

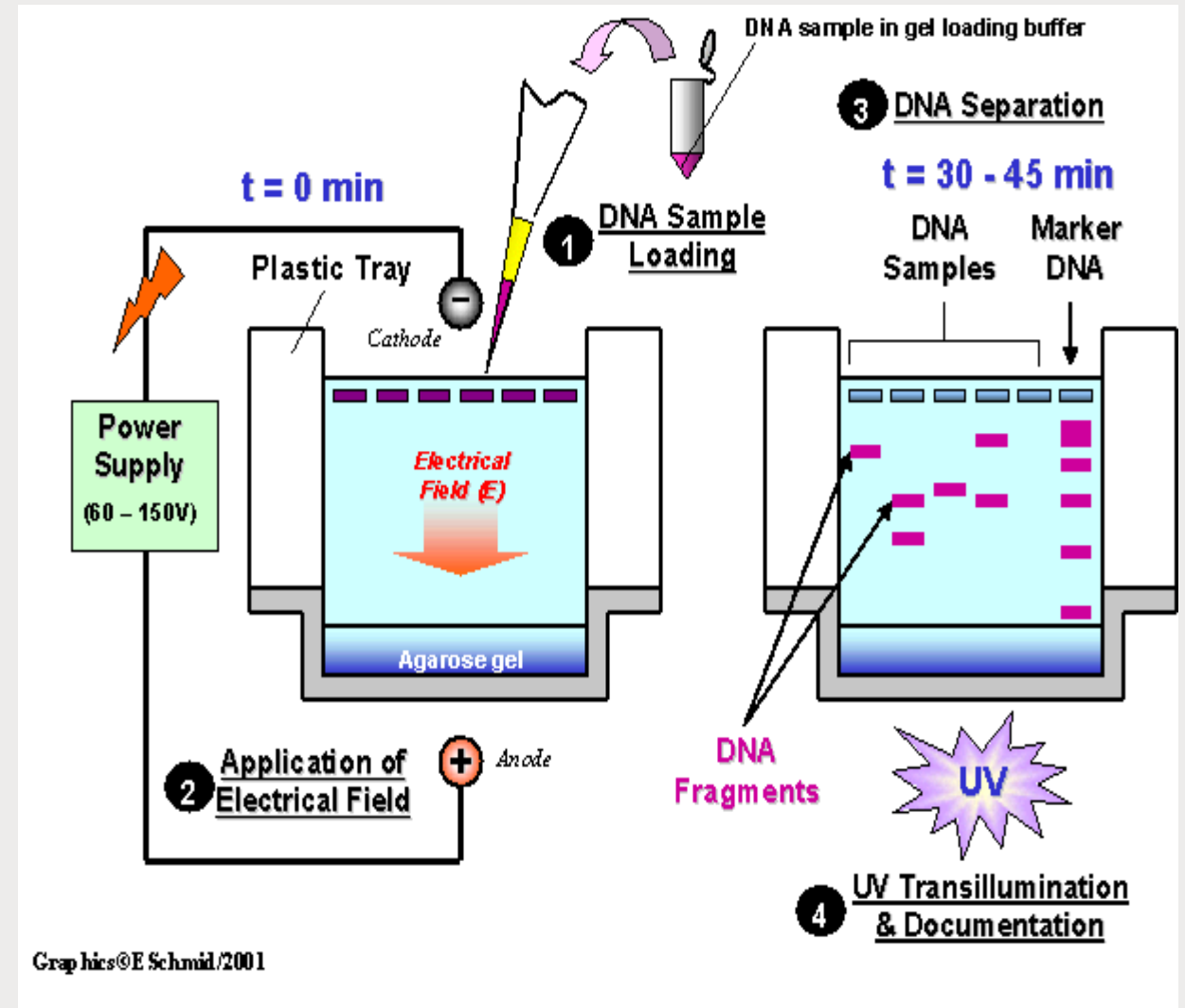


DETECTION AND CHARACTERIZATION OF MOLECULAR AMPLIFICATION

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 \log_{10} of the length of the DNA strand.

