Name _____ **Instructor** _____ **Lab Section**_____

Objectives: To gain a better understanding of:	**Background material may be found in**
• Use of Bacteria in Biotechnology	• Chapter: 10.1-10.16, 10.22 – 10.23
• DNA & Plasmid Structure and Function	• Chapter: 11.1-11.3
• Fundamental Biotechnology Techniques	• Chapter: 12.1-12.3, 12.6 - 12.13
	Biology: Concepts and Connections, 8th ed.

Biotechnology is a term that applies to a range of biological techniques that use molecules and cells to make medicines, foods and other products that are useful to humans. In 1953, James Watson and Francis Crick published their discovery of the DNA (deoxyribonucleic acid) double helix and the first accurate description of the fundamental structure of DNA. In less than 50 years, science leapt from the discovery that DNA contains genetic information to the ability to effectively manipulate the size or behavior of laboratory animals by altering their genetic makeup. Scientists and doctors are now applying human gene therapies in hopes of curing cancer and treating AIDS. The criminal justice system is now using DNA fingerprinting to identify criminals in cases of rape and murder. It is the universality of the genetic code in organisms as diverse as viruses, bacteria, plants, and animals that provide some of the most convincing evidence supporting the theory of evolution. The universal nature of DNA has also allowed information obtained from studies of organisms such as yeast and fruit flies to be applied to humans. In the following exercises you will employ some of the most basic molecular techniques used in laboratories around the world.

♦ THE USE OF MICROPIPETTORS

A micropipettor is a device used to accurately measure and distribute small volumes of solutions for a variety of molecular biology techniques. The following exercise is designed to familiarize you with the proper and accurate use of a micropipettor. Mastery with this tool is essential to the success of your upcoming biotechnology experiments.

DIRECTIONS FOR USING MICROPIPETTORS

Your instructor will review these instructions prior to the beginning the lab exercises.

CAUTIONS WITH REGARD TO THE USE OF THE MICROPIPETTORS

- **Set pipette volume only within the range specified for that micropipettor.** Do not attempt to set a volume beyond the pipet's minimum or maximum values.
- **When using a micropipettor, first apply a tip**. Forgetting to do this would cause liquid leakage into the barrel. Since a micropipettor works by air displacement, its internal mechanism must remain dry.
- **Never flame the micropipettor tip**. It is made of plastic and would melt.
- **Always keep a micropipettor in a vertical position when there is fluid in the tip.** Do not allow liquid to accidentally run back into the barrel of the micropipettor.
- **Use your thumb to control the speed at which the plunger rises after taking up or ejecting fluid. Release the plunger slowly**. Releasing the plunger too quickly will cause liquid to be sucked into the barrel.

SETTING AND USING THE MICROPIPETTOR

1. **Check that you have the correct micropipettor for the volume you are measuring. There are <u>three different sizes</u> in the lab:**
   - a "**P-20**" is used for volumes that range from **1 to 20 µL,**
   - a "**P-200**" is used for volumes that range from **10-200 µL,**
   - a "**P-1000**" is used for volumes that range from **100-1000 µL.**

2. **Dial in the desired volume. <u>Do you understand how to read the scale? *If not -- ASK!*</u>**

3. Push the end of the pipet into the proper-size tip. **The small tips are for P-20's and P-200's; the larger tips are for P-1000's.**

4. **Micropipettors have a two-position plunger with friction stops**. **Depressing the plunger to the first stop measures the desired volume**. **Depressing the plunger to the second stop introduces an additional volume of air meant to blow out any solution remaining in the tip**

♦ PIPETTING SMALL VOLUMES

In our experiments today you will be working with extremely small volumes. It will be useful to first become comfortable with measuring such small volumes.

