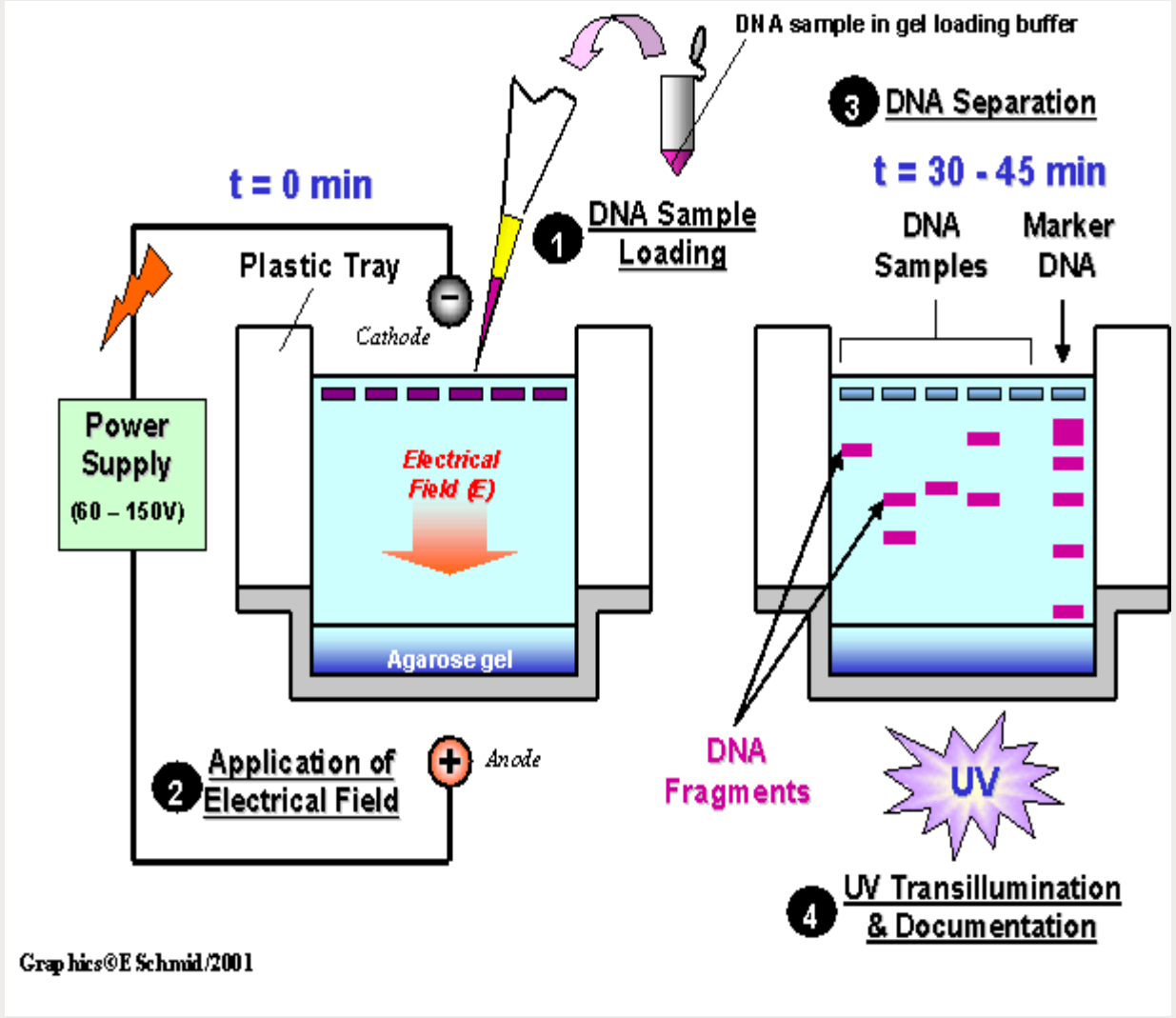
DETECTION AND CHARACTERIZATION OF MOLECULAR AMPLIFICATION

Products: Agarose Gel Electrophoresis, Southern Blot Hybridization, Restriction Enzyme Digest Analysis, and Enzyme-Linked Immunoassay

Agarose Gel Electrophoresis

- Agarose is a polysaccharide
- The pore size of agarose is responsible for much of its DNA separation properties.
- DNA molecules migrate at a rate that is inversely proportional to the log10 of the length of the DNA strand.

