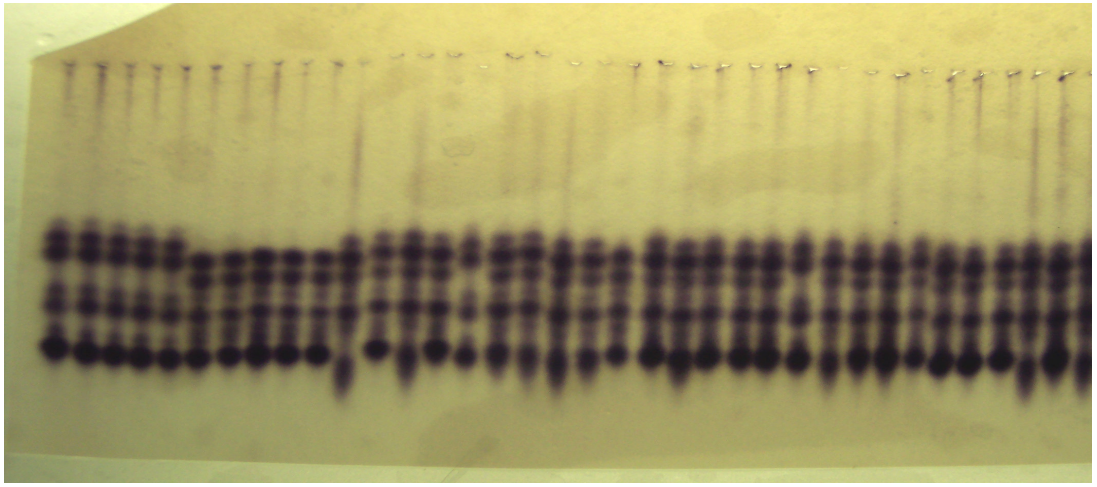
ACP

Origin



MDH

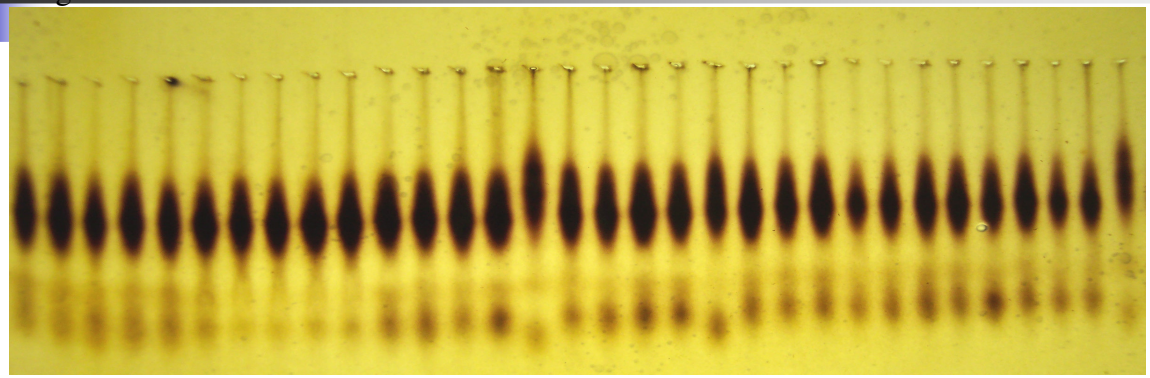
Origin



Sample 542743

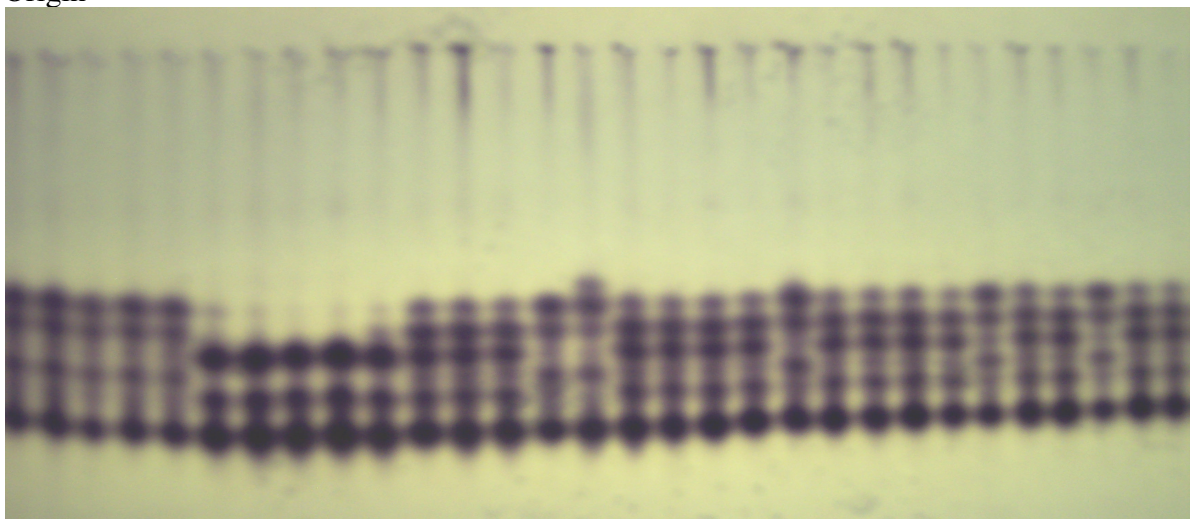
ACP

Origin



MDH

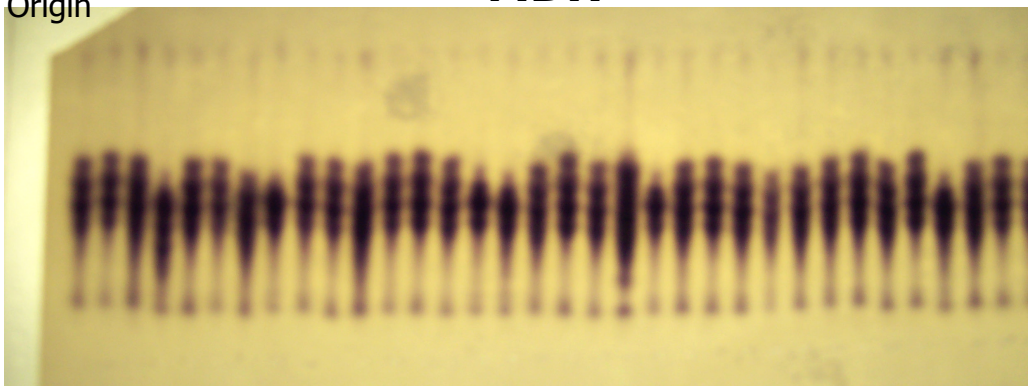
