

SEED 2009

## Day 2: Colony Streaking

**Learning Objective:** This exercise will teach sterile technique and single-colony isolation. The lab will familiarize you with one of most important tools in molecular biology, antibiotic selection. It will also demonstrate the importance of careful labeling.

### Background: The Importance of Selection

In molecular biology, we cannot practically see and isolate individual microbes. But since we want to make changes to organisms, it is essential that we have a way to separate the microbes we want from those we do not. This is done by a technique known as **selection**. Selection provides a way in which microbes can be selected by their characteristics (**phenotype**). There are several common ways in which selection is practiced which mainly center around an organism's ability to tolerate a substance in its surroundings or live without a vital nutrient. The most common are **antibiotic resistance** and **auxotrophy**. The former depends on the ability of a microbe to survive the effects of an antibiotic in the growth medium, where the latter is a microbe's inability to grow without a nutrient supplement (such as a vitamin or an amino acid). These **selection markers** are used to in conditions such that only microbes which have been given the ability to tolerate the presence or absence of a compound can survive and reproduce. However, these traits are not entirely unique and so a molecular biologist must exercise proper **sterile technique** to avoid contamination. Additionally, using the concept of **monoclonal colonies** allows the scientist to be sure that the microbes being used are all of the same **genotype**.

**The exercise:** You will be working with three different cell types or strains. They each have a particular antibiotic resistance (ampicillin, kanamycin, or chloramphenicol) and color (red, green, or no color fluorescence) phenotype. Your job will be to determine the resistance and color phenotypes of unknown bacterial cultures (which will be some combination of the original three cell types).

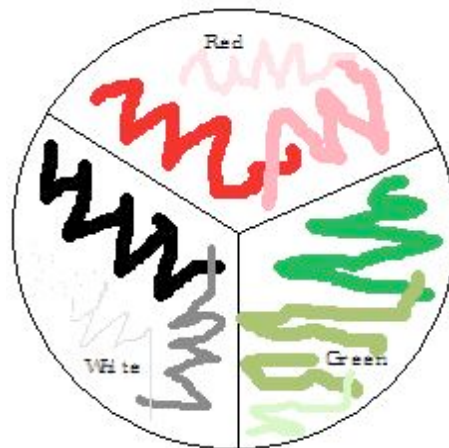
**Materials:**

- 2 LB-Ampicillin plates (Blue Mark)
- 2 LB-Kanamycin plates (Green Mark)
- 2 LB-Chloramphenicol plates (Black Mark)
- 6 LB plates
- 3 "standard" bacterial cultures (in 15mL tubes)
- 4 "unknown" bacterial cultures (in 15 mL tubes)
- Sterile toothpicks and wooden sticks
- Lab Pen
- Straight Edge

## Protocol

### Part I—Identity of Standards

1. Take one of each an LB, LB-Amp, -Kan, and -Chl plate; split the plate into three sections using a marker (hint: think about where it makes most sense to make your markings!)
2. Label the sections of the plate with the names of the known “standard” strain phenotypes (Red, Green, White)
3. Streak each “standard” bacterial culture onto one section of each of the three plates.
  - a. To streak to single colonies, use a **sterile** toothpick or wooden stick. Dip the end in the culture medium.
  - b. Move the stick across the plate in a zig-zag fashion being careful not to cross back over your path. After a good distance, discard the used stick.
  - c. With a new sterile stick, carefully draw the stick across the very end of the zig-zag and start a new zig-zag. It is important that you only cross the old zig-zag at the end because you are trying to spread out the individual bacteria.
  - d. Repeat step c with a new sterile stick



### Part II—Composition of Unknowns

1. Streak each “unknown” bacterial culture onto its own LB plate (no antibiotic). **\*\*The goal is to get *single colonies!*\*\*** (think about what the results of your experiment may look like otherwise!)  
Make sure you label your plate
2. Take the other LB-Amp, -Kan, and -Chl plates; split them into four sections and label them
3. Streak each “unknown” bacterial culture onto one section of each of the three plates
4. Repeat on “Extra Credit” Sample if time allows

Part III—Sources of Contamination

1. Divide an LB plate into four sections and label them
2. Using a sterile toothpick, scrape the inside of your cheek. Use the toothpick to streak one section of the LB plate.
3. Test other sources of contamination. Some ideas to try are: your hands and fingers, your pipette, your pen, and tap water.

Put all of the plates (labeled with your group members initials) in the 37C incubator

By next week, you should be able to fill in the following tables with check marks...

	<b>Kan<sup>R</sup></b>	<b>Cam<sup>R</sup></b>	<b>Amp<sup>R</sup></b>
<b>Red</b>			
<b>Green</b>			
<b>White</b>			

	<b>Mixed Culture #1</b>	<b>Mixed Culture #2</b>	<b>Mixed Culture #3</b>	<b>Mixed Culture #4</b>
<b>Red</b>				
<b>Green</b>				
<b>White</b>				

...and this table with numbers...

	<b>Mixed Culture #1</b>	<b>Mixed Culture #2</b>	<b>Mixed Culture #3</b>	<b>Mixed Culture #4</b>
<b>Number of Red Colonies</b>				
<b>Number of Green Colonies</b>				
<b>Number of White Colonies</b>				
<b>Total Number of Colonies</b>				
<b>Fraction of Red Colonies</b>				
<b>Fraction of Green Colonies</b>				
<b>Fraction White Colonies</b>				