

## Microbial Genetics

### Third Step

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### Lab.6

#### Identifying Mutants (Replica Plating)

**Replica plating** is a microbiological technique in which one or more secondary Petri plates containing different solid (agar -based) selective growth media are inoculated with the same colonies of microorganisms from a primary plate (or master dish), reproducing the original spatial pattern of colonies.

With bacteria mutants can be identified more easily, because bacteria produce very large populations very quickly.

Mutant can be identify by 2 methods:-

- 1- Positive (direct) selection involves the selection of mutant cells and rejection of non mutant cells. For example:  
plating out bacteria on a medium containing penicillin. Survivors, which are penicillin - resistant mutants, can be isolated.

- 2- Negative (indirect) selection or replica plating:

This technique has been discovered by Esther Lederberg & her husband, Joshua Lederberg in 1951. It is used for detect, for example, auxotrophs that have nutritional requirements not possessed by the parent (non mutant) cell. To isolate auxotrophs, colonies growing on a master plate containing a complete medium can be transferred by a sterile velvet pad is pressed onto the master plate, and the colonies are transferred simultaneously to a minimal medium, which lacks essential nutrients such as the required amino acid. An auxotrophic mutant will fail to appear on the minimal medium.

### **Principle :-**

Replica plating involves creation of exact copy of master plate( exact number and exact location of each colony). The exact copy of master plate can be created with the help of sterile velvet leather, cotton or chamois leather stamp or clothes. These clothes or stamp acts as microneedle for inoculation of microbes when pressed against master plate bacterial culture. Impressing a petri plate on to the velveteen. Organisms will be transferred from their positions on the fabric pile to the nutrient medium in the plate. then we can transfer these colonies to another media (plate) to investigate mutants.

### **Materials:-**

Sterile velvet Master plate(isolated colonies of *Salmonella typhimurium* growing on Loria agar contain histidine ( $\text{his}^+$ ). plate of Loria agar or nutrient agar with histidine (control plate: which is an exact copy of the master plate) plate of Loria agar or nutrient agar without histidine ( test plate :by which you will check the presence(+) or absence(-) the mutant .

