

Molecular Methods for Seed Quality Testing



Introduction

“Subeejam Sukshetre Jayate Sampadayte”

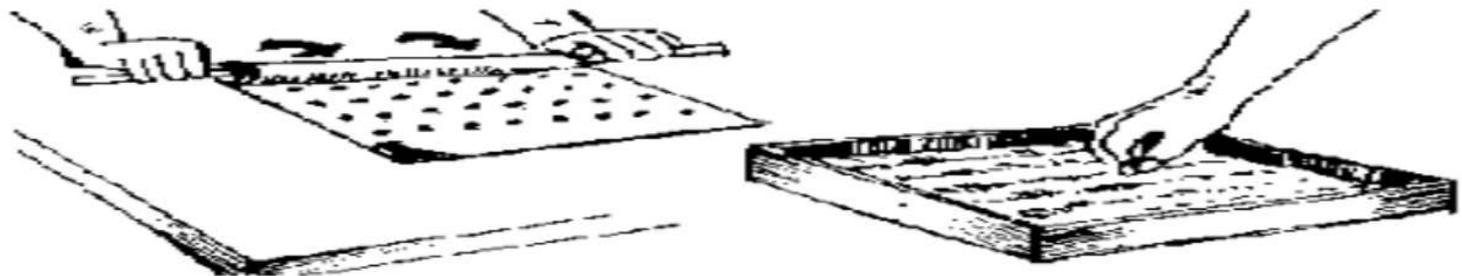
- High quality seed is determined by its parent’s genetic makeup, physical integrity , purity , its health and physiological quality.
- Farmers continue to adopt precision agricultural practices and depend on the performance of every single seed put into the ground— the only way to achieve that is by seed testing.

Every inch of the field counts, and seed testing plays a significant role.



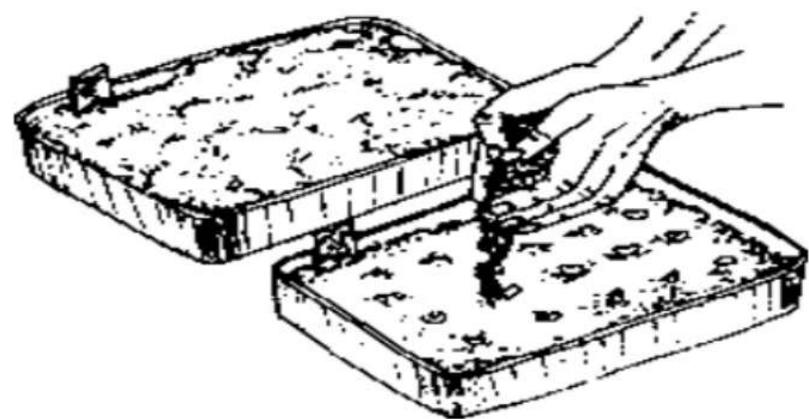
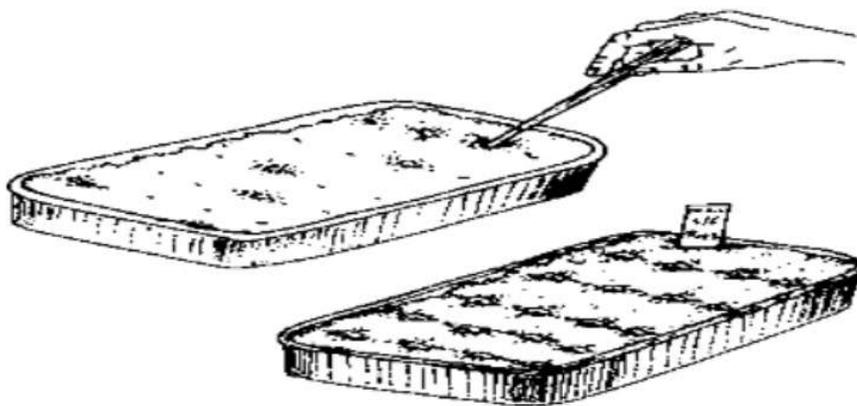
Seed Technology encompasses:

1. Development of superior varieties
2. Production
3. Evaluation
4. Processing
5. Storage
- 6. Testing**
7. Certification/quality control
8. Marketing and distribution
9. Seed pathology
10. Seed entomology
11. Seed physiology
12. Seed ecology



Seed Testing

- ✓ Seed testing is the art and science of evaluating seed quality for agricultural purposes (Copeland and McDonald, 2001).
- ✓ Seed tests tell us if a crop of seeds is worth collecting, if handling procedures are correct, and how many potential seedlings are available for regeneration.
- Seed quality in India is legally controlled by The Seeds Act, 1966.