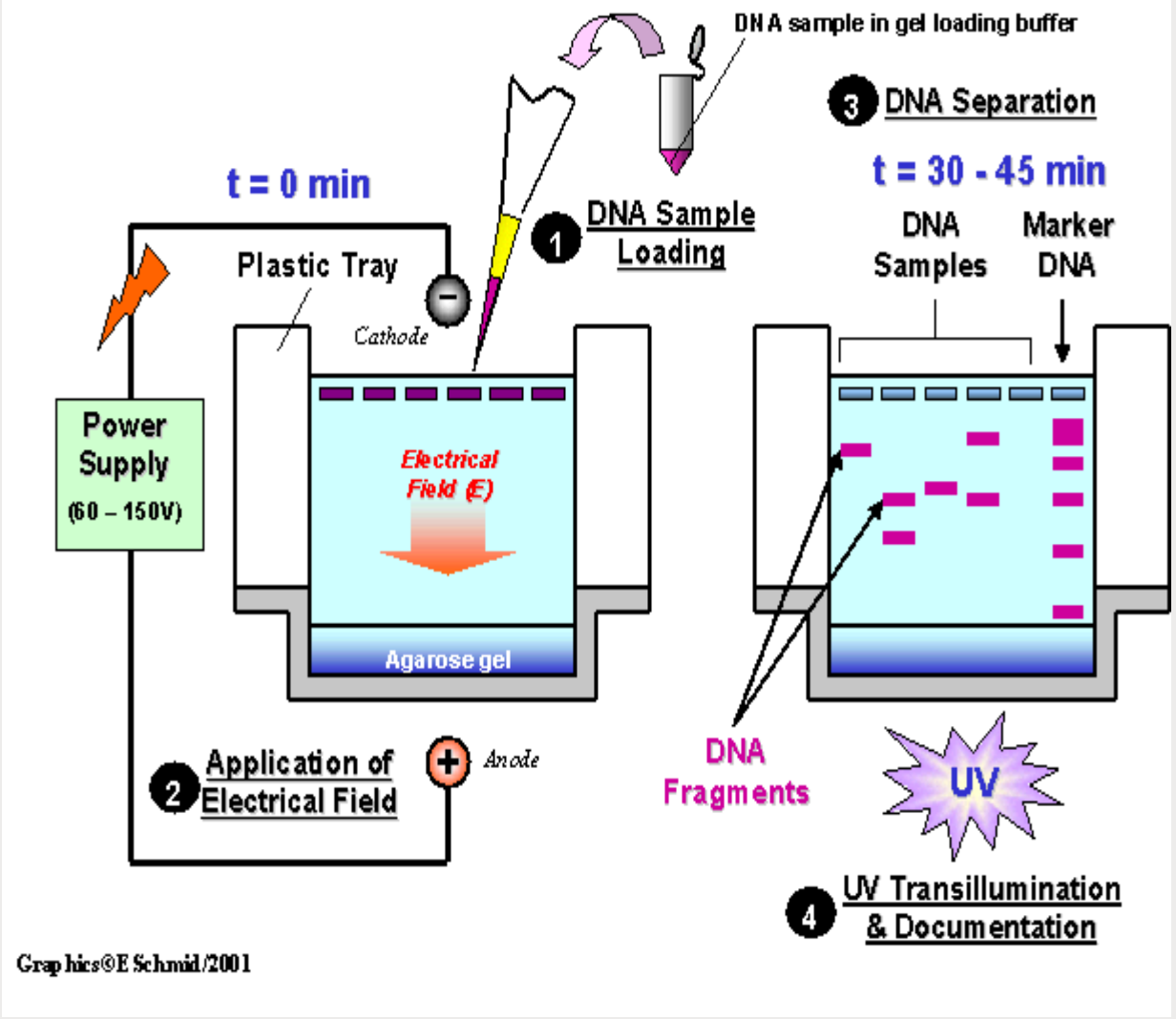


Agarose Gel Electrophoresis

Technical Considerations:

