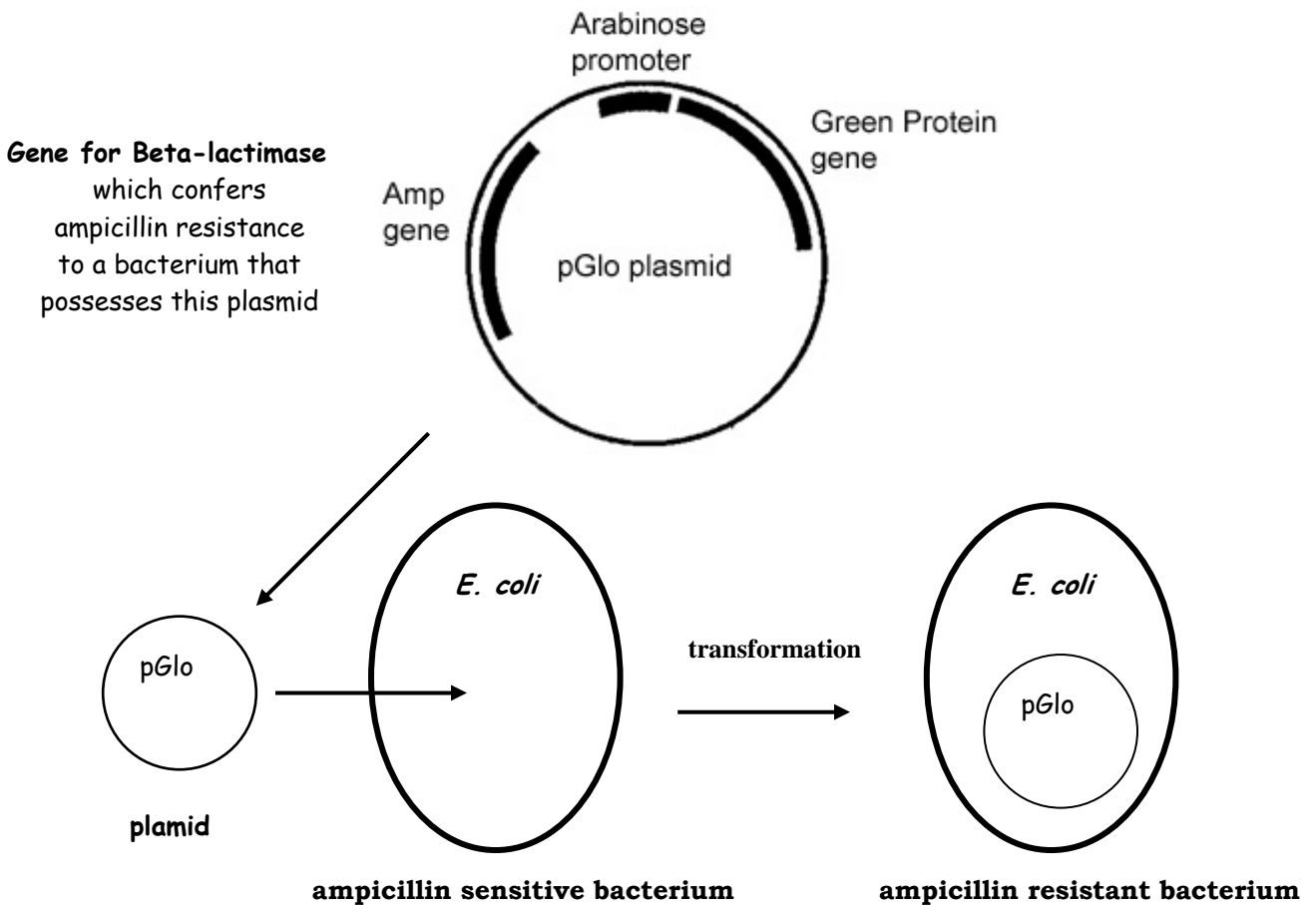


◆ **TRANSFORMATION OF *E. COLI* WITH PLASMID DNA**

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 genotype and 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.