Why do we need to test seeds?

- ❖ Seeds – **basic, vital** and **crucial** input for crop production
- ❖ Quality control of seeds depends on the different seed testing protocols which determine the **genuineness** of the cultivar.
- ❖ Evaluates the **planting value** and the **authenticity** of the certified lot.
- ❖ Assess the seed quality attributes of the seed lots which have to be offered for **sale**.





SEED QUALITY CLASSIFICATION

**GENETIC
QUALITY**

01

**PHYSICAL
QUALITY**

02

**HEALTH
QUALITY**

04

03

**PHYSIOLOGICAL
QUALITY**



Genetic purity

- ❖ Genetic purity is defined as true to type plants / seeds conforming to the characteristics of the variety as described by the breeders.
- ❖ It refers to the percentage of contamination by seeds or genetic material of other varieties or species.



Methods to assess genetic purity

1. Morphological
2. Conventional grow out test
3. Chemical tests
4. Gel electrophoresis
5. Polymerase Chain reaction
6. Molecular markers(DNA)



Traditional approach to purity testing

Morphological traits

Seed morphology



Examination of Seedlings



In lab or greenhouse



In field



CHEMOTAXONOMY

The use of biochemical methods to analyze various components of seeds. Chemotaxonomists have recognized two groups of compounds that are generally useful in classification of plant species:

1. **Episemantic or secondary metabolites**
(pigments or fatty acid etc.)
2. **Semantides or sense carrying molecules**
(Proteins, Nucleic Acids)

Methods of testing based on –

1. Analysis of secondary metabolites
2. Protein analysis
3. Nucleic acid analysis

1. Analysis of secondary metabolite

These tests range from simple colour tests to complex chromatographic separations of phenols, anthocyanin, flavonoids and other compounds.

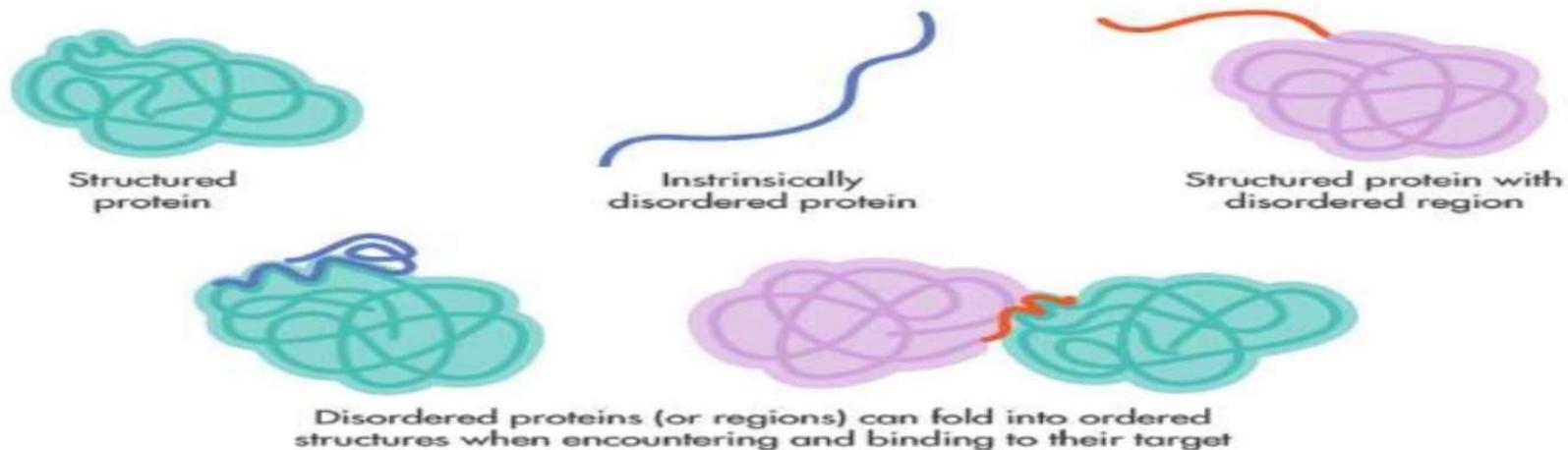
1. Phenol test- Wheat
2. Peroxidase test- Soybean
3. Potassium hydroxide – bleach test- Sorghum
4. Fluorescence test - Ryegrass
5. NaOH test- Wheat
6. Anthocyanin test- Rice, Soybean

2. Protein analysis

Proteins are the direct gene products, therefore the analysis of seed, seedling proteins and enzymes is most successful and widely used. Hence much attention has been focused on seed storage proteins.

