Protocol: Basic Skills

A. Streaking a Plate and Sterile Technique

In this exercise, we will take bacteria from liquid culture and test their ability to grow on different petri plates. One of the bacterial strains contains a drug resistance plasmid for Ampicillin resistance and the other contains a drug resistance plasmid for Kanamycin resistance, while the third does not contain a resistance plasmid. Each student should also streak for single colonies on one petri plate. On the other plates, simply make a squiggle of culture on the plate.

Before you begin, which plates from the table below do you predict will contain growth? Mark the Table with a "+" or "-".

Each group of three or four students:

	Bacterial Strain/Plasmid:		
Petri Plate:	MM294/pAMP	MM294/pKAN	MM294
LB + Amp (2 red lines)	Expt. (streak plate)*	?	?
LB + Kan (1 green line)	?	Expt. (streak plate)*	?
LB only (black or none)	_____? control	_____? control	_____? control (streak plate)*

*Each member of the group should streak one plate for single colonies, using the suggested streak pattern.

Label the petri plates properly: Label the bottom of a petri plate (the half with the agar). Remember to label before you inoculate. Label along the outside edge so you can see the colonies in the middle. Write your lab day, initials of your group, and the strain of bacteria that you put on the plate.

B. Measurements and Micropipetting

Each student will practice with the small and large volume Micropipettors. Do the exercises outlined below. You should do *at least two* exercises with each pipettor size. Check your volumes to be sure they add up to the correct total. Take as much time as you need to learn; this is important for everything we do.

Exercise using the P1000

Tube	**Sol. I**	**Sol. II**	**Sol. III**	**Sol. IV**	**TOTAL**
A	350 uL	250 uL	400 uL	---------	1000 uL
B	220 uL	280 uL	270 uL	230 uL	1000 uL
C	250 uL	350 uL	200 uL		900 uL

Exercises using the P200

Tube	Sol. I	Sol. II	Sol. III	Sol. IV	TOTAL
D	25 uL	100 uL	45 uL	30 uL	200 uL
E	60 uL	30 uL	70 uL	40 uL	200 uL
F	50 uL	30 uL	45 uL	25 uL	150 uL

Exercises using the P20

Tube	Sol. I	Sol. II	Sol. III	Sol. IV	TOTAL
G	2.5 uL	7 uL	4.5 uL	6 uL	20 uL
H	6 uL	3 uL	4 uL	7 uL	20 uL
I	3 uL	2.5 uL	4 uL	5.5 uL	15 uL

When you are confident that you can accurately use the P20, then bring one of the completed tubes G or H and your P20 and demonstrate to the instructor.

C. Set Up a Restriction Digest

You may have the opportunity to set up a restriction digest for next week's lab. The Instructor will help you complete the list below:

Plasmid DNA _____

Restriction Enzyme _____

10x Buffer _____

water _____

HINT: to set up the digest, add water, 10X buffer, and plasmid DNA. Always add restriction enzyme last! Spin the eppendorf tube briefly in the microfuge (pulse) to pool ingredients on the bottom of the tube. Be sure your initials are legible on the top and the side of the tube. Put the tube into a floating rack in the 37°C water bath.