A- Gel performance

- characteristics of the gel (agarose concentration, class, and grade)
- electrophoresis running conditions (voltage applied to the gel, loading and running buffers used, and duration of the electrophoretic run)
- characteristics of the nucleic acid fragments (quantity, size, and conformation)

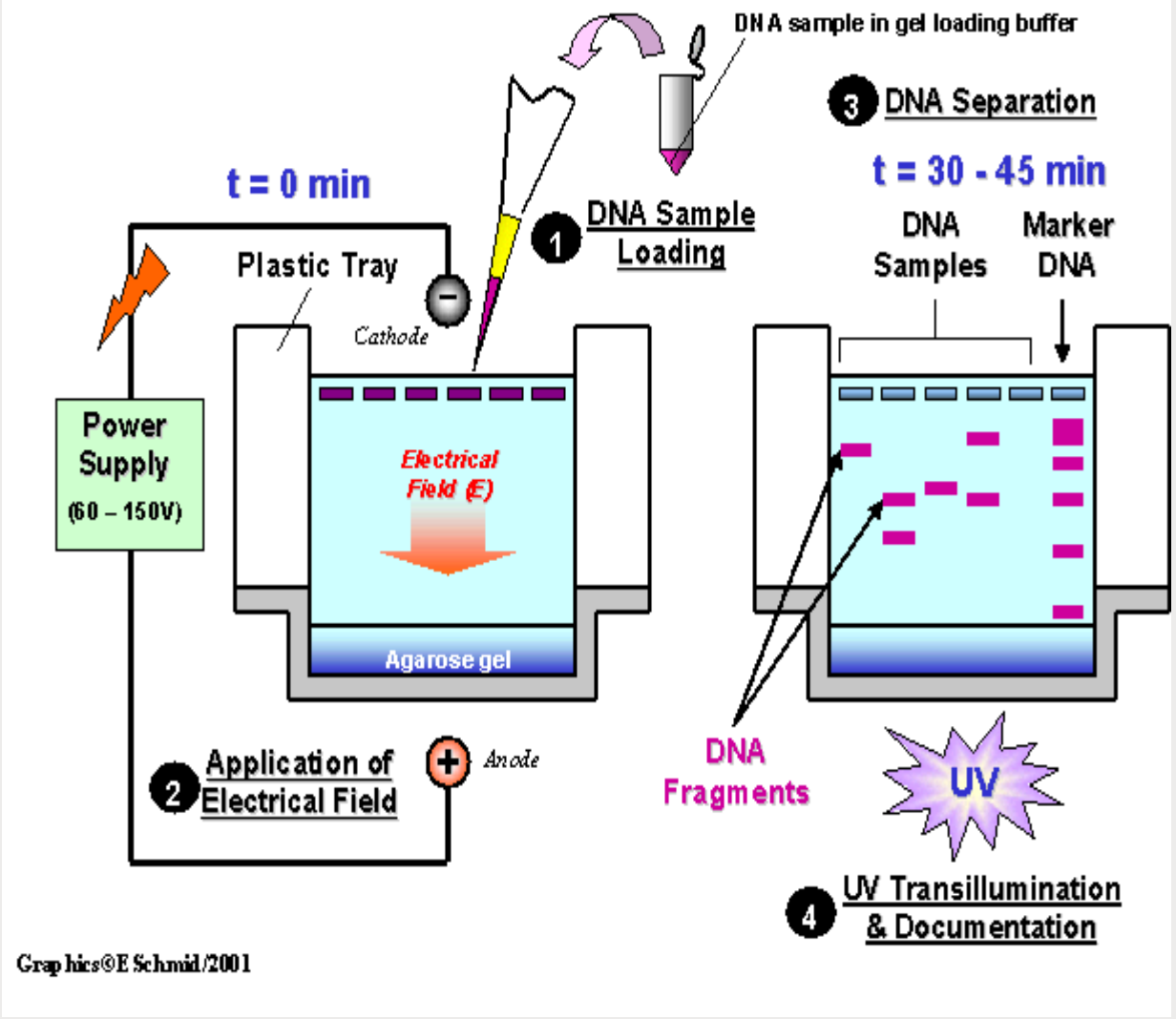


Agarose Gel Electrophoresis

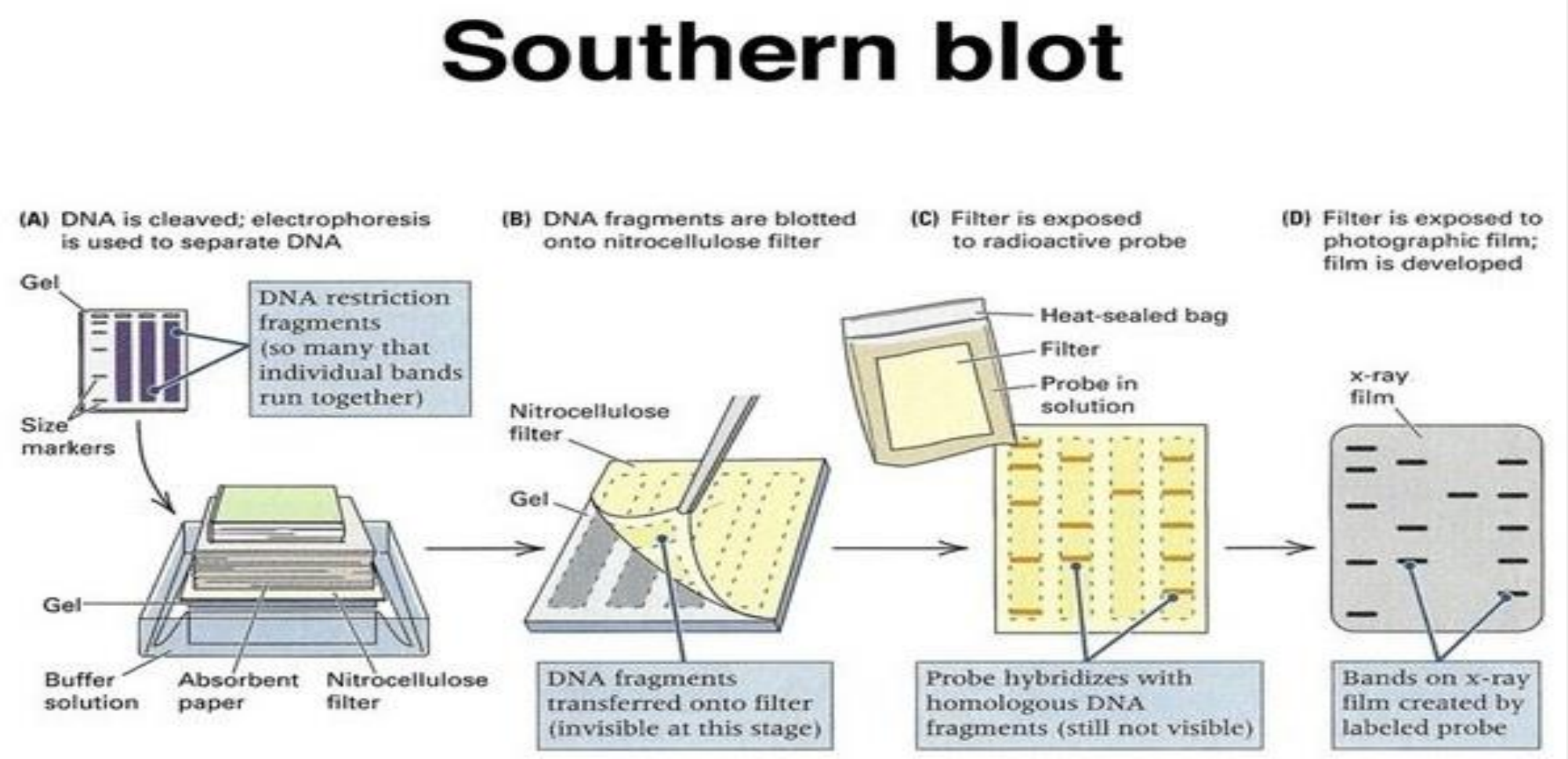
Technical Considerations:

B- Target detection

- Ethidium bromide (EtBr)
- SYBR Green I
- GelStar
- Methylene blue



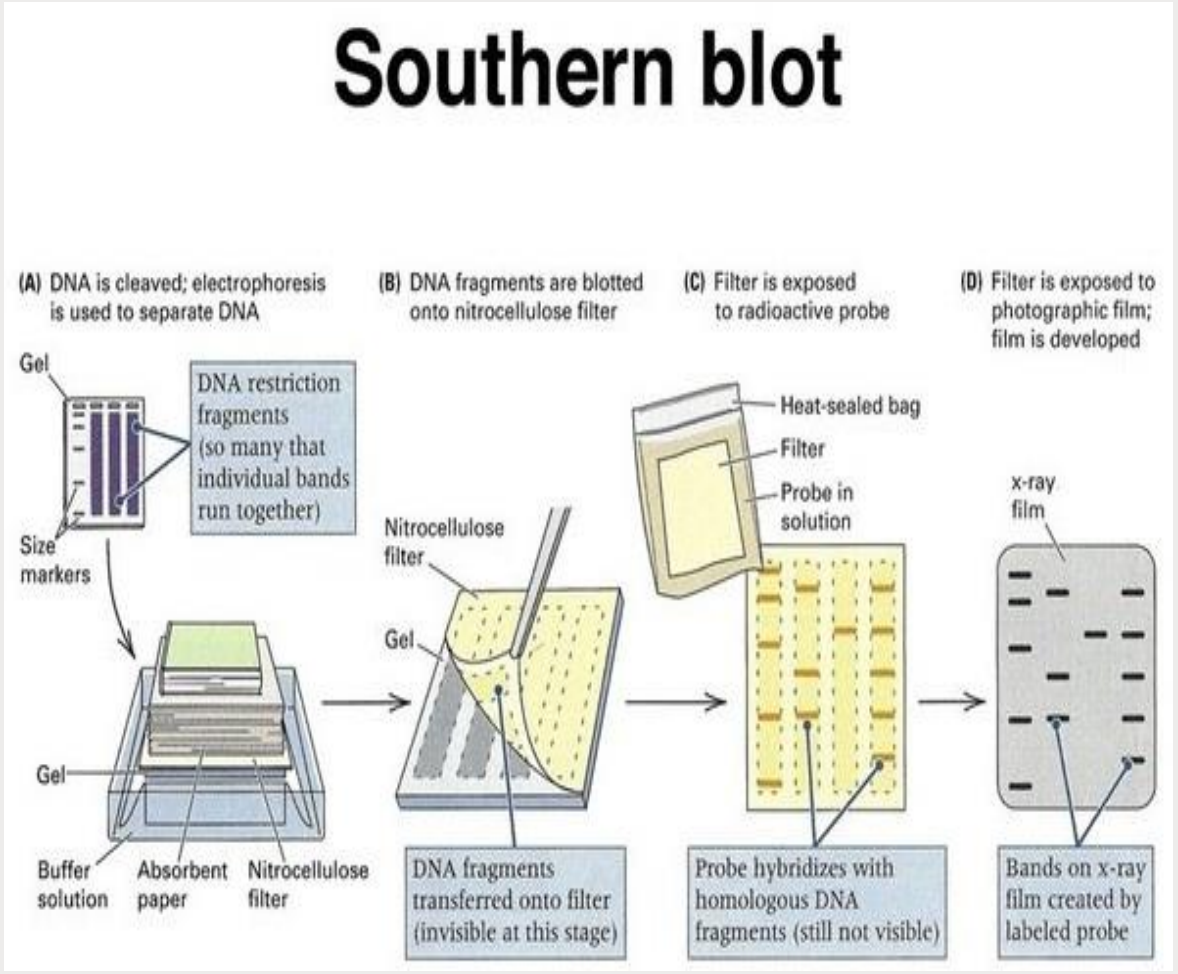
Southern Blot Hybridization



Southern Blot Hybridization

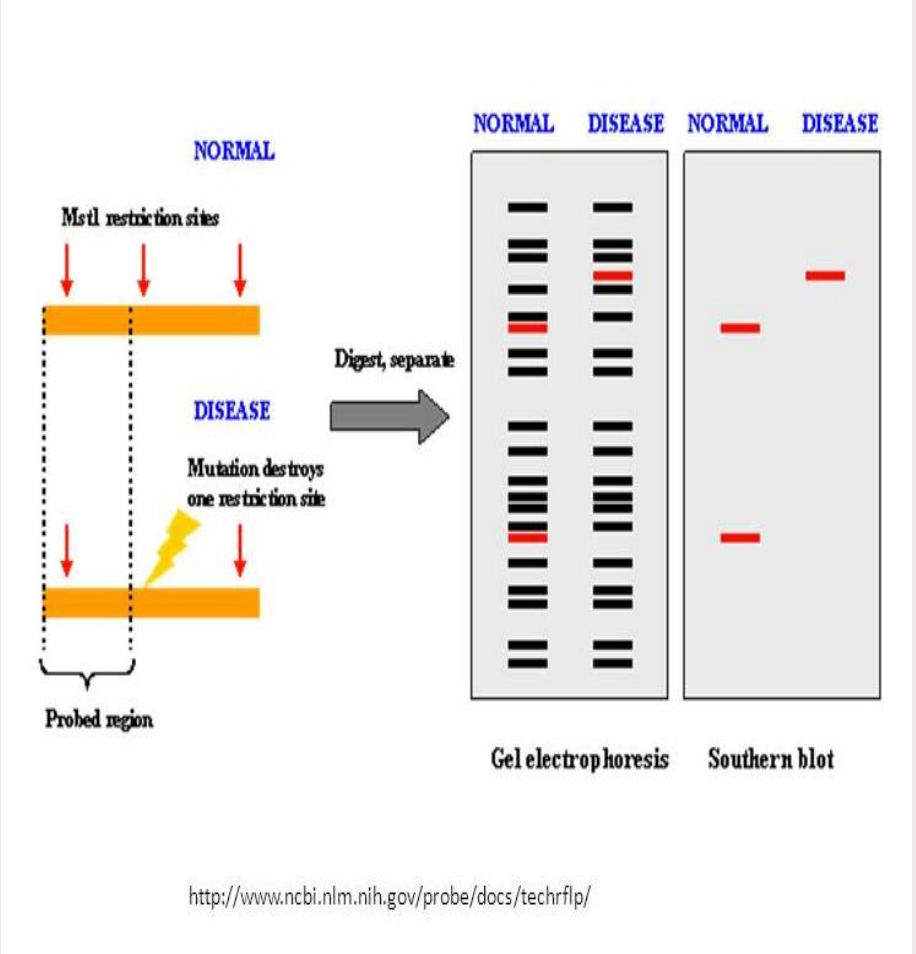
Technical Considerations:

- The type of membrane
- Transfer buffer
- Transfer method
- Hybridization conditions
- Probe system
- Probe sequence used



Restriction Enzyme Digest Analysis

- **Restriction fragment length polymorphism (RFLP) analysis**
- specific double-stranded DNA sequence
- Visualization and detection