There are two primary methods:

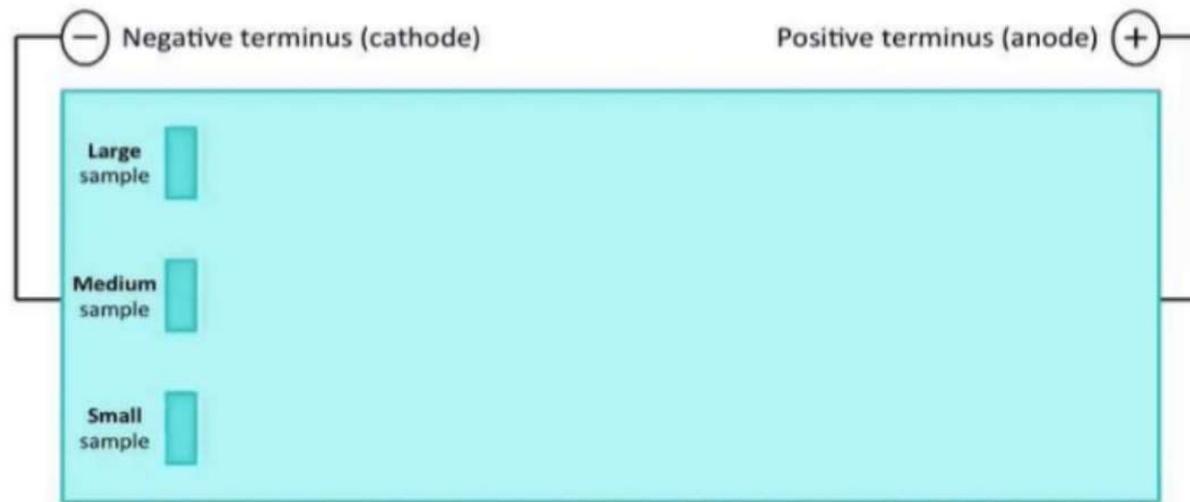
1. Gel electrophoresis
2. Isoelectric focusing



Gel electrophoresis

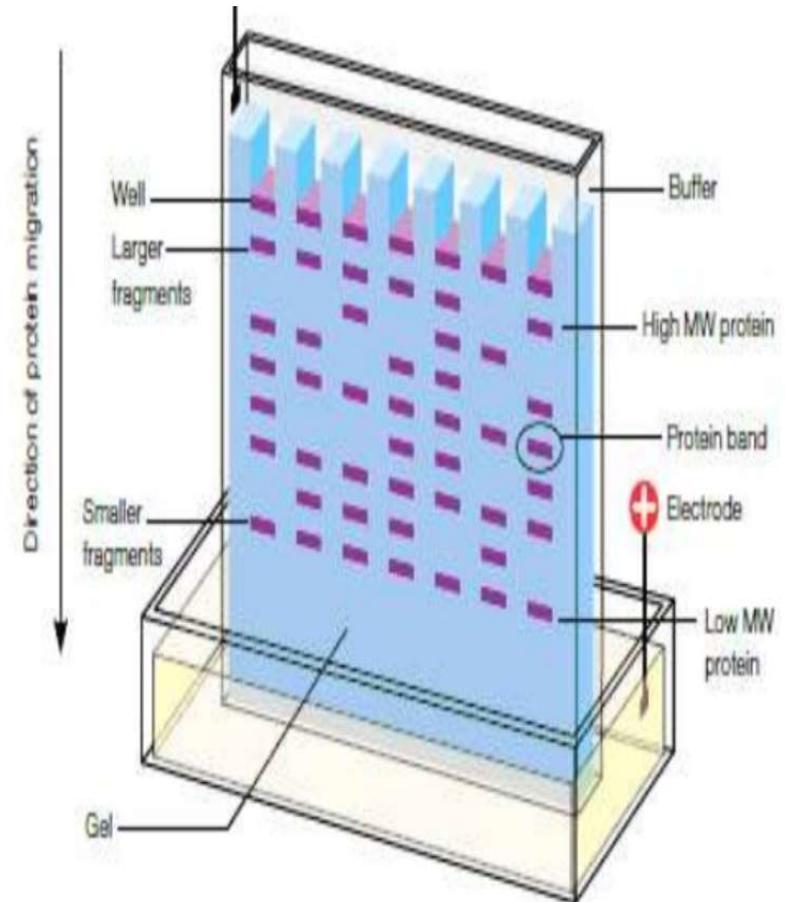
The term gel electrophoresis refers to a technique used for separation and analysis of DNA, RNA and proteins based on their size and charge.

