RAMA UNIVERSITY

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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

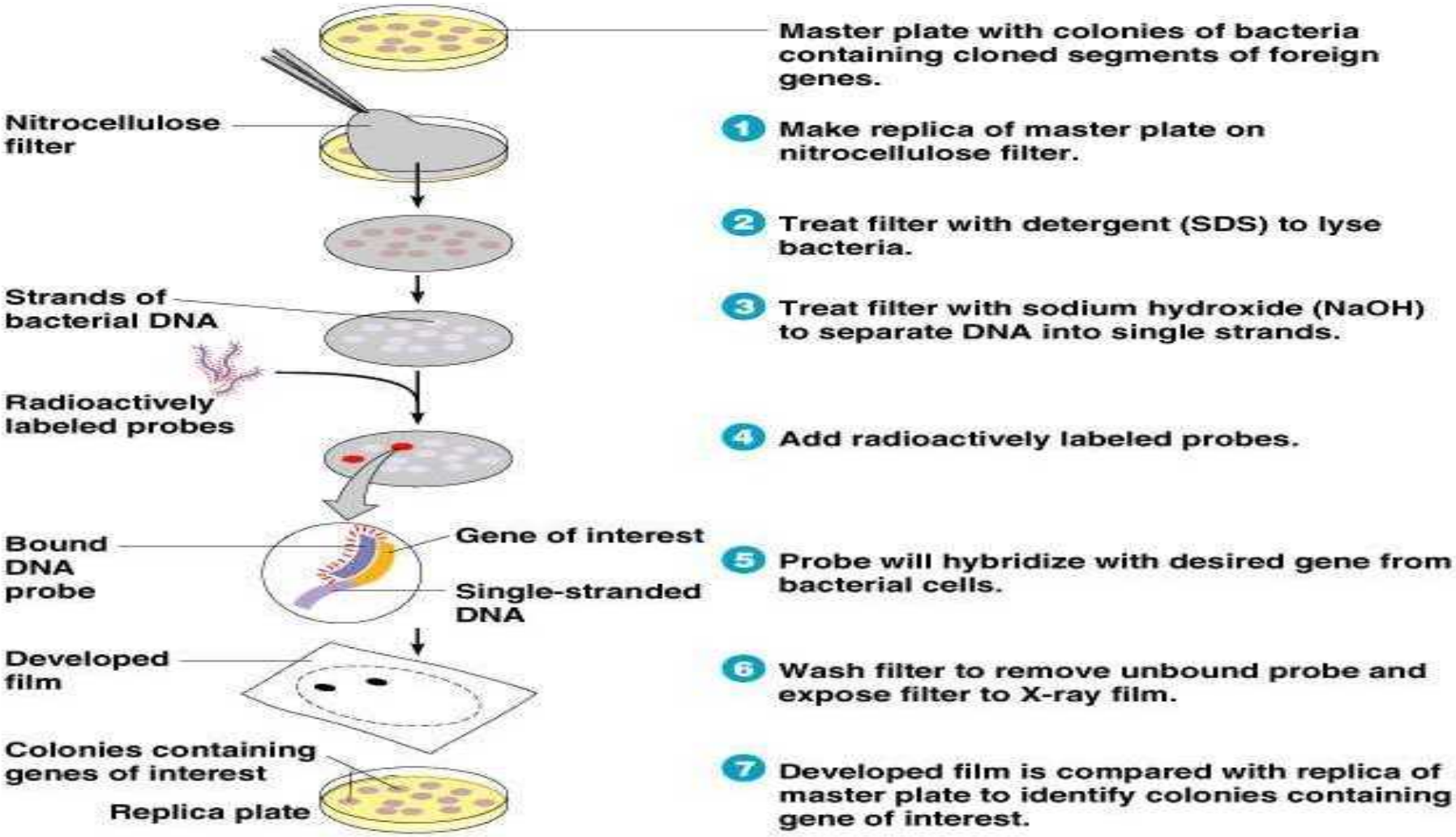
Filter is baked at **80˚c** – to fix the DNA – impregnation

Hybridization with **radioactive probe –** DNA sequence used in transformation

Washing off – unhybridized probe

Hybridized colonies – visualized by **autoradiography**

Only transformed colonies will show autoradiograph



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.