Origin



Sunflowers

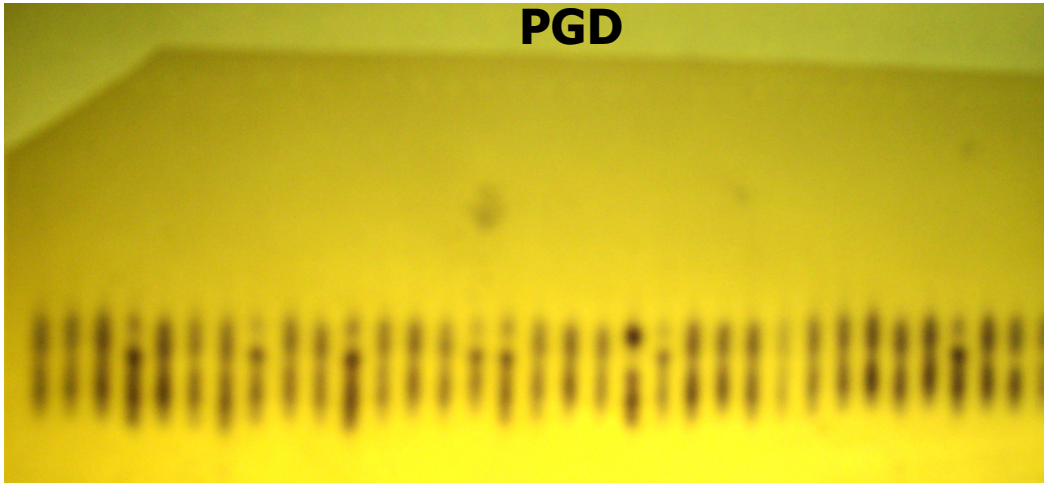
MDH

Origin



Control at position 20

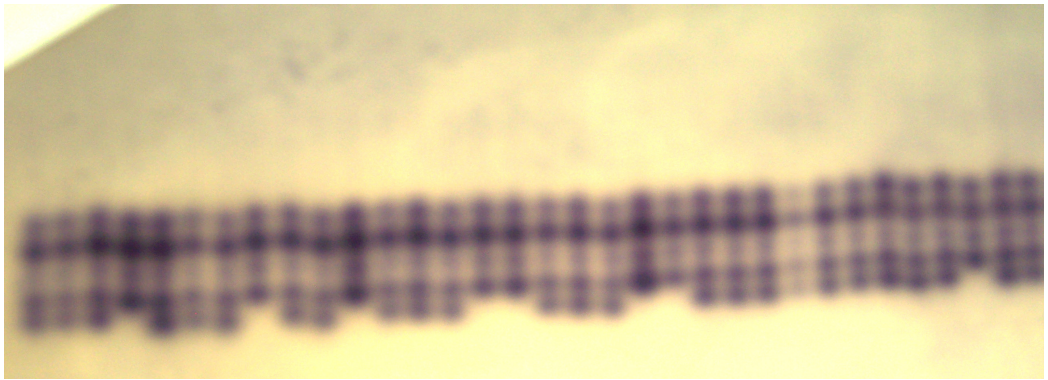
PGD



Sunflowers

Origin

PGM



Control at position 20

PHI

Questions????