❑ PRACTICING WITH THE P-20

1. Obtain a piece of wax paper.

2. In your reaction tube rack (small brightly colored rack) you will find a small tube of colored water.

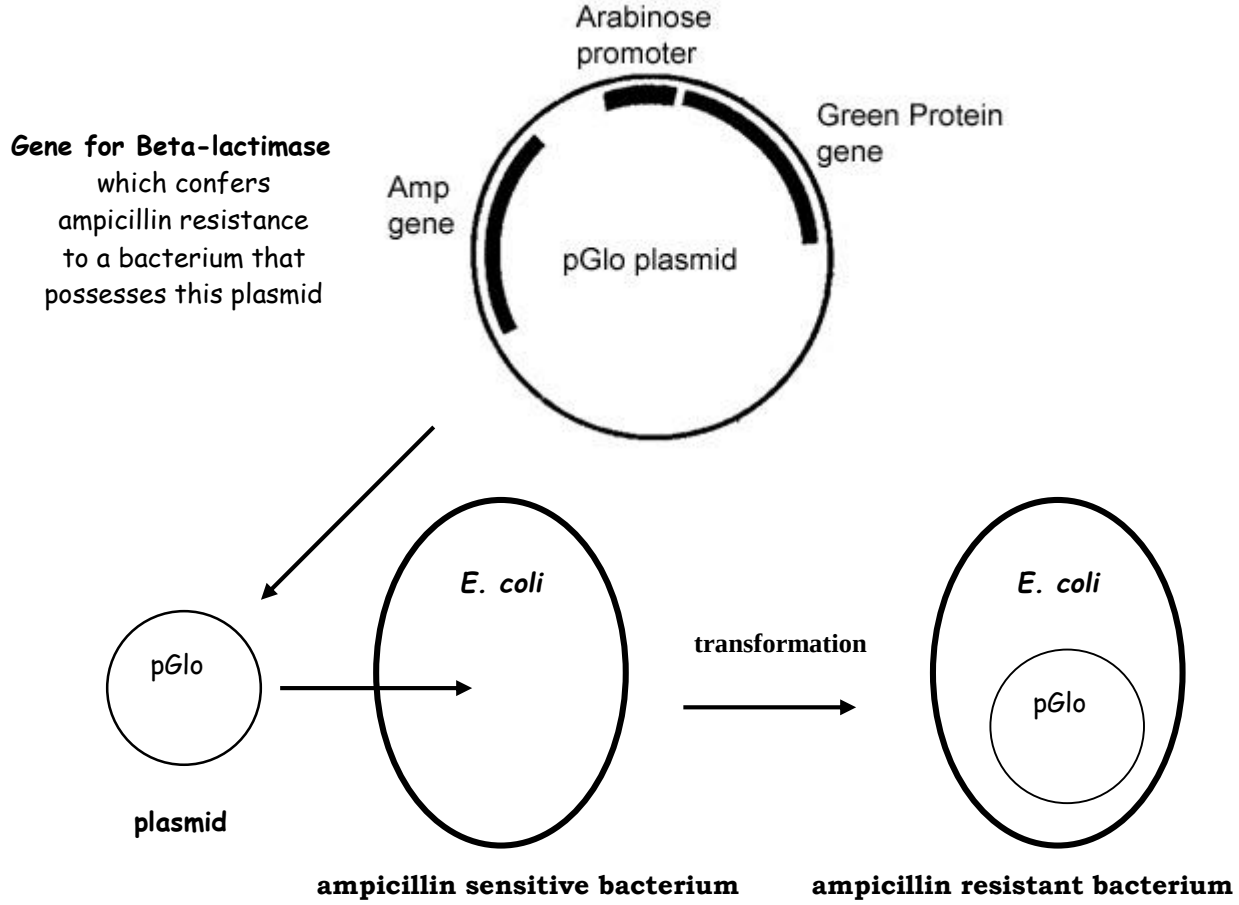
3. Dial the P-20 micropipettor to 10 µL, then draw up 10 µL of water. Look at the pipet tip and note how small this volume is.