Lab #5 Transformation of E. coli with pGlo Plasmid

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 Fluorescent Protein (GFP)** 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 GFP 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 self-evident as you proceed.

◆ Day 1 Making Competent Cells

Exposure of *E. coli* cells to cold calcium chloride (CaCl_2) alters the cell membrane in a way that favors the uptake of exogenous plasmid DNA. Cells treated in this way are said to be competent for transformation. The processes of making competent cells and transformation must be done under sterile conditions. Use common sense and don't contaminate your samples.

1. Obtain two 15 ml centrifuge tubes. Pipet 5 ml of a log phase *E. coli* culture into each. Log phase cells are found at the teacher's station.
2. Spin the tubes for 5 minutes at setting #2 in the low-speed bench-top centrifuge
3. The cells will form a pellet at the bottom of the tube. Pour off the supernatant into the "waste" beaker, while keeping the pellet.
4. Add 5 ml of ice-cold 50mM CaCl_2 to each tube and vortex until all the cells are uniformly suspended (no clumps)
5. Incubate the tubes on ice for 20 minutes.
6. Spin the cells for 5 minutes at setting #2 in the low-speed bench-top centrifuge again
7. Pour off the supernatant into the "waste" beaker, while keeping the pellet.
8. Add 0.5 ml of ice-cold 50mM CaCl_2 to each tube and vortex until all the cells are uniformly suspended (no clumps)
9. Transfer the contents of both tubes into one microcentrifuge tube. Label the top of the tube "CC" for competent cells.
10. Place the competent cells back on ice and keep them there until needed. The cells are now competent for the uptake of plasmid DNA.

