



FACULTY OF ENGINEERING & TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

COLONY HYBRIDIZATION

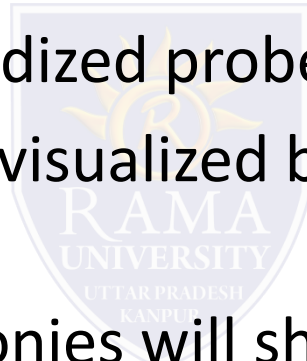
- Technique to identify bacterial colony with foreign gene - genetic engineering
- Transformed colonies are detected - radioactive DNA/RNA used in the probe

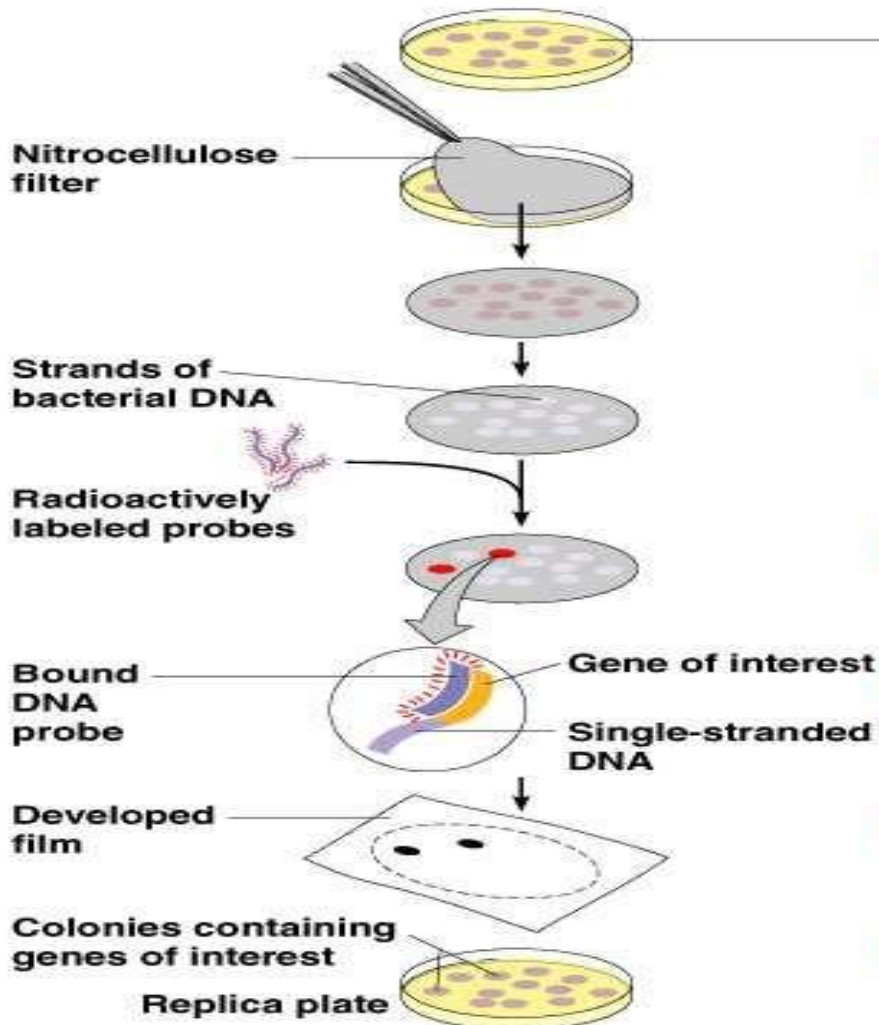


STEPS

- Transformed colonies - agar plate - **master plate**
- Colony of master plate are **replica plated** on nitrocellulose membrane
- A reference point is marked on both for further comparison
- **After colonies appear - Alkali** - lyse the bacterial cell - denature DNA
- Filter - Proteinase K - digest & remove protein
- Filter is baked at **80° c** – to fix the DNA – impregnation

- Hybridization with **radioactive probe** – DNA sequence used in transformation
- Washing off – unhybridized probe
- Hybridized colonies – visualized by **autoradiography**
- Only transformed colonies will show autoradiograph





Master plate with colonies of bacteria containing cloned segments of foreign genes.

- 1** Make replica of master plate on nitrocellulose filter.
- 2** Treat filter with detergent (SDS) to lyse bacteria.
- 3** Treat filter with sodium hydroxide (NaOH) to separate DNA into single strands.
- 4** Add radioactively labeled probes.
- 5** Probe will hybridize with desired gene from bacterial cells.
- 6** Wash filter to remove unbound probe and expose filter to X-ray film.
- 7** Developed film is compared with replica of master plate to identify colonies containing gene of interest.

Plaque hybridization

Plaque hybridization is a technique used in Molecular biology for the identification of recombinant phages.

The procedure can also be used for the detection of differentially represented repetitive DNA.

The technique (similar to colony hybridization) involves hybridizing isolated phage DNA to a label probe for the gene of study.

This is followed by autoradiography to detect the position of the label.

The plaque hybridization procedure has some advantages over colony hybridization due to the smaller and well defined area of the filter to which the DNA binds.