4. Now eject the water in the pipet on to the wax paper to form one small spot. Again note the small size of the spot.

5. Repeat this procedure with volumes of 8, 6, 4, 2, and 1 µL. There should be a total of 6 spots on the paper.

♦ TRANSFORMATION OF *E. COLI* WITH PLASMID DNA

Transformation is a process in which cells take up foreign DNA from their environment. Under proper conditions, a cell that is incubated with **plasmid DNA** can absorb the plasmid into its cytoplasm. A plasmid is a small ring of DNA, separate from the chromosome that is often found in bacteria and yeast. Plasmids can function as vectors to shuttle genes into and out of cells. Expression of genes on the plasmid will then alter, or "transform", the phenotype of the cell. Cells that take up the plasmid are called **transformants**. Transformation can occur in either prokaryotic or eukaryotic cells. In today's experiment, we will transform a harmless enteric bacterium, ***E. coli***, with the **pGlo plasmid**.



Transformation of cells with DNA is a very inefficient process. Only a small fraction of cells in a liquid culture will uptake DNA and become transformed (1 out of 100,000 on average). Because our goal is to isolate the transformants, we need a way to separate the few transformants from the majority of untransformed cells. This is accomplished through a process called **selection**.

The most common means of selection occurs through the use of **antibiotics**. As a rule, plasmids contain genes that confer antibiotic resistance to their host cell. For example, *E. coli* is sensitive to (killed by) an antibiotic called **ampicillin**. Ampicillin interferes with the formation of bacterial cell walls and thus kills newly divided cells that must form new cell walls. The pGlo plasmid contains an **ampicillin resistance gene**. This gene encodes an enzyme, β lactimase, which enzymatically degrades ampicillin. Therefore, bacteria that take up the plasmid (transformants) become resistant to ampicillin. ***When a culture of bacteria, containing both transformants and untransformed cells, is exposed to ampicillin, the transformants survive, but the untransformed cells die.*** This produces the desired result of isolating transformants away from untransformed cells.

The pGlo plasmid also contains the **Green Protein Gene.** This gene, which is derived from a jellyfish called *Aequorea victoria*, encodes a fluorescent protein that glows green when exposed to ultraviolet light. The pGlo plasmid also contains the **arabinose promoter**. A promoter is a short region of DNA that regulates the expression of a gene. In other words, the promoter determines whether the gene will be turned on or off. The arabinose promoter controls the expression of the green gene in the pGlo plasmid. When arabinose (a simple sugar) is present in the growth medium, the gene is expressed. However, when arabinose is absent from the growth medium the green protein gene is not expressed.

After completion of the selection process, you will be asked to analyze the results of the transformation experiment. In other words, did you successfully transform *E. coli* with the pGlo plasmid? You will also be asked to analyze the expression of the green protein gene by exposing your cultures to UV light. Proper analysis requires the use of both positive and negative controls in the experiment. Positive and negative controls are reactions carried out under controlled conditions that tell us whether or not the experiment is working and provide a reference for comparison with the experimental reaction. The nature of the controls for this experiment will become clear as you proceed.

♦ TRANSFORMATION PROCEDURE

1. Label one 1.5 ml plastic tube as **pGlo**. Label a second 1.5 ml tube as **control**.

2. Using a p1000, add **250 µL cold $CaCl_2$ solution** to each tube. **Place tubes on ice.**

3. Using a sterile inoculating loop, transfer four large colonies (or a large clump) of *E. coli* into the tube labeled **pGlo** as demonstrated by your instructor.

4. Immediately resuspend the cells by pipeting up and down with the p200 micropipettor. **Continue pipeting until you obtain a uniform suspension** (no clumps).

5. Return the tube to the ice. Do one tube at a time because if the solution sits, $CaCl_2$ will precipitate onto the bacteria and make it difficult to re-suspend.

6. Using a sterile inoculating loop, transfer four large colonies (or a large clump) of *E. coli* into the tube labeled **control**.

7. Immediately resuspend the cells by pipeting up and down with the p200 micropipettor. **Continue pipeting until you obtain a uniform suspension** (no clumps).

8. Return the tube to the ice.

9. Using a p20 micropipettor with a new sterile tip, add 10 µL of the **DNA** to the tube labeled **pGlo**. Mix by gently tapping the tube with your finger. Return the tube to the ice.

10. **Incubate both the pGLO tube and the control tube on ice for 15 minutes**.