◆ Day 1 - Transformation Procedure

1. Obtain 3 microcentrifuge tubes and label them 1-3
2. Add 190 μl of competent cells to each tube.

Lab #5 Transformation of *E. coli* with pGlo Plasmid

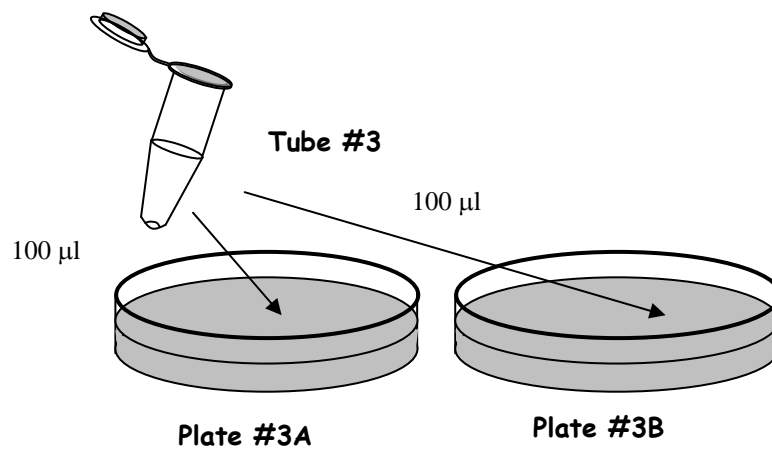
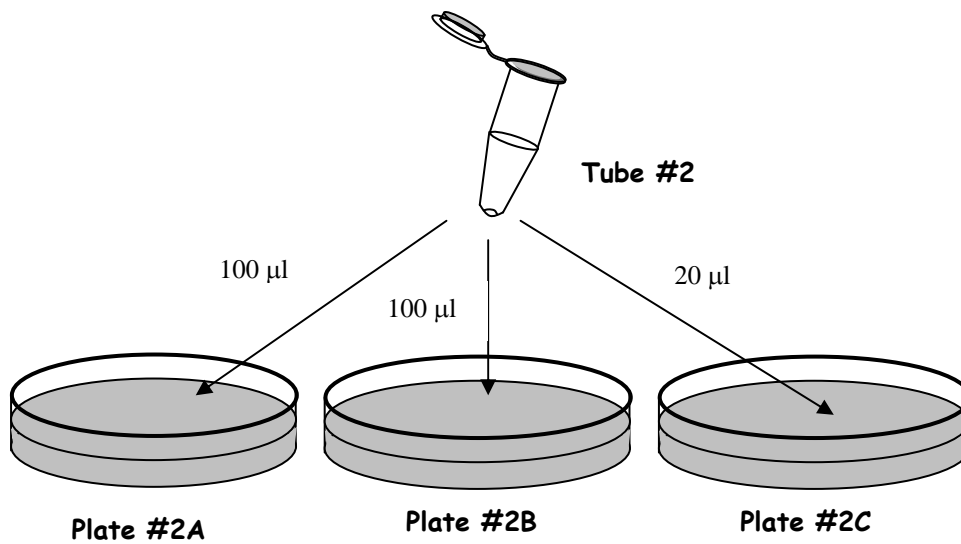
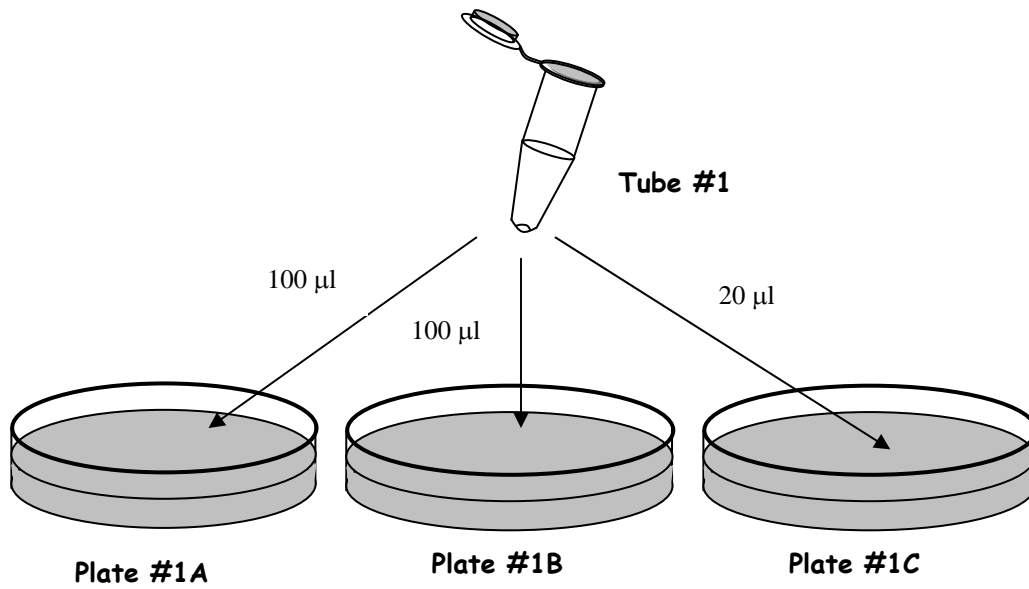
3. Return the tube to the ice.
4. Find the plasmid DNA samples in your ice bucket. You have 2 different concentrations; 0.001 $\mu\text{g}/\mu\text{l}$ and 0.01 $\mu\text{g}/\mu\text{l}$.
5. Add 10 μL of 0.001 $\mu\text{g}/\mu\text{l}$ pGlo plasmid DNA to tube #1. Mix by gently tapping the tube with your finger. Return the tube to the ice.
6. Add 10 μL of 0.01 $\mu\text{g}/\mu\text{l}$ pGlo plasmid DNA to tube #2. Mix by gently tapping the tube with your finger. Return the tube to the ice.
7. Tube #3 does not receive DNA. Its is our negative control
8. Incubate tubes 1-3 on ice for 20 minutes.
9. Following the 20 minutes ice incubation, heat shock the cells by placing them in a 42⁰C water bath for 90 seconds.
10. Place the cells back on ice for 1 minute.
11. Move the cells to room temperature.
12. Add 0.8 ml of LB broth to each tube.
13. Incubate the tubes in a 37⁰C water bath for 30 minutes
14. Use the time of this incubation period to label your agar plates.
 - a. Obtain 3 LB-Amp plates. Label them as 1A , 2A and 3A
 - b. Obtain 4 LB amp-ara plates. Label them as 1B, 1C, 2B and 2C
 - c. Obtain 1 LB plate. Label it as 3B
 - d. Label all plates with the date and your group's initials. Labeling should be on the bottom towards the side so as not to obscure results.
15. After the 30 minute incubation is complete you will use a glass spreader to spread cells on the appropriate plate. The table below indicates which cells will be spread on what plate and at what volume. Ask the instructor to explain if you cannot interpret the table. Also the diagram on page 4 shows which cells go on which plates.
16. You must work carefully 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 pipet the designated volume of cell suspension on to an agar plate.
 - 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.