- The suffix phoresis means “migration” or “movement”, while the prefix electro indicates the use of electricity as a mean to separate molecules.



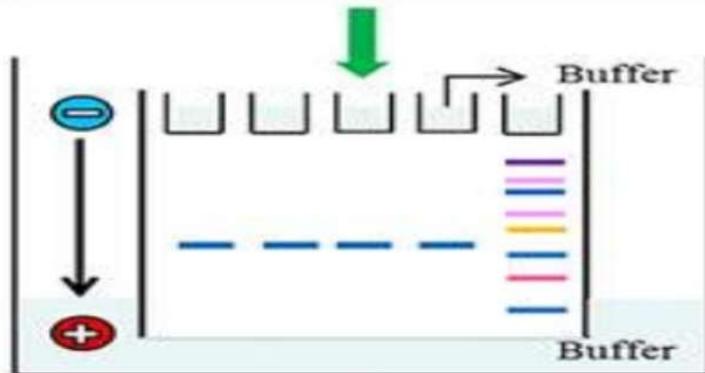
SDS-PAGE (Sodium dodecyl sulphate- Polyacrylamide gel electrophoresis)

- Denaturing and reducing sodium dodecyl sulphate (SDS)-PAGE- widely used electrophoresis technique
- SDS-PAGE separates proteins primarily by mass because the ionic detergent SDS denatures and binds to proteins to make them uniformly negatively charged.
- Thus, when current is applied, all SDS- bound proteins in a sample will migrate through the gel toward the positively charged electrode.
- Proteins with less mass travel more quickly through the gel than those with greater mass - sieving effect of the gel matrix.

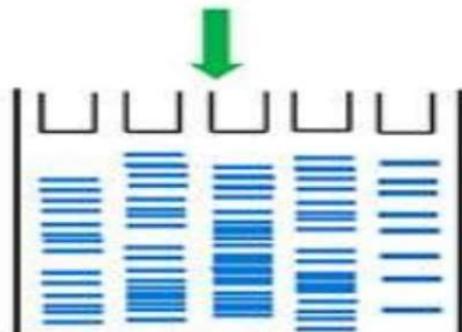




Protein samples and marker
loaded in vertical SDS-PAGE system



Direction of migration of samples
in vertical SDS-PAGE system



SDS-PAGE gel after Coomassie
blue staining

Isoelectric focusing(IEF)

Isoelectric focusing (IEF)- separates molecules by differences in their isoelectric point (pI).

Performed on proteins in a gel- takes advantage of the fact - overall charge on the molecule of interest is a function of the pH of its surroundings.

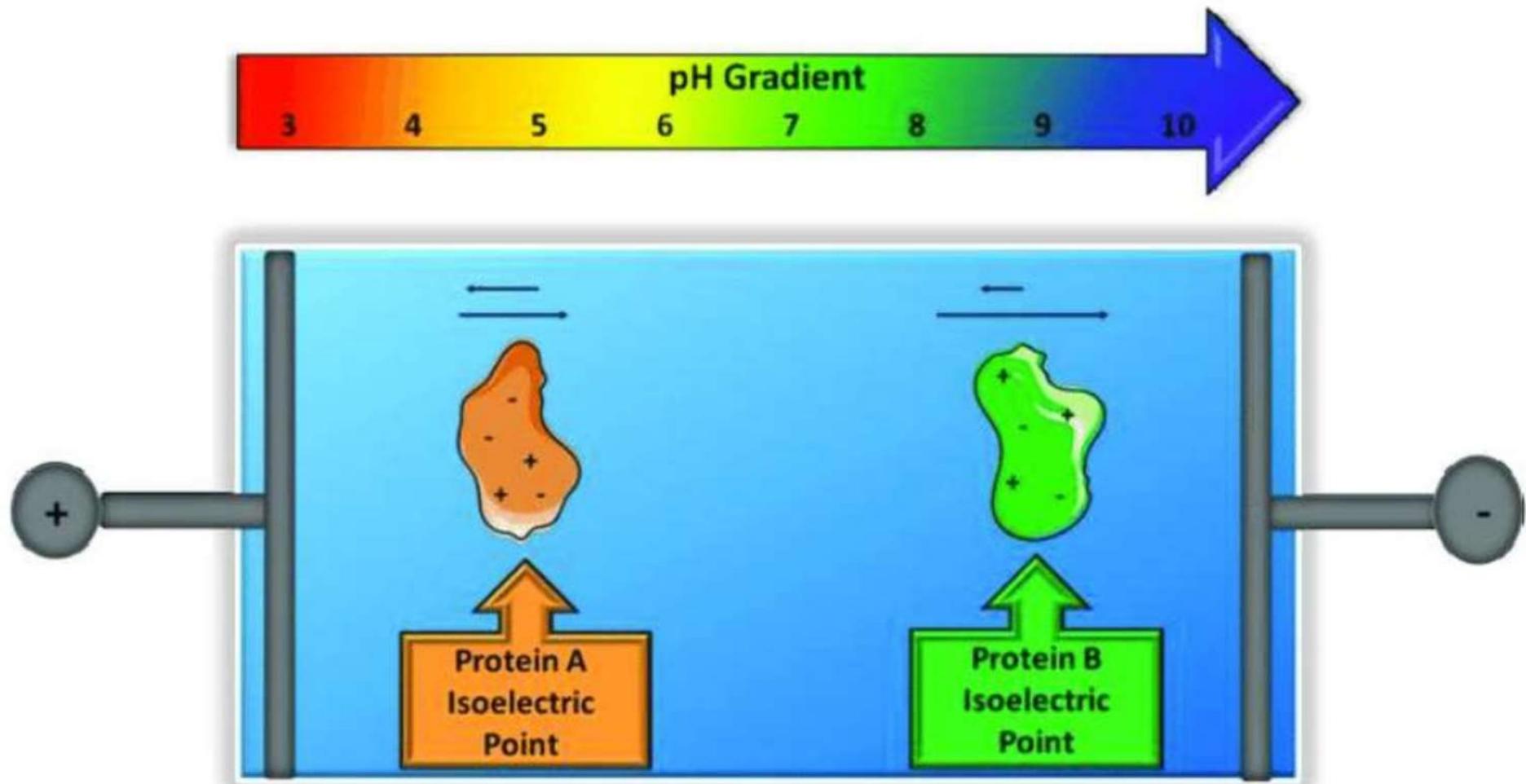
A protein that is in a pH region below its isoelectric point (pI) will be positively charged and so will migrate toward the cathode (negatively charged electrode).

As it migrates through a gradient of increasing pH, the protein's overall charge will decrease until the protein reaches the pH region that corresponds to its pI.

At this point - no net charge and migration ceases.

As a result, the proteins become focused into sharp stationary bands with each protein positioned at a point in the pH gradient corresponding to its pI.