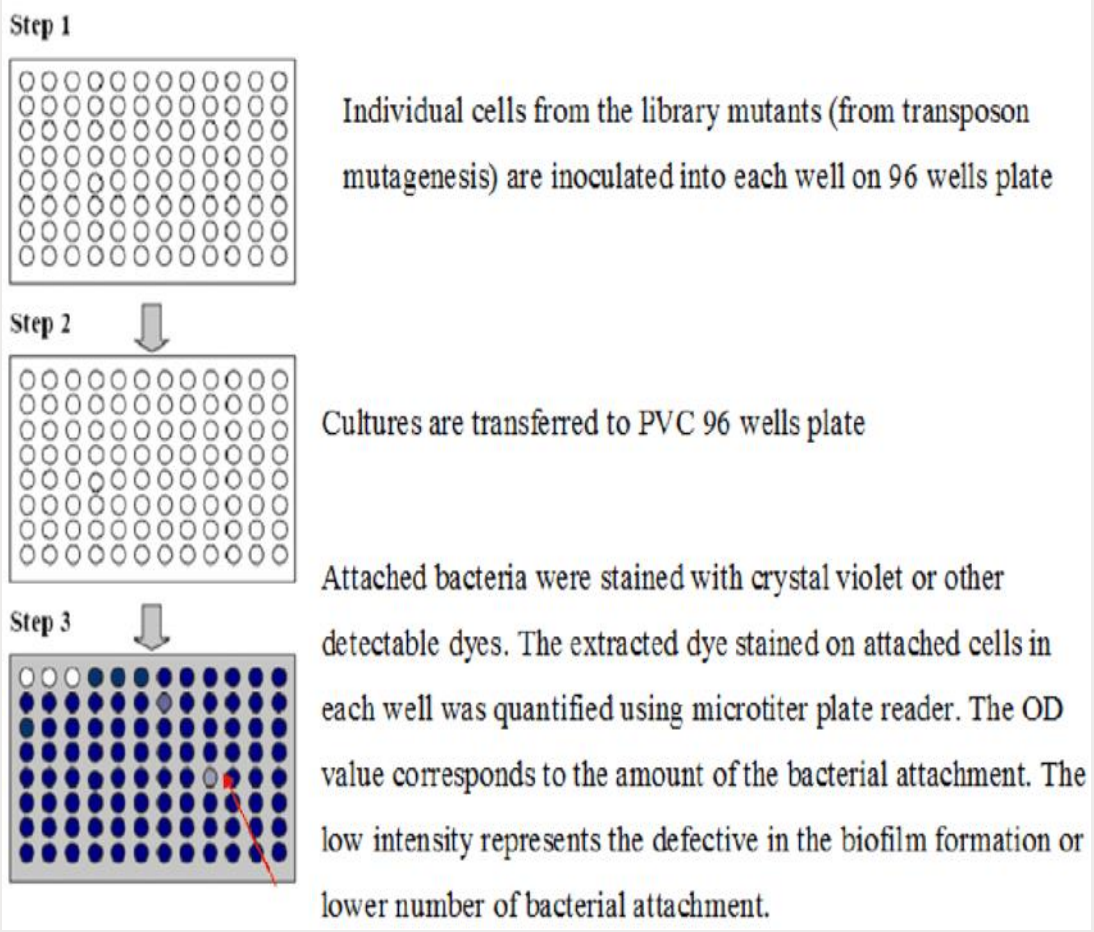


Restriction Enzyme Digest Analysis

- **Technical Considerations:**
- Concentration of enzyme
- Concentration of buffer,
- Concentration of target nucleic acid
- Temperature (usually 37 °C) of reaction
- Duration of reaction
- Purity of target nucleic acid
- Supercoiling

Enzyme-Linked Immunoassay

- **Solid-Phase Enzyme-Linked Immunoassay (EIA)**
- Probe-based methods for the detection and identification of PCR products have been developed for several different solid phases, including nylon membranes, microwell plates, microparticles, and oligonucleotide microarrays
- Microtiter plate assays



Enzyme-Linked Immunoassay

- **Technical Considerations:**
- Hybridization probe design (probe sequence and labeling method)
- Choice of microtiter plate
- Technique for affixing probe to the microwell surface
- Hybridization and wash conditions
- Means of detecting hybridized amplicon