11. Use the time of this incubation period to label your LB agar plates. Obtain **two LB-Amp plates**. Label one as **#1 pGlo**. Label the other **as #3 control**. Label both with your name and the date. LB stands for Luria-Bertani medium which is a special nutrient solution (food supply) for the bacteria. These two plates contain the antibiotic drug, ampicillin.

12. Obtain **one LB-amp-arabinose plate** and label it as **#2 pGlo**. Label with your name and the date.

13. Obtain **one LB plate** (it contains neither ampicillin nor arabinose) and label it as **#4 control**. Label with your name and the date.

14. Heat shock the cells by placing both the **pGLO tube and the control tube** into a 43^0C water bath for 90 seconds. Just dip the base of the tubes in the water without completely submerging them.

15. Return the tube to ice. Incubate on ice for <u>one</u> minute.

16. Place tubes in a rack at room temperature.

17. Using your p1000 and a clean (sterile) tip, add 250 µL of Nutrient broth to each tube. Mix gently by tapping the tube with your finger.

18. Incubate the tubes at room temperature for 15 minutes.

19. Obtain a glass spreader and beaker of ethanol.

20. Now it is time to spread the bacterial cultures on the nutrient agar plates. You must work carefully (that is, apply sterile technique) so that you do not accidentally introduce unwanted microorganisms to your plates.

    The general procedure is as follows.
    a. First sterilize your glass spreader by dipping it in alcohol and passing it over the flame of your Bunsen burner.
    b. Then, using the P-200, pipet 100 µL of a cell suspension on to an agar plate.

    Produce the following 4 plates:
    Plate #1 100 µL of **cells from the pGlo tube** on the LB-Amp plate labeled **#1 pGlo**.
    Plate #2 100 µL of **cells from the pGlo tube** on the LB-Amp-arabinose plate labeled **#2 pGlo**
    Plate #3 100 µL of **cells from the Control tube** on the LB-Amp plate labeled **#3 control**.
    Plate #4 100 µL of **cells from the Control tube** on the LB plate labeled **#4 control**.

    c. Quickly spread the cell suspension across the plate until the cells are uniformly distributed across the surface of the agar. Move quickly, but gently. Do not tear the surface of the agar.
    d. Return the glass spreader to the alcohol. Flame the glass spreader again before setting it on the bench.
    e. In order to avoid cross contamination of your samples you must use a clean pipet tip for each new culture.

21. Incubate the plates at room temperature for 15 minutes.

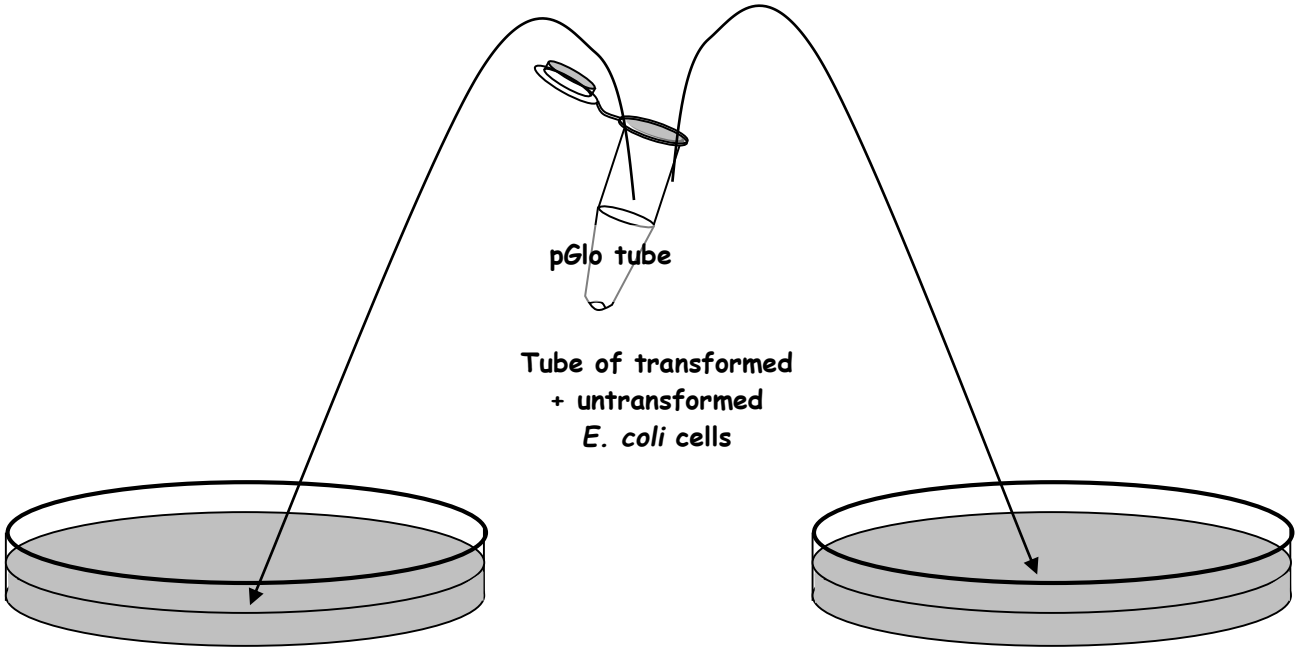
22. Then incubate the plates upside down in the 37^0C incubator for 12-24 hrs.