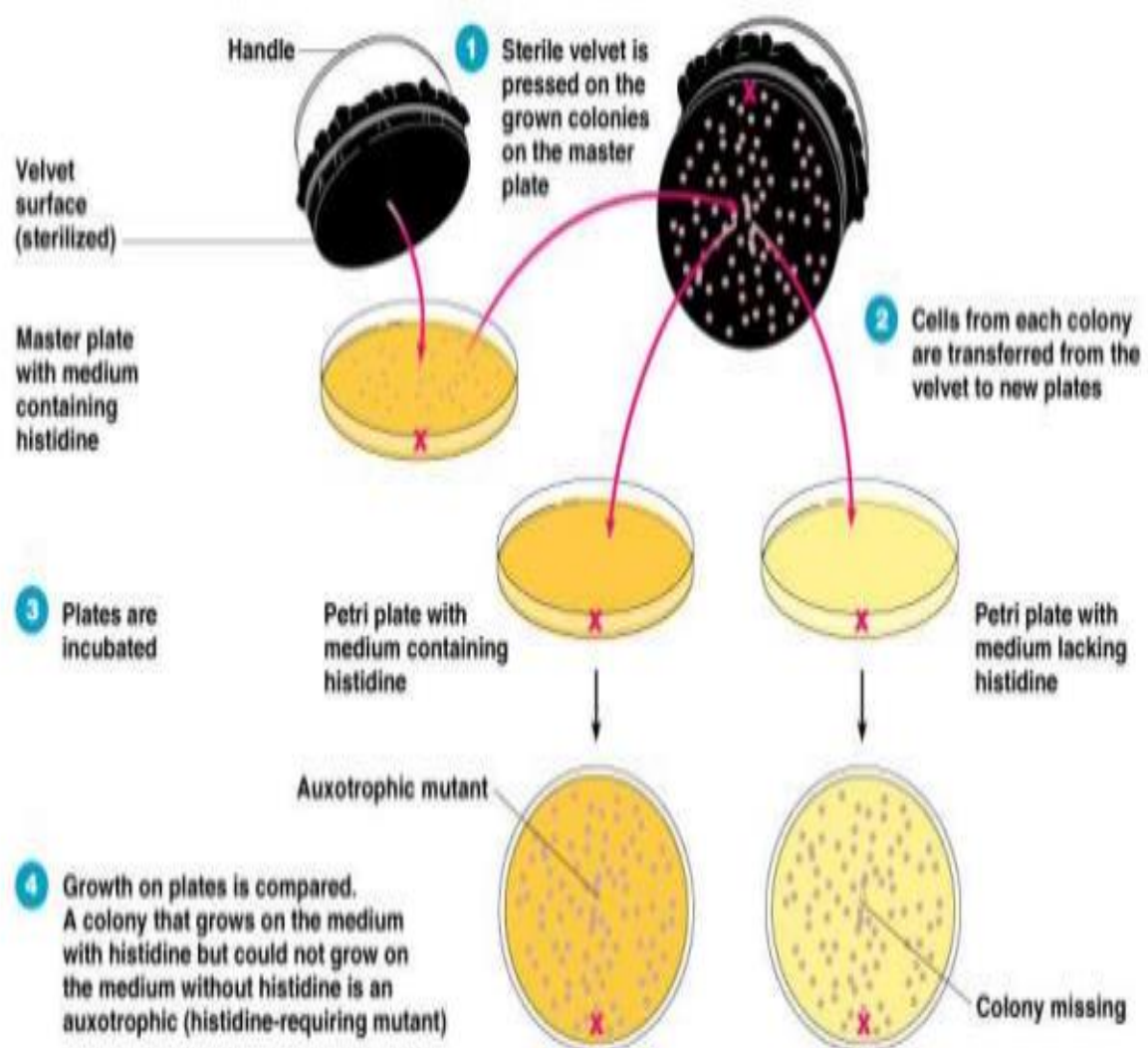
### **Procedure :-**

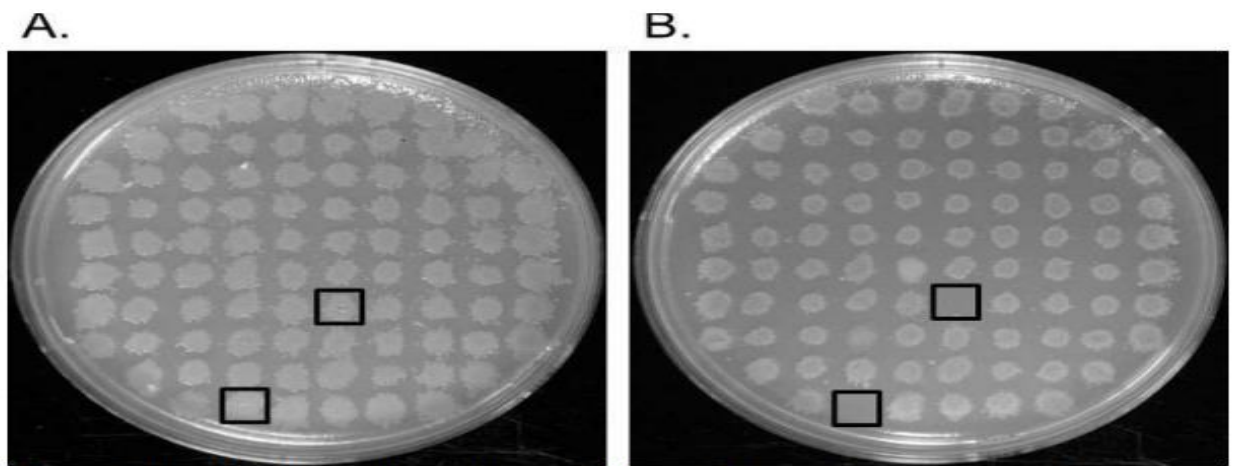
- 1- Mount a piece of sterile velvet by stretching it on a cylindrical metallic block (slightly smaller than Petri dish).



- 2- Place the block with velvet side facing upwards.

- 3- Invert the master plate with bacterial cells growing on media containing histidine. and gently press against the velvet. The number of projecting fibres of the velvet act as inoculating needles sampling every clone of the cells on the agar.
- 4- Remove the master plate.
- 5- Now take new plate of Luria agar with histidine (control plate) and place it inverted on the stamp. press it gently and then remove it.
- 6- Repeat step5 using new plate of Luria agar without histidine (test plate).
- 7- Incubate the plates in incubator 37°C for 24hr.
- 8- Examine the number and location of each growing colony and compare them to the colonies on the master plate.
- 9- colony that appears on the control plate( a copy of master plate) and disappear from the test plate represent mutant (auxotroph) bacteria and the replica test is positive(+).





**Figure 3:** Reading the results of Replica plating technique .A: Control plate( Replica test negative). B: Test plate (Replica test positive).