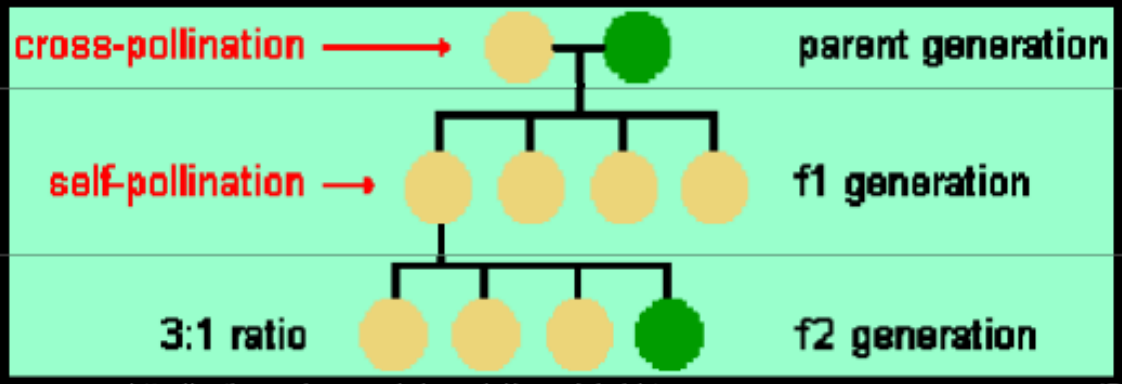
Genetic Markers – Description

- Recognizes the characteristics of the phenotype and/or genotype of particular individual
- Measurable characters – e.g. Seed size, disease resistance
- Their inheritance can be followed through generations



Mendel Theory

http://anthro.palomar.edu/mendel/mendel_1.htm

Genetic marker types

- Morphological marker
- Molecular marker
  1. Protein marker (biochemical marker)
  2. DNA marker

DNA-based Molecular marker

- A DNA sequence that is readily detected and whose inheritance can be easily monitored.
- The uses of molecular markers are based on the naturally occurring polymorphism.
- A marker is a gene of known function and location, that allow the studying of the inheritance of the gene.
- A marker must be a polymorphic ie, it must exist in different forms so that chromosomes carrying mutant gene can be distinguished from the chromosome with the normal gene by a marker.
- NB: polymorphism involves existence of different forms of same gene in plants or population of plants.

