Name: _____ TOC#

Transforming E. Coli with pGLO Plasmids, a lab

Day One

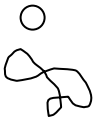
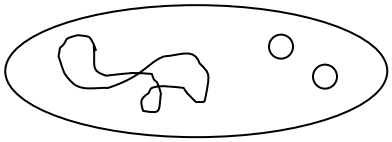
Transformation Background:

Transformation is a process of transferring genetic information from one organism to another. In bacteria, a small circular piece of DNA known as a plasmid (Table 1), transfers genetic information between bacteria, allowing these microbes to gain antibiotic resistance and adapt to new environments.

This natural process can be modified by humans to increase our quality of life. In agriculture, genes are added to help plants survive difficult climatic conditions, insect damage and increase their nutrients. Toxic chemical spills are often controlled" by transformed bacteria. Currently, many diabetics rely on insulin made from bacteria transformed with the human insulin sequence. Scientists use transformation as a tool to work on ways to treat other human diseases and conditions.

In order to successfully transform bacteria, you will need to add $CaCl_2$, transformation solution (TS), to neutralize both the bacterial cell wall and membrane charges, then, quickly shock them with a temperature change in order for them to uptake the pGLO plasmid. After the stressful event, you will provide nutrient broth to restart their growth. Deviating from the protocol listed below may decrease your success in obtaining transformed bacteria.

Table 1: Illustration of a bacterial cell with chromosome and plasmids

Symbol	Bacterial structure	Illustration of *E. coli* K-12
	Plasmid containing a few genes Circular bacterial chromosome	

The Act of Transformation:

This transformation procedure involves three main steps. These steps are intended to introduce the plasmid DNA into the E. Coli cells and provide an environment for the cells to express their newly acquired genes.

To move the pGLO plasmid DNA through the cell membrane you will:

1. Put solution in ice (makes plasmid stick to the cell wall)
2. Use a transformation solution of $CaCl_2$ (weakens cell wall)
3. Carry out a procedure referred to as a heat shock (forces uptake of plasmid)

For the transformed cells to grow in the presence of ampicillin you must:

1. Provide them with nutrients and a short incubation period to begin expressing their newly acquired genes.

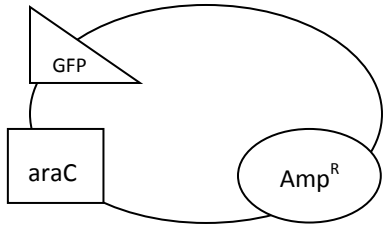
What is the process that you will complete during this lab?

Plasmid Background:

In this lab, you will be using non-pathogenic E. *coli* bacteria and pGLO, a plasmid modified with three genes. The pGLO plasmid contains the genetic codes for (see Table 2):

1. a green fluorescent protein (GFP) from the bioluminescent jellyfish, *Aequorea victoria*
2. ampicillin resistance (amp^R)
3. a special gene regulation system (araC) requiring the sugar arabinose in the media to induce GFP expression

Table 2: pGLO plasmid and its three important genes.

Symbol	Type of gene	pGLO plasmid with inserted genes
Amp^R	Ampicillin resistance gene. When expressed in the presence of ampicillin, bacteria can survive.	
araC	araC gene. In the presence of arabinose, araC creates a protein induces the expression of GFP	
GFP	GFP gene. If araC produces the inducer, GFP produces the green fluorescent protein	

This means the GFP gene will only 'turn on' if arabinose is in the bacteria's environment. If pGLO transformation is successful and the bacteria are growing in arabinose, the colonies will appear neon green under UV light. These fluorescing green bacteria must contain the pGLO plasmid with the GFP gene as well as the other genes found on the pGLO plasmid. For this reason, the green fluorescent protein (GFP) gene is often used as a "reporter gene" to identify expression of other genes of interest.

As a class draw and take notes on the diagram that depicts how the genes in the plasmid interact with each other

The Lab Objective:

1. With the tools and lab protocol provided, you will be able to perform genetic transformation.
2. By the end of the lab activity and analysis you will understand one method of biotechnology (transformation) that scientists use to genetically modify organisms.

Your task:

1. Complete genetic transformation by following lab procedures
2. Determine