Quadrant II – Transcript and Related Materials

Programme	**:**	**Bachelor of Science (Third Year)**
Subject	**:**	**Zoology**
Semester	**:**	**VI**
Course Code	**:**	**ZOD 104**
Course Title	**:**	**Animal Biotechnology**
Title of the Unit 4	**:**	**Transformation methods and techniques.**
Module name	**:**	**Screening: Colony hybridization and Plaque hybridization.**
Name of the presenter:		**Ms. Filomena Pereira**

NOTES

COLONY HYBRIDIZATION - Method of screening. Used to identify the colonies in a plate, which contain specific DNA sequences (desired DNA). These colonies are obtained from bacterial cells into which sequence was introduced through genetic engineering. This technique was developed in 1975 by M. Grusten and S. Hogness.

Procedure: 1) Bacterial cells subjected to transformation are plated on agar plate, this is the **master plate.**

2) Colonies of master plate are replica plated on nitrocellulose filter membrane.

- For replica plating, a block of wood is covered with velvet cloth.
- Block is sterilized and lowered into the master plate till velvet touches the colonies.

- Block is withdrawn and lowered onto a nitrocellulose filter so that bacterial cells stuck onto velvet are transferred onto the filter.
- Nitrocellulose filter disc then placed on the surface of gelled nutrient medium.

3) Both master plate and nitrocellulose membrane are incubated to develop colonies.

- Cells growing on nitrocellulose filter disc are nourished through the diffusion of nutrients from gelled medium.

4) Filter disc is removed and treated with alkali (0.5 N NaOH) to use the bacterial cells and denature DNA.

- Filter neutralized by tris-hydroxymethyl buffer.

5) Filter is dipped in proteinase k solution that removes proteins from the filter.

- Denatured DNA remains bound to the filter.

6) Filter is baked at 80 degree celcius to fix DNA properly to the membrane.

- This yields DNA print of bacterial colonies in same position as the master plate.

7) Filter is hybridized with radioactive probes in suitable conditions.

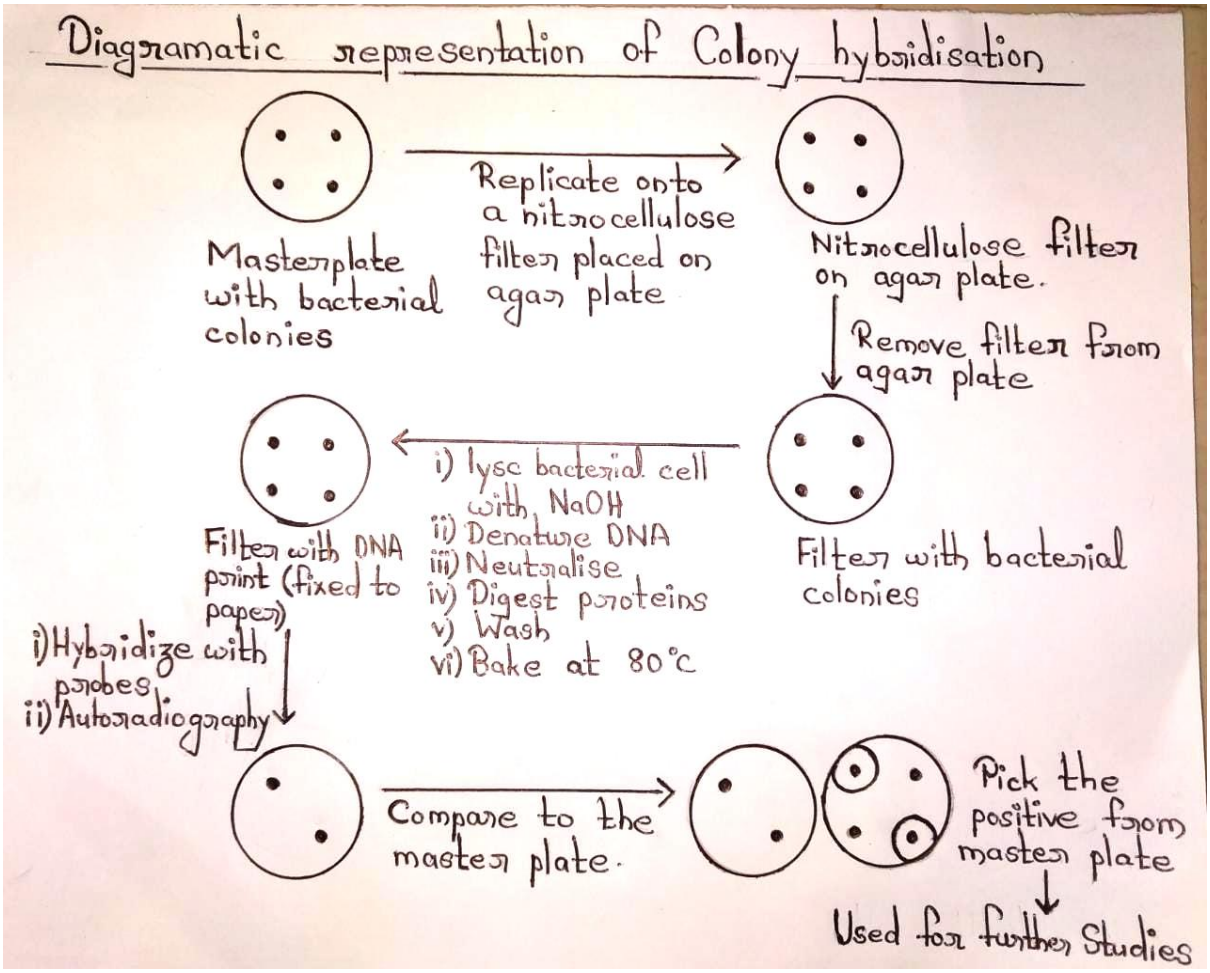
- Probe represents the sequence of DNA segment used for transformation.
- Probe can be labelled nonradioactively too.
- Unhybridized probes removed by repeated washing.

8) Colonies whose DNA hybridizes with the probe are detected by autoradiography.

9) Positions of colonies showing up in autoradiography are compared with master plate to identify colonies.

- Colonies showing positive radiograph are picked from the master plate and used for further studies.

Diagrammatic representation of Colony hybridization:



PLAQUE HYBRIDIZATION- is a variation of colony hybridization. In this method, nitrocellulose filter is applied to the surface of the agar plate so filter comes in

direct contact with the plaques. In 1977, W. D. Benton and R. W. Davis devised this technique for screening of bacteriophages from plaque forming units.

Procedure:

1) Lawn of *E.coli* is prepared.

2) Phage particles (with foreign DNA) are plated on bacterial lawn.

3) Incubated for 6-8 hours.

4) Plate is cooled at 4 degree celcius for one hour to stiffen the agar.

5) Nitrocellulose sheet is now placed on agar surface and left for 30 seconds to minutes.

6) Reference marks made both on filter as well as plate.

7) Both phage particles and unpackaged recombinant phage DNA from plaques will stick to the filter.

8) Nitrocellulose filter then lifted carefully and placed on the filter paper soaked in alkali to denature DNA.

9) Plate stored at 4 degree celsius for later isolation of desired recombinant phage.

10) Filter neutralized by transferring it in neutral buffer.

11) Filter baked at 80 degree celsius to fix DNA.

12) Hybridization with labeled nucleic acids (probes) allows detection of plaques having desired recombinant DNA.

13) Wash unhybridized probes with saline and use hybridized DNA for studies.

14) Widely used procedure for library screening.