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Chapter 17

Replica plating technique for identifying *E. Coli* auxotrophic mutants: Principles, methods, and application

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1. Introduction:

Replica plating is a key microbiological method for replicating microbial colonies onto secondary Petri plates, enabling efficient phenotype screening. Colonies are transferred from a primary (master) plate to secondary plates with selective growth media, preserving their spatial arrangement for comparative analysis (Lederberg and Lederberg, 1952; Kageyama et al., 2011; Reddy et al., 2019). A velveteen-covered disk picks up colonies from the primary plate and imprints them onto the secondary plates. This technique is used to screen for phenotypes such as auxotrophy and antibiotic resistance. For instance, a missing colony on a secondary plate indicates sensitivity to a substance in the selective media, aiding in identifying specific microbial traits (Davis, 1950; Hall, 1982; Tripathi et al., 2013; Tripathi et al., 2018).

1.1. Utilization in Negative Screening:

Replica plating is used for negative screening to select colonies sensitive to specific substances. For example, transferring colonies from a primary plate to an ampicillin-containing plate isolates ampicillin-sensitive colony. These colonies die on the selective plate, but their presence is inferred from their positions on the primary plate.

1.2. Significance in Experimental Rigor:

Replica plating enhances experimental rigor by offering insights into the viability and age of cells on the original plate. This method improves the reliability of outcomes, particularly in evaluating microbial responses to selective pressures, ensuring accurate and consistent results in phenotype screening. Essential Materials for Replica Plating Experimentation.

2. Materials required:

1. *E. Coli* XL-1 blue primary cultures: Essential for starting the experiment.
2. Luria Broth (LB)
3. LB-agar media: Solution A and Solution B, which are minimal media:
4. Solutions of amino acids (1 mg/mL): contains the essential amino acids leucine, lysine, tryptophan, and histidine for the growth and metabolism of microorganisms.
5. Ampicillin 50 µg/mL solution
6. Laminar flow
7. Spectrophotometer
8. Incubator shaker:
9. Static incubation
10. Petri dishes
11. Conical Flasks
12. Velvet fabric: Micropipettes: Accurate tools for delivering tiny amounts of liquid.
13. Toothpicks

3. Procedure:

To conduct a replica plating experiment efficiently and accurately, follow these meticulous steps:

3.1. Initiation of Primary Culture:

Start by inoculating 1% (v/v) of an overnight culture of *E. coli* XL-1 Blue into fresh medium to start a primary culture.

3.2. Centrifuge Tube Preparation:

When the culture is at an optical density (O.D.) of 0.6–0.7 at 600 nm, aliquot 200 µL of the culture into 16 centrifuge tubes: 8 tubes A1 to A8 and 8 tubes B1 to B8.

3.3. UV Exposure:

Put the tubes designated as A (A1 to A8) under UV radiation for 30 minutes and the tubes designated as B (B1 to B8) under UV radiation for 20 minutes.

3.4. Labeling LB Agar Plates:

Mark two LB agar plates A and B. The plates must be divided into 8 sections and marked as A1 to A8 on plate A and B1 to B8 on plate B.

3.5. Inoculation of Bacterial Culture:

Streak the bacterial culture from each centrifuge tube onto the correspond in section on the plates using autoclaved toothpicks.

3.6. Incubation:

Incubate the plates overnight at 37°C to allow colony growth.

3.7. Grid Marking on LB Agar Plates:

Mark two new LB agar plates as A and B respectively, dividing them into 5 × 5 grids labeled A1 to A25.

3.8. Colony Picking:
Pick single colonies from sections A1, A2, etc., and place them on the corresponding cells in the grid on both plates.

3.9. Repeat Colony Picking:
Repeat the colony picking process on the second plate.

3.10. Incubation:
Incubate the plates overnight at 37°C to facilitate colony growth.

3.11. Master Plate Formation:
Obtain colonies on each cell of the grid the next day, resulting in the formation of master plates.

3.12. Preparation of Minimal Media Plates:
Prepare six types of minimal media plates: Control, Leu+, His+, Lys+, Trp+, and Amp+, each supplemented with the respective amino acid or antibiotic.

3.13. Replica Plating:
Utilizing an autoclaved velvet cloth wrapped around the base of a conical flask as a stamp, pick up a replica of the master plate and stamp it onto all six minimal media plates.

3.14. Incubation:
Incubate the plates overnight at 37°C to promote microbial growth.

3.15. Observation:
Examine the plates the following day for the presence of mutants, noting any variations or unique phenotypes observed. By meticulously adhering to these steps, the replica plating experiment can yield valuable insights into microbial behavior and genetic variation.

4. Precautions:
1. Complete every step in an aseptic manner.
2. Because the cloth's fibers function as an inoculation needle, the velvet side should be placed up against the master plate.
3. Sunlight exposure must be prevented at all costs.

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