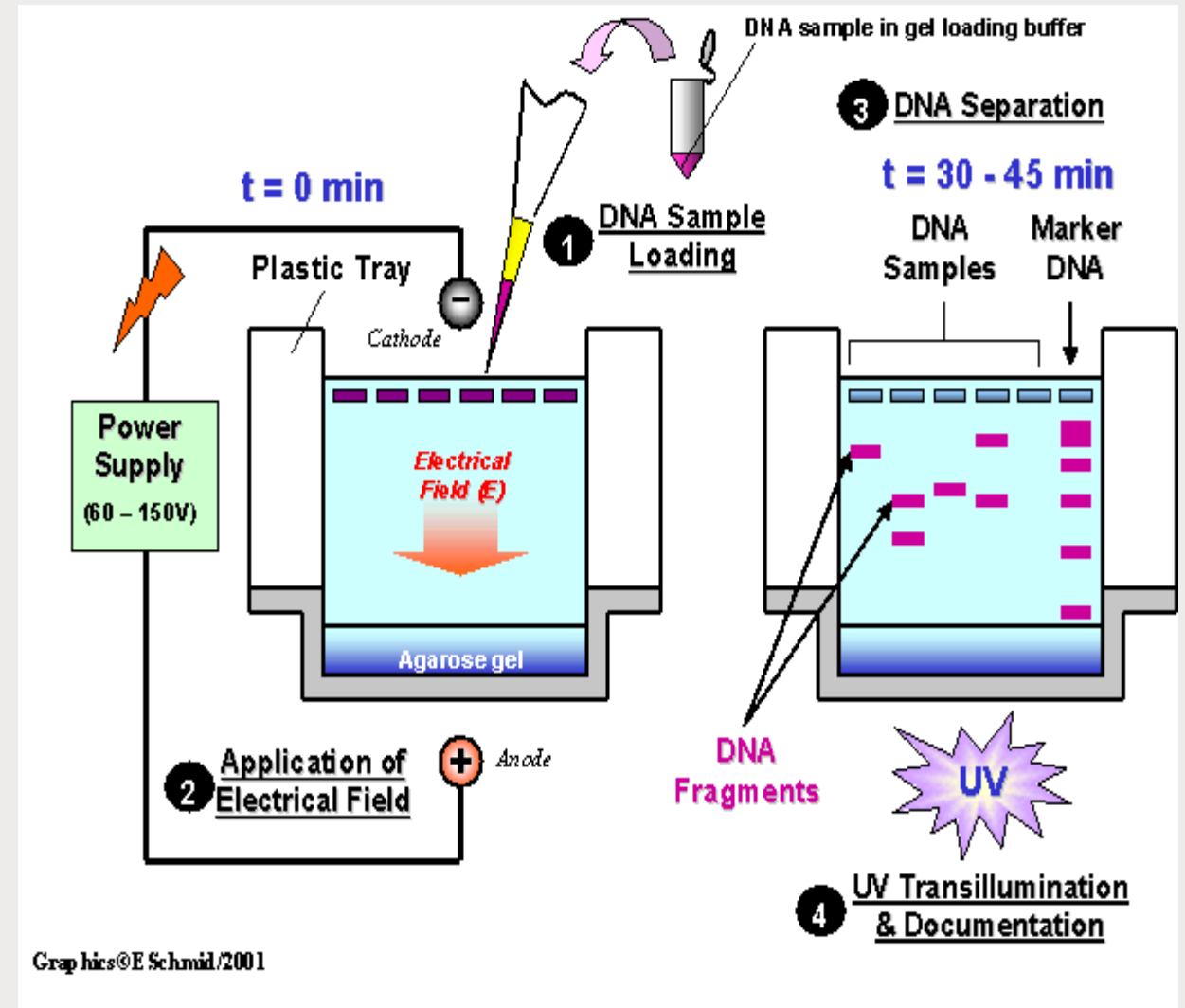


Agarose Gel Electrophoresis

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)