RFLP steps

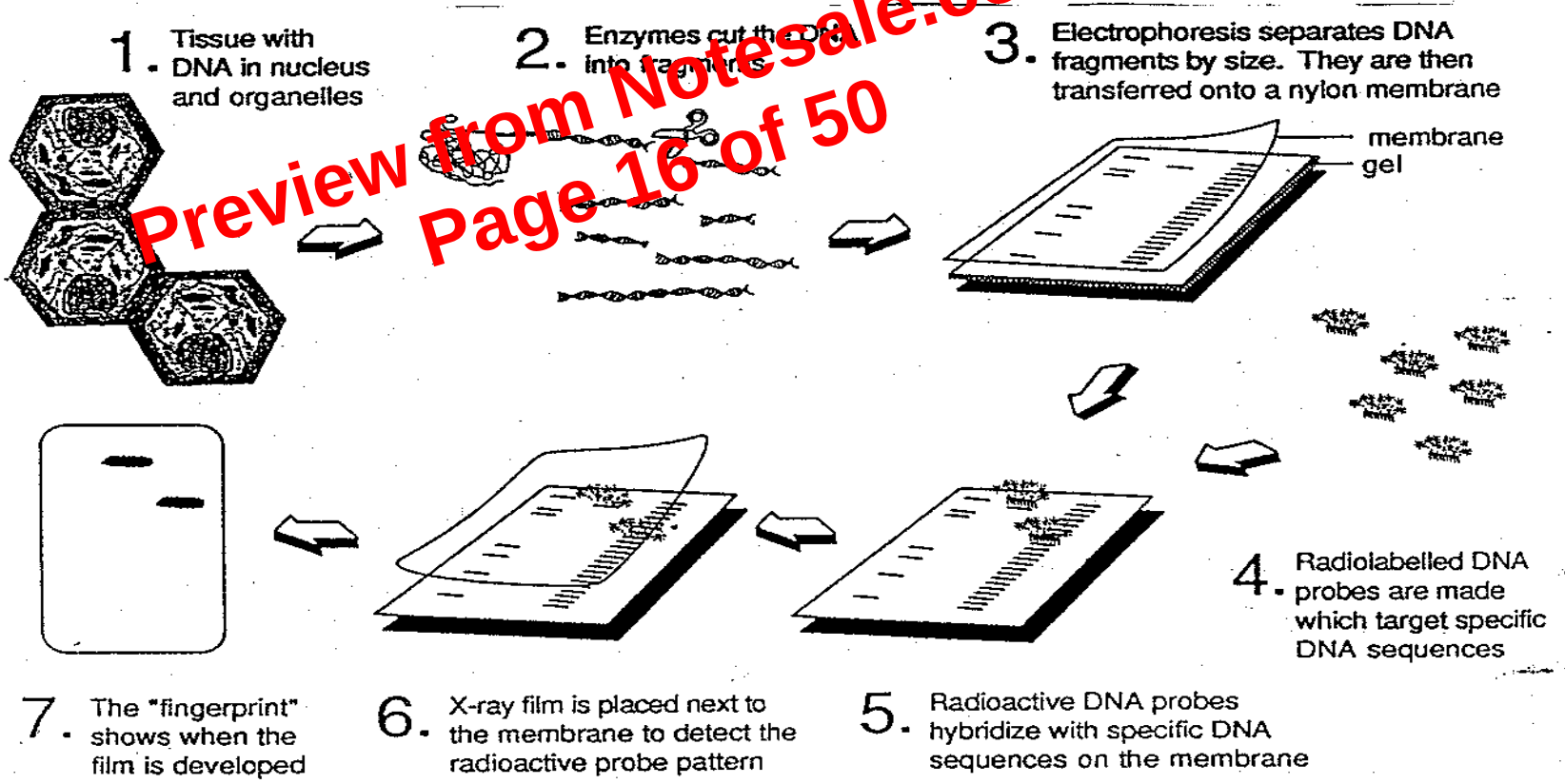
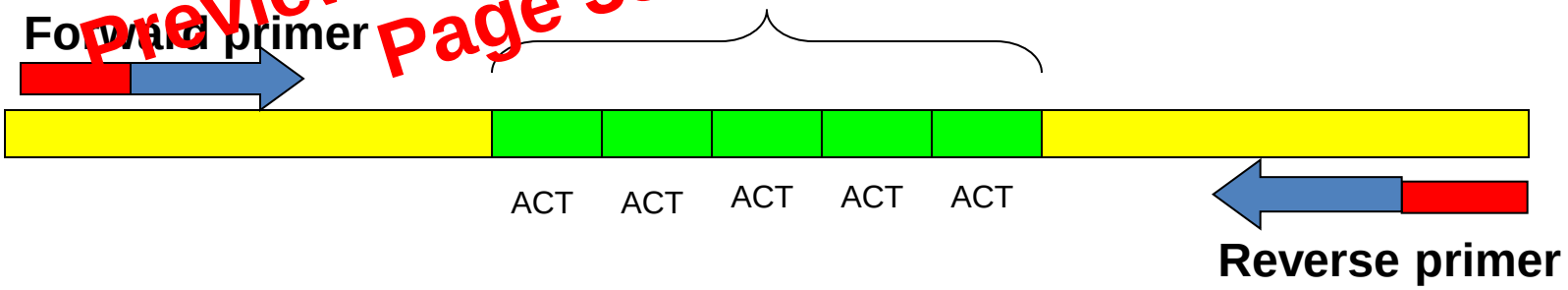


Figure 1. Schematic representation of DNA isolation, restriction nuclease digestion, electrophoresis, and Southern hybridization.

DNA fingerprinting- The powerful new tool for the Genetic Detective. 1990. Peter M. Gresshoff *et al.* Tennessee Farm and Home Science.

Primers anneal to the flanking regions of microsatellites



1. PCR will amplify the region with **ACT repeats**
2. PCR fragments separated by capillary electrophoresis

AFLP (Amplified Fragment Length Polymorphism)

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- AFLP is based on selectively amplifying a subset of restriction fragments from a complex mixture of DNA fragments obtained after digestion of genomic DNA with restriction endonucleases.
- Polymorphisms are detected from differences in the length of the amplified fragments by polyacrylamide gel electrophoresis (PAGE)
- The technique involves four steps: (1) Restriction of DNA and ligation of oligonucletide adapters, (2) Preselective amplification , (3) Selective amplification (4) Gel analysis of amplified fragments.
- AFLP involves the restriction of genomic DNA, followed by ligation of adaptors complementary to the restriction sites and selective PCR amplification of a subset of the adapted restriction fragments. These fragments are viewed on denaturing polyacrylamide gels either through autoradiographic or fluorescence methodologies .

Basic steps of AFLP fingerprinting

5. **Amplification:** DNA fragments with *MseI-EcoRI* ends will be selected as DNA template for amplification.

   - Two PCR primers complementary to the two adaptors are used in amplification.

   -The PCR primers are labeled with radioactive or fluorescence dye for detection of DNA bands on gels.

The aim of this step is to restrict the level of polymorphism and to label the DNA. For this second amplification, we added three more nucleotides at the 3' end of the primer sequence used for the pre-amplification (= adapters sequence + 3 nucleotides). These two additional nucleotides make the amplification more selective and will decrease the number of restriction fragments amplified (polymorphism). Moreover, one of the primers (usually the *EcoRI* primer) is labeled with a fluorescent dye, and will allow the visualization of DNA during the migration.