

BASIC TECHNIQUES FOR BACTERIAL GENETICS.

A. Streaking Plates.

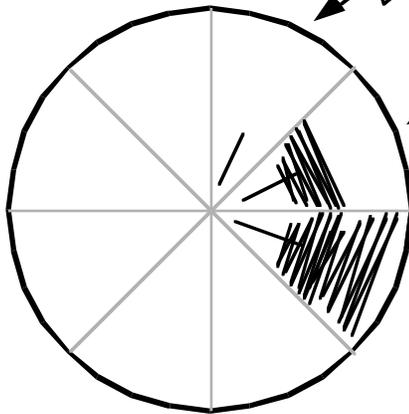
When picking and streaking lots of bacterial colonies it is often quicker to use sterile toothpicks and sticks instead of using a wire loop that must be sterilized between each colony by passing it through a flame. Using the technique shown below, eight colonies can be streaked for isolation on a single plate. (Save the used toothpicks and sticks to be reautoclaved.)

(1) Use a sharpie to draw sectors on the bottom of an agar plate. Follow steps 2-4 for each colony to be streaked out.

(2) Pick bacteria with sharp toothpick and make a single line in center of plate sector.

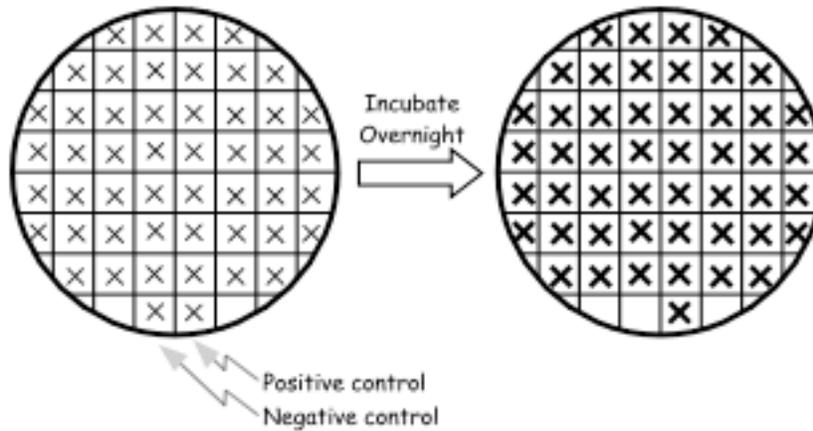
(3) Streak from the middle of the line with the edge of a sterile stick. Keep streak closely spaced and stay within border of plate sector.

(4) Using another sterile stick, streak out from the middle of the previous streak. Keep streak closely spaced and stay within border of plate sector.



B. Picking and patching colonies.

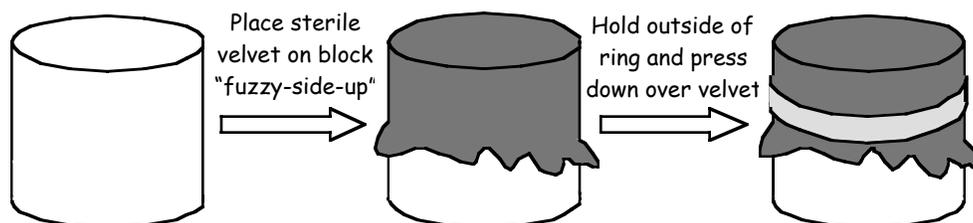
When checking out bacterial strains, it is often useful to patch many colonies on a single petri plate so they can be tested simultaneously by replica printing onto a series of media. To do this, place a fresh master plate over a "patching grid". (A replica patching grid is supplied at the back of the manual). Touch the top of each colony with a sterile toothpick and draw a small x on the new master plate. (Only use each toothpick once. Save the used toothpicks to be reautoclaved). Many patches can be placed on a single plate. After patching, incubate the plate overnight to let the patches grow up. The next day this plate can be used as a master for replica-printing. Always mark each plate at the top of the patch grid since the patch grids are symmetrical.



C. Replica printing.

This technique transfers cells from an array of colonies (or patches) on one plate, to a series of "replica" plates. Thus, each of the replica plates is inoculated by cells in the same arrangement as on the original ("master") plate. The transfer is done as follows:

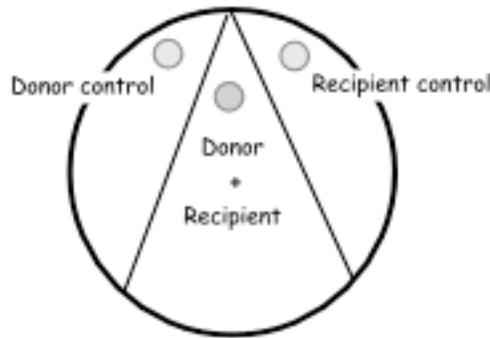
1. Mark the top of the master plate and each replica plate. Always make the last plate replicated a control that all the colonies can grow on. This insures that failure of a colony to grow is not simply due to inefficient transfer.
2. Place a sterile velvet over the replica-printing block (fuzzy side up) and push a ring down over the sides of the block.



3. Press a plate containing an array of bacterial colonies (or patches) onto the surface of the sterile velvet. Press just hard enough that the fabric pattern become visible in the agar. Carefully lift the plate straight up and remove it to avoid smudging the print. Most of the cells on the plate will be transferred to the surface of the velvet.
4. Once a replica of the master plate has been formed on the velvet, press each of the fresh plates to be inoculated onto the surface of the velvet which carries cells. Many replicas can be made from a single master. (Save the velvets. If the velvets are washed and autoclaved, they can be used many times.)

D. Plate matings.

1. Grow fresh overnight cultures of the donor (F+) and recipient (F-) strains. (If the F-factor is unstable it may be necessary to grow the donor in selective media. In addition, if the F-factor is temperature sensitive then the donor must be grown at 30°C.)
2. Plate on a medium that selects for the phenotype of the exconjugants. Divide the plate into 3 sections.



3. Place a small drop of the donor and recipient cultures on the plate as shown above. In the center section place the drops of the donor and recipient directly on top of each other. Allow the drops to dry onto the plate.
4. With a sterile stick, streak the plate from the drops out. (Save the used sticks to be reautoclaved.)
5. Incubate 1-2 days.

E. Spreading plates

It is often necessary to spread a small amount of a supplement on the surface of a plate. This is done by using a spreader (a glass rod or Pasteur pipet bent to form an equilateral triangle which should be about 2/3 the diameter of the petri dish). Immediately prior to use, dip the spreader in a beaker of ethanol then pass it through a bunsen flame to ignite the alcohol. (Do not leave the spreader in the flame so long that it heats up). After the alcohol has "burned off", thoroughly spread the liquid on the surface of the plate until it is uniformly covered.