Source of cells	Plate #	Volume plated	Ampicillin	Arabinose
Tube 1	1A	100 μl	+	-
Tube 1	1B	100 μl	+	+
Tube 1	1C	20 μl	+	+
Tube 2	2A	100 μl	+	-
Tube 2	2B	100 μl	+	+
Tube 2	2C	20 μl	+	+
Tube 3	3A	100 μl	+	-
Tube 3	3B	100 μl	-	-

17. Incubate the plates at room temperature for 15 minutes

Lab #5 Transformation of *E. coli* with pGlo Plasmid

18. Then incubate the plates upside down in the 37°C incubator overnight.
19. The instructor will store the plates at 4°C until the next lab period.
20. Properly dispose of bacteria-contaminated items as described by the instructor.
21. Wipe down your bench with a disinfectant solution.



Day 2 ANALYSIS OF BACTERIAL GROWTH PATTERNS AND GFP EXPRESSION

The pattern of bacterial growth on your plates will vary between samples. You may observe isolated colonies on some plates. Bacterial colonies, which appear as round, slightly raised, tan spots, are derived from a single cell that has undergone multiple rounds of cell division. You may also observe a “lawn” of growth. When bacterial growth is very dense individual colonies are indistinguishable from each other. Rather the bacteria appear as a thin mat (lawn) of growth. You may also observe plates with no bacterial growth at all. In this case the agar should appear relatively clear in comparison to plates with growth.

The arabinose promoter controls transcription of the green fluorescent 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.

Procedure:

Look at your plates in visible light and under UV light. Describe the pattern of growth and expression of the green fluorescent protein gene for each of the 4 plates. Then provide a rationale for the 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?)

Plate #1A

Plate #1B

Plate #1C

Plate #2A

Plate #2B

Plate #2C

Plate #3A

Plate #3B

Determination of Transformation Efficiency

The efficiency of transformation is measured as the number of transformed colonies obtained per microgram (μg) of plasmid DNA used in the experiment. Generally, an increase in DNA concentration will result in higher transformation efficiency. However, excessive DNA concentration can actually reduce transformation efficiency.

To determine transformation efficiency you must calculate the mass of pGlo plasmid DNA spread on the plate. Do this by following these steps:

1. Determine the mass, in μg , of pGlo DNA used in the transformation experiment.
2. Determine the fraction of cell suspension spread on the plate
3. Multiply the mass of DNA by the fraction spread to determine the mass of pGlo spread on the plate (multiply the values from steps 1 and 2 above)
4. Divide the number of colonies counted by the mass of pGlo spread on the plate (value from step 3)

What was the transformation efficiency of the experiment conducted in Tube #1? Show the calculation below.

What was the transformation efficiency of the experiment conducted in Tube #2? Show the calculation below.

Did an increase in concentration of plasmid DNA improve the efficiency of transformation?

Lab #5 Transformation of E. coli with pGlo Plasmid

Notes: