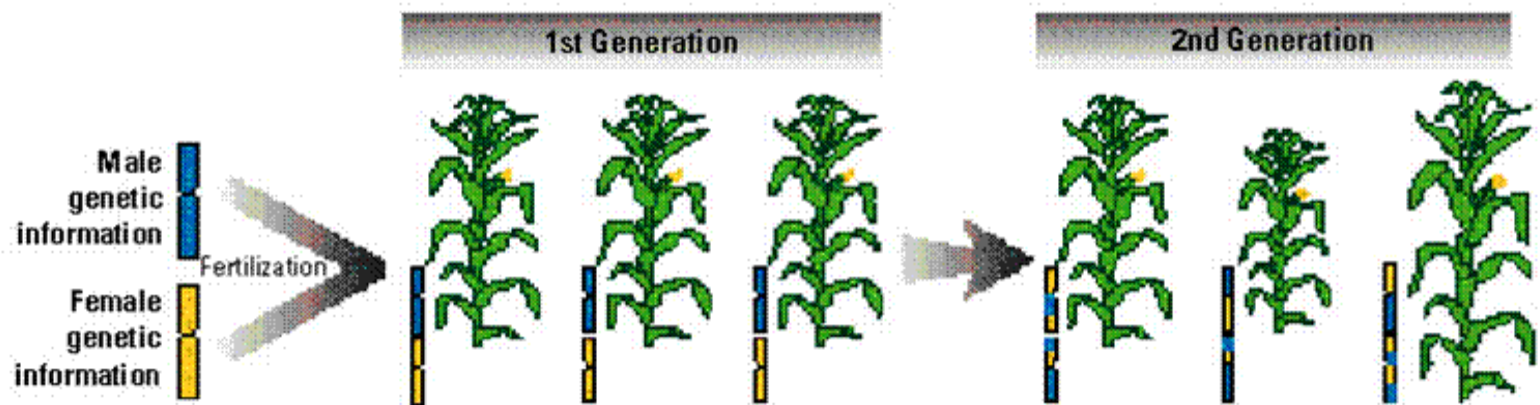


**Isolation of plant genomic
DNA from its F1 progenies
and its visualization in
agarose gel electrophoresis**

F1 generation

- In F1 generation: F1 plants are grown to produce sufficient F2 seeds
- The F1 plants are planted and assessed for their behaviour with regard to easily observable characters and the selfed plants that occur accidentally are identified and removed .



Sexual Reproduction: Hybrid maize that has been produced through sexual reproduction displays identical genetic makeup. The depiction of the second generation represents the use of seed recycled from hybrids, a common practice in many developing countries, and its varied results.

F1 generation progeny

- Phenotypic selection of the best performing clones as parents. Such selection can only be effective if the pedigree of clones is correctly identified.
- Progeny testing, in which individual parents are evaluated on the basis of the performance of their offspring, will not be valid if some of the offspring are illegitimate
- The use of genomic tools is one of effective method to overcome this problem of progeny identity.
- Genomic tools such as molecular markers have been used to trace errors in progeny established for plant breeding
- In progeny testing, true progeny are detected by the presence of DNA sequences corresponding to both alleles contributed by the two parents.

DNA extraction

- Extraction of large quantity and high quality DNA is often a limiting factor in genetic analysis of plant traits important to agriculture.
- To maintain a low-cost, high yield, high quality, and high throughput method genomic DNA is prepared from leaves and dry seeds.
- The DNA extraction method combines a traditional CTAB DNA extraction and modified DNA clean-up procedure.
- DNA clean-up procedure uses silica-coated magnetic beads into a new platform that reduced significantly the use of the expensive magnetic beads while producing high yield and high quality of genomic DNA.

DNA Extraction

- DNA was extracted from young leaves harvested after flowering, The leaves (30 mg) were ground manually with mortar with extraction buffer
- Extraction buffer--Cetyl-Trimethyl Ammonium Bromide (CTAB) hot extraction buffer [100 mM Tris-HCl, pH 8.0, 1.4 M NaCl, 20 mM EDTA, 2% CTAB, 4% (w/v) PVP (polyvinyl pyrrolidone), 10 mM β -mercaptoethanol and sodium bisulfite (NaHSO₃)].
- The mixture was incubated at 65°C for 1 h, then mixed with 500 μ l of chloroform/ isoamyl alcohol (24: 1). Centrifuged at 13,000 rpm for 15 min.
- The supernatant was recovered and mixed with 2/3 volume of isopropanol at room temperature (30 min). The resulting pellet was washed in 1 ml of ethanol (76%), dried and then suspended in 100 μ l of TE buffer [10 mM Tris-HCl, pH 8.0, and 1 mM EDTA, pH 8.0].
- The DNA was quantified by spectrophotometer and stored at 4°C.
- The quality and concentration of the extracted DNA of each sample was determined by taking the readings directly from a Nano- drop spectrophotometer ND-1000-v3.7 (Thermo Scientific) at absorbance ratios of 260/280 and 260/230.



Cutting the tissues in small pieces

1



Grind it in mortal and pestle with liquid Nitrogen and CTAB

2



Boil the sample at 60°C for 30 minutes

Collect the homogeneous Mixture in a tube

3



Centrifuge the Sample

4

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Collect the pellets and Remove supernatant

8



Centrifuge the Tube

7



Add chilled ethanol

6



Collect the supernatant

5



Add 70% ethanol

9



Centrifuge the Tube

10



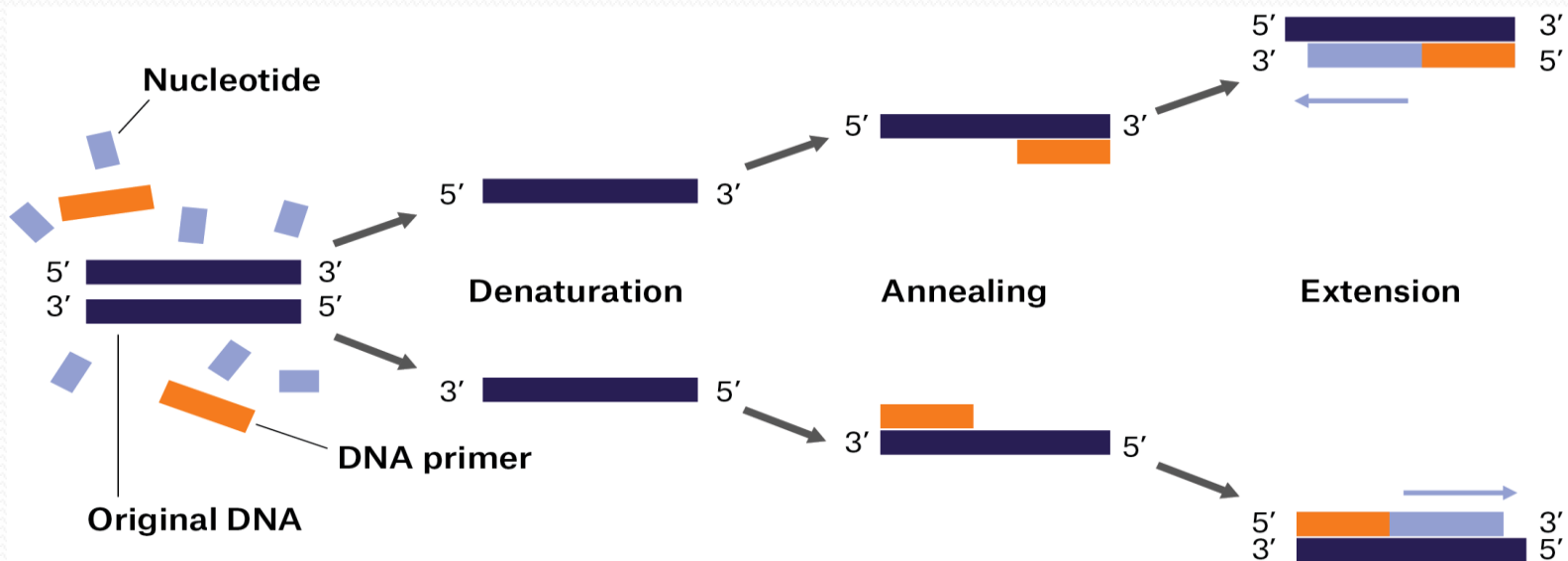
To pellet add TE buffer

11

Amplification by PCR

- The extracted DNA was amplified by PCR.
- The PCR program included:
 - an initial denaturation at 95°C for 5 min,
 - 35 cycles of 30 sec at 95°C, 1 min at the annealing temperature and
 - 1 min 72°C, followed by a terminal phase of 7 min at 72°C.
- PCR reactions were carried out in an Eppendorf Mastercycler Gradient thermocycler.

Amplification by PCR



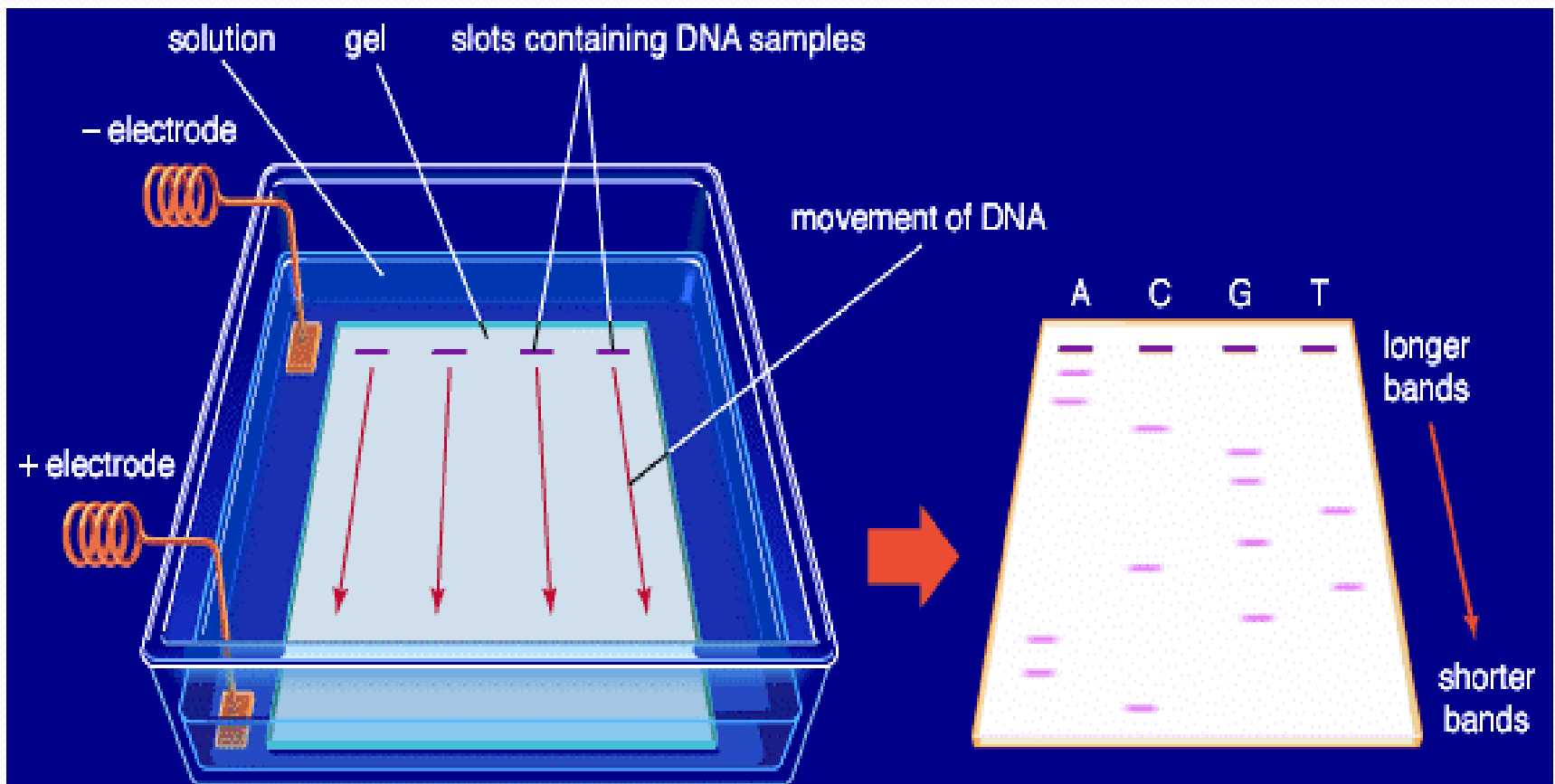
Molecular markers

- Several types of molecular markers, microsatellites or simple sequence repeats (SSRs) markers have been utilized in characterization and clarification of parentage-offspring relationship as well as validation of genotype identity
- Efforts have been made to verify the progeny generated from crosses using gel electrophoresis to separate the DNA fragments after running a PCR for SSR analysis
- The progeny are identified by comparing their banding pattern of the alleles with that of the parents.

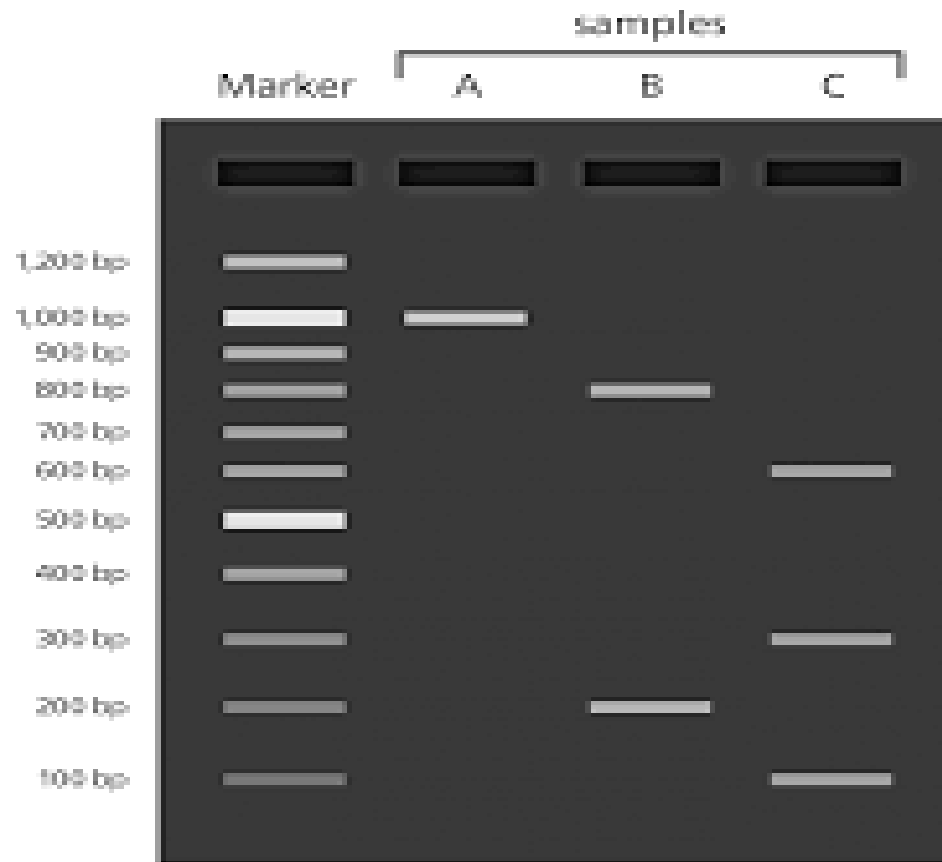
Gel electrophoresis

- Gel electrophoresis was used to determine the quality of the DNA on 0.8% agarose gel in $1 \times$ TAE (*i.e.* 40 mM Tris- acetate buffer in 1 mM EDTA, pH adjusted to 8.3 with acetic acid).
- Electrophoresis was run at 80V for an hour. The gel was stained with gel red 3 \times (Biotium) and visualised under UV light (Syngene Bioimaging System).
- The DNA samples were diluted to a working concentration of 50 ng/ μ l by addition of appropriate amount of sterile water.

Determination of DNA by gel electrophoresis



Detection of DNA by using molecular markers



DNA extraction from sample
(2 lanes per specimen)

(Examination of purity of
extracted DNA)

Amplification of DNA by PCR
method using extracted DNA as
template

Extract DNA from target sample for examination using a DNA extraction kit available on the market.

Purity of extracted DNA is examined as necessary. If purity is low, it is not possible to detect.

Amplify the target gene (a part of it) using the target detection primer for examination under PCR method. It is possible to amplify from microscopic amounts of DNA template.

Electrophoresis of DNA amplified
by PCR using agarose gel

Perform agarose gel electrophoresis of DNA amplified by PCR method using ATTO "Submerged electrophoresis chamber". It is separated into bands according to the size of DNA.

Dyeing agarose gel with ethidium
bromide

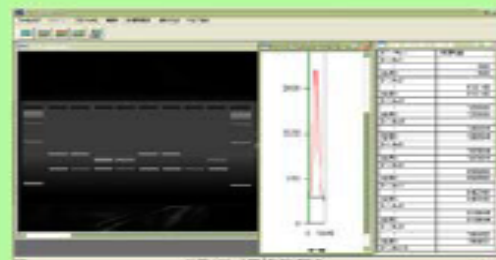
Dye gel using ethidium bromide that is connected specifically with DNA and emits fluorescence by irradiating UV light in order to visualize DNA in gel.

Fluorescent detection imaging of
dye agarose gel using gel
imaging device

Detect and image gel dyed with ethidium bromide using ATTO "PrintGraph".

Determination based on the
captured image (determination
using 2 lanes per specimen)

Determine from the captured pattern.



- Determination example of result Positive: Sample A, B, C, Negative: Sample D
Determination is made according to the presence of the target gene for examination (band) based on whether a band of marker DNA is detected or not.