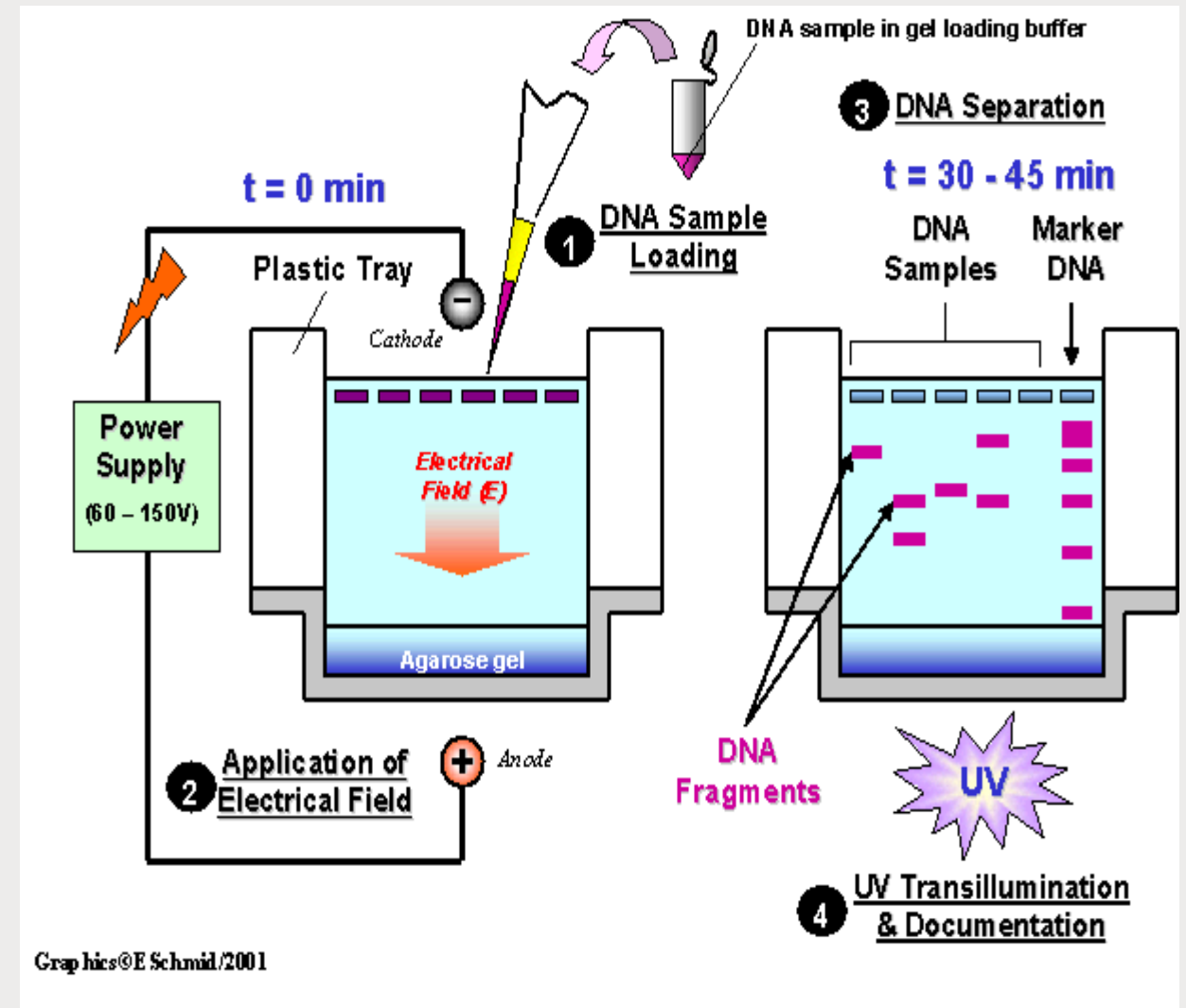


Agarose Gel Electrophoresis

Technical Considerations:

B- Target detection

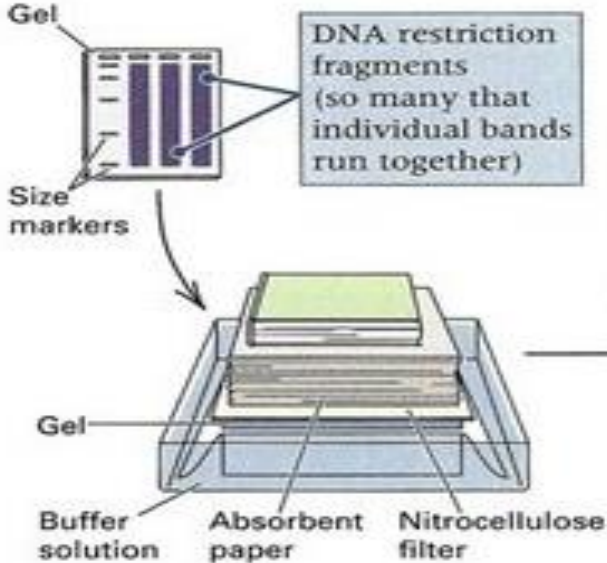
- Ethidium bromide (EtBr)
- SYBR Green I
- GelStar
- Methylene blue



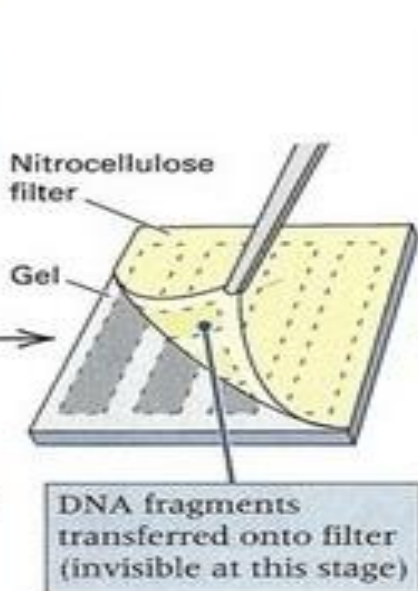
Southern Blot Hybridization

Southern blot

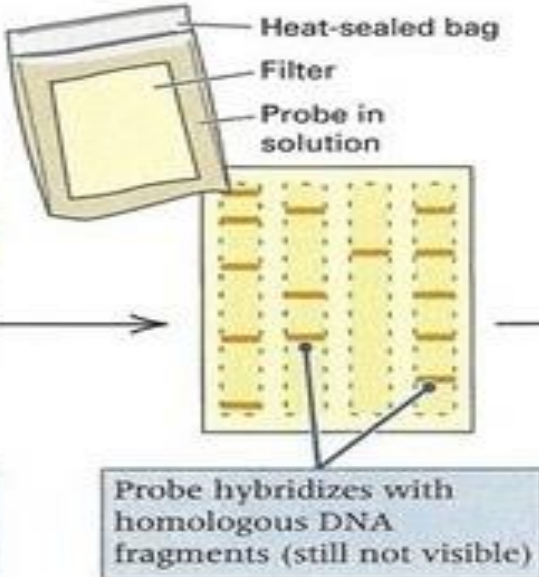
(A) DNA is cleaved; electrophoresis is used to separate DNA



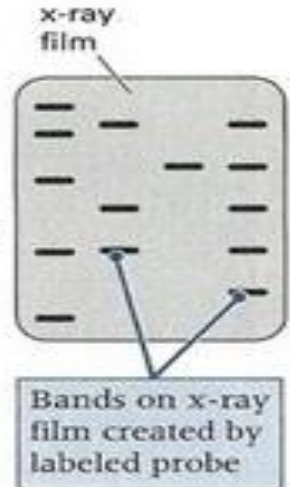
(B) DNA fragments are blotted onto nitrocellulose filter