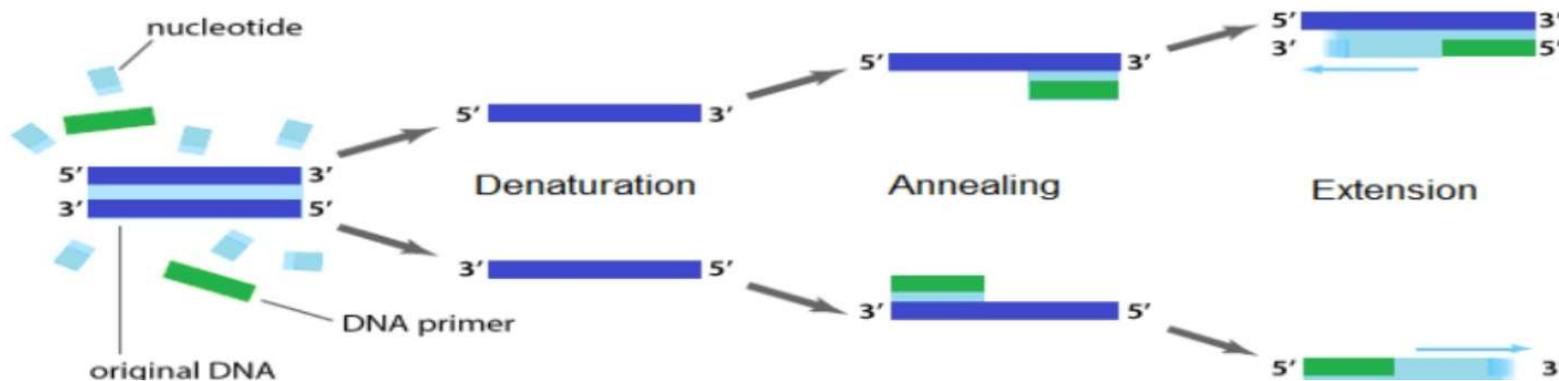
Isoelectric Focusing of Proteins



Nucleic acid analysis

Polymerase chain reaction

- Polymerase chain reaction (PCR) is an efficient and cost effective molecular tool to copy or amplify small segments of DNA or RNA.
- PCR was originally developed in 1983 by the American biochemist Kary Mullis. He was awarded the Nobel Prize in Chemistry in 1993 for his pioneering work.



CYCLE 1

Denaturation
95°C

Annealing
55-65°C

Extension
72°C



Polymerase



Primers

Taq DNA Polymerase
(<https://pubmed.ncbi.nlm.nih.gov/2001000/>)

Starting point for the
DNA replication

CYCLE 1

Denaturation
95°C

Annealing
55-65°C

Extension
72°C



ATGTTAGA

TACAATCG



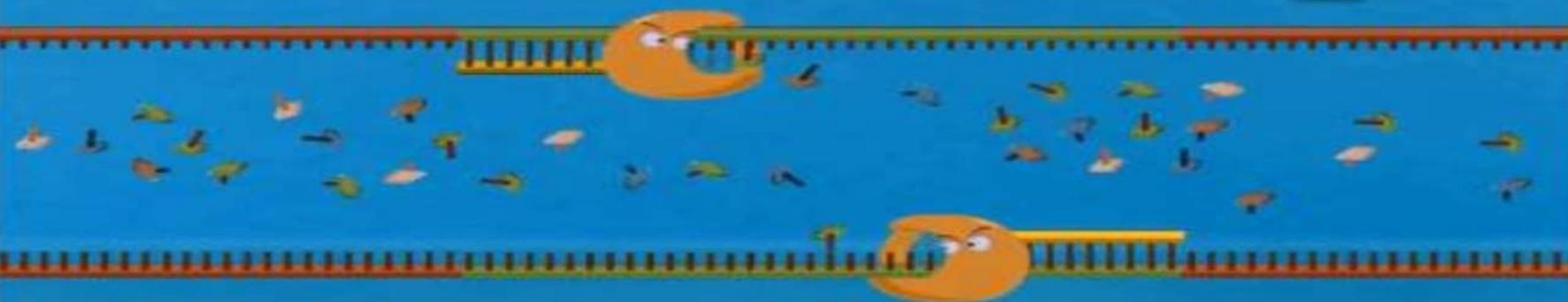
GAGTCAACT

CYCLE 1

Denaturation
95°C

Annealing
55-65°C

Extension
72°C



DNA Markers

Grow out test

- Based on the identification of morphological characteristics at various stages of plant growth.
- Subject to influence by environmental factors and is time-consuming.

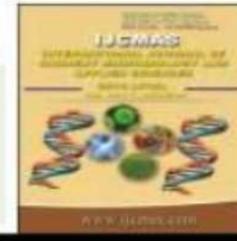
DNA markers

- Based on the genotype of the hybrids eliminating environmental variations .
- Based on identification of genotype specific profiles



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Utilization of SSR Markers for Seed Purity Testing in Popular Maize Hybrids

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Pushpa Kharb¹ and Mehar Chand Kamboj²

- ❖ SSR markers- Simple sequence repeats – Repeating Sequence of 2-5 nucleotides.
- ❖ Microsatellite markers can be used for fingerprinting of hybrids, assessing variation within parental lines and testing the genetic purity of hybrid seed lot.
- ❖ DNA markers systems, PCR based co-dominant **SSRs (microsatellites)** are preferred for genotyping – reproducibility and abundance .



**THANK
YOU**