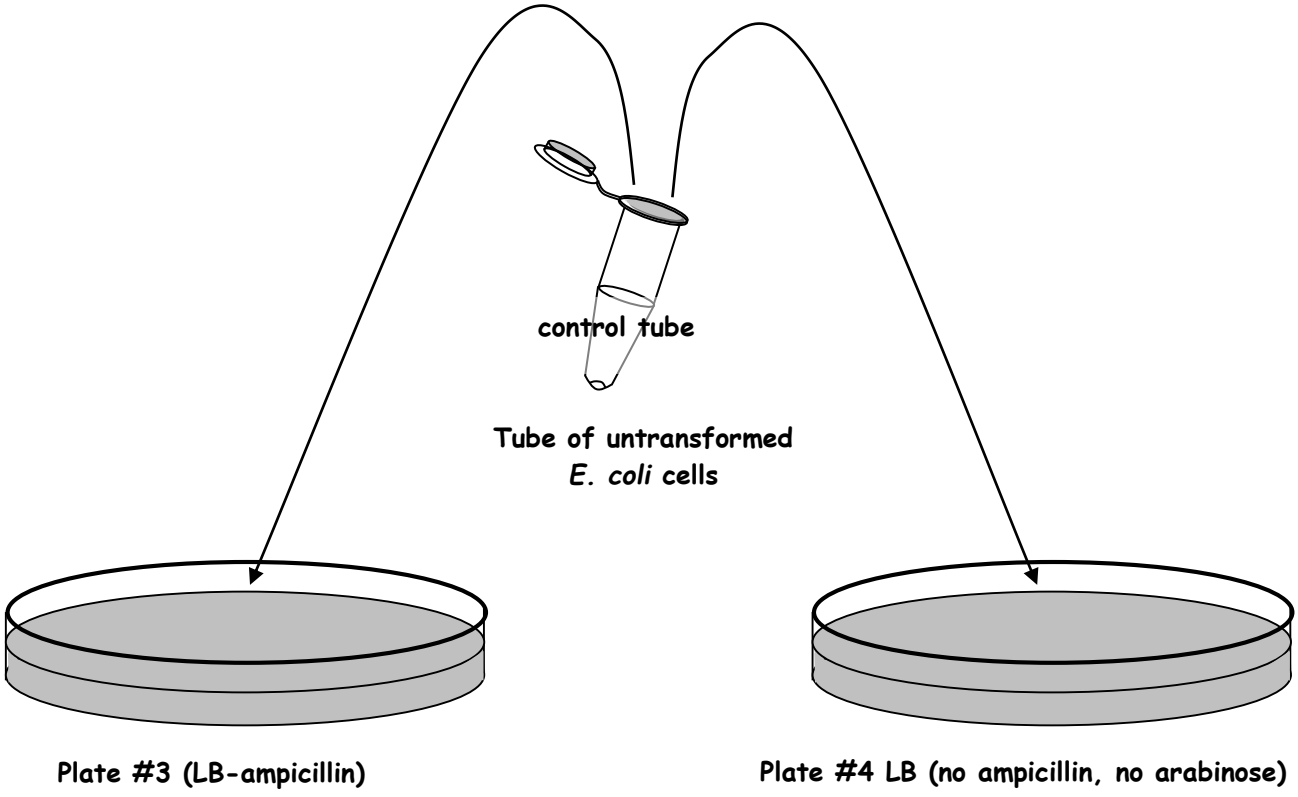
23. Store the plates at 4^0C until the next lab period.