(C) Filter is exposed to radioactive probe



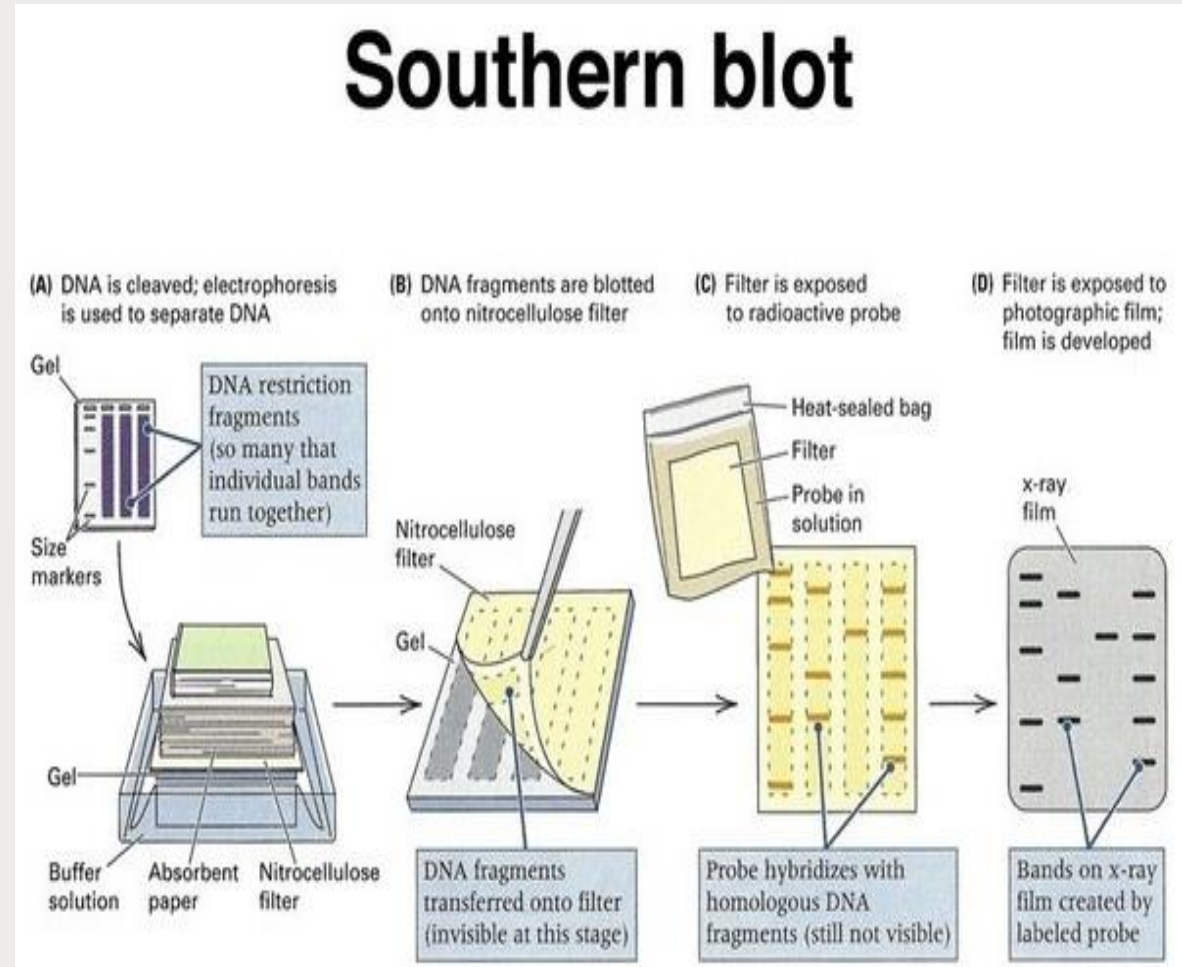
(D) Filter is exposed to photographic film; film is developed



