

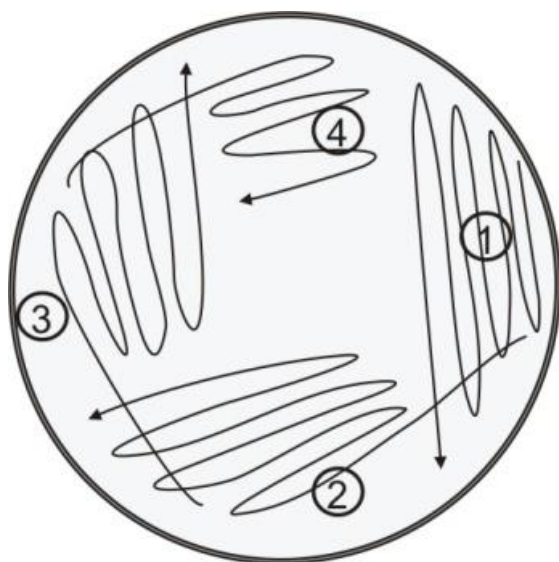
Choose colonies and label using these Colony Labeling Instructions

First, choose and clearly label your 5 mutant colonies with a unique code that we'll use to track it for the remainder of our research. The code will tell us from which ancestor the colony was derived, whether it is a derived (mutant) or ancestral colony type, and then given each derived colony a unique number code. The formula is GroupNumber-Mutant or Ancestor- Unique2DigitNumber. Example: A2-M-04 or B4-A-01.

NOTE: If your mutant transformation plates do not contain 5 colonies, discuss your options with the TA. If you don't have any colonies, you will process colonies from another group with overage, and be responsible for those colonies for the remainder of the project. Be careful to label with the other group's ID code and record those numbers in your notebooks. Also, the ancestor should you sequence should match the mutants you are working with.

Streak-planting for colony isolation and storage

1. Obtain an LB agar plate with Carbenicillin and IPTG for each sample.
2. Label the bottom of the plate along the edge with team number (or your initials), plasmid name and plate type.
3. Sterilize your bench with 70% ethanol and wiping it down with a paper towel.
4. Circle 5 colonies which you are most interested in. Label them according to the colony labeling instructions.
5. Using a sterile toothpick, touch one of the circled colonies and to pick up a tiny amount of cells. (*You may not even be able to observe the colony changed shaped with the naked eye)
6. Gently spread the bacteria over a section of the plate, as shown in the diagram below, to create streak #1.
7. Using a fresh, sterile toothpick, drag through streak #1 and spread over the second section to create streak #2
8. Using a third and fourth fresh, sterile toothpick to create streak #3 and #4.
9. Stack plates upside down into the metal petri dish holder and incubate at room temperature.



<https://ecurrent.fit.edu/blog/panther-voices/isolate-bacterial-colonies-streak-plate-method/#prettyPhoto>



Streak plate of Legionella allows the researcher to isolate individual colonies, each representing a single clonal lineage or genotype. Image credit: CDC/James Gathany - CDC [Public Health Image Library](#) (ID#: 79).