24. Properly dispose of bacteria-contaminated items as described by the instructor.

25. Wipe down your bench with a disinfectant solution.

GO TO THE NEXT PAGE TO SEE A DIAGRAM OF HOW THE FOUR PLATES ARE PRODUCED


pGlo tube
Tube of transformed
+ untransformed
E. coli cells
Plate #1 (LB-ampicillin)
Plate #2 (LB-ampicillin + arabinose)


control tube
Tube of untransformed
E. coli cells
Plate #3 (LB-ampicillin)
Plate #4 LB (no ampicillin, no arabinose)

ANALYSIS OF BACTERIAL GROWTH PATTERNS

POSSIBLE TYPES OF BACTERIAL GROWTH ARE:

- **NO GROWTH** = NO BACTERIAL GROWTH CAN BE OBSERVED (the agar should appear relatively clear in comparison to plates with growth)
- **ISOLATED COLONIES** = SMALL, INDIVIDUAL "PATCHES" (COLONIES) OF BACTERIA CAN BE OBSERVED AT VARIOUS LOCATIONS ON THE PLATE (Bacterial colonies, which appear as round, slightly raised, tan spots, are derived from a single cell that has undergone multiple rounds of cell division)
- **LAWN GROWTH** = MOST OF THE SURFACE OF THE PLATE IS COVERED IN A THIN FILM (A LAWN) OF BACTERIA GROWTH; INDIVIDUAL COLONIES ARE NOT OBSERVED (When bacterial growth is very dense, individual colonies are indistinguishable from each other and the bacteria appear as a thin mat (lawn) of growth)

ANALYSIS OF EXPRESSION OF THE GREEN PROTEIN GENE

The arabinose promoter controls transcription of the green protein gene. In the presence of arabinose the gene is expressed and the green fluorescent protein is synthesized. When exposed to ultraviolet (UV) light, the green protein fluoresces brilliant green light.

Your results will not be available until next week. We will examine your plates then, but today you will be asked to examine and describe the pattern of growth and expression of the green protein gene for each of the 4 instructor's plates. Then provide a rationale for the expected result (why do we see lawn growth, colony growth or no growth on each of the various plates? Did each plate demonstrate expression of the green protein gene? Why or why not?)

QUESTIONS

PLATE #1 **Type of Growth: _____** **Glowing green?: _____**

Explanation of results:

PLATE #2 **Type of Growth: _____** **Glowing green?: _____**

Explanation of results:

PLATE #3 **Type of Growth: _____** **Glowing green?: _____**

Explanation of results:

PLATE #4 **Type of Growth: _____** **Glowing green?: _____**

Explanation of results:

QUESTIONS

1. **How** does ampicillin kill bacterial cells?

2. How do ampicillin-resistant *E. coli* combat the lethal effects of the ampicillin antibiotic?

3. What is a promoter, and how does it affect gene expression?

4. What is the relationship between arabinose, the arabinose promoter, and the expression of the green protein gene?

Clean-up:

_____ Place pGlo and Control tubes in the tip waste container.

_____ The following should remain on the tray on your table: 3 micropipets (p-1000, p-200, p-20); 2 boxes of tips; rack with 3 tubes food coloring (red, green, yellow); waxed paper; tip waste container.

_____ Return all other supplies to correct trays on the side counter.

LABORATORY NOTES

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