Southern Blot Hybridization

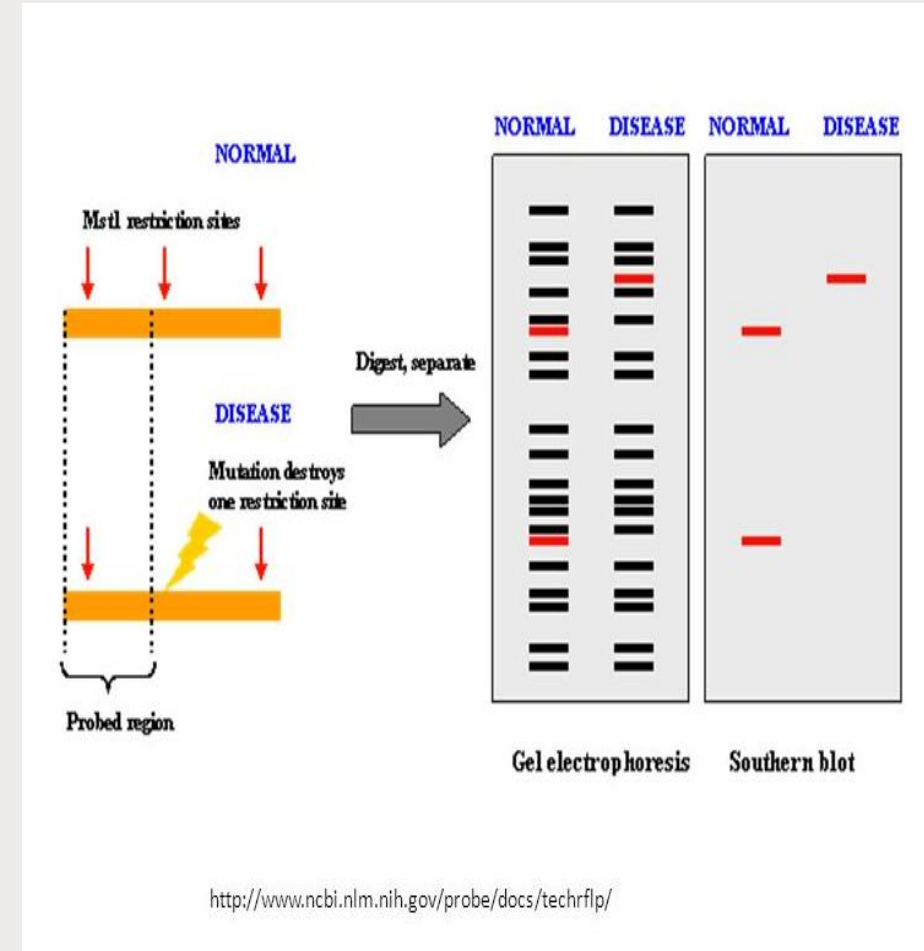
Technical Considerations:

- 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



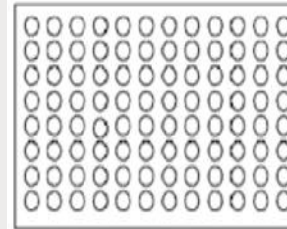
Restriction Enzyme Digest Analysis

- **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

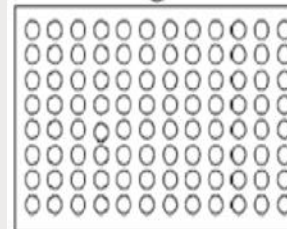
- **Solid-Phase Enzyme-Linked Immunoassay (EIA)**
- Probe-based methods for the detection and identification of PCR products have been developed for several different solid phases, including nylon membranes, microwell plates, microparticles, and oligonucleotide microarrays
- Microtiter plate assays

Step 1



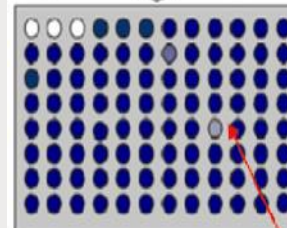
Individual cells from the library mutants (from transposon mutagenesis) are inoculated into each well on 96 wells plate

Step 2



Cultures are transferred to PVC 96 wells plate

Step 3



Attached bacteria were stained with crystal violet or other detectable dyes. The extracted dye stained on attached cells in each well was quantified using microtiter plate reader. The OD value corresponds to the amount of the bacterial attachment. The low intensity represents the defective in the biofilm formation or lower number of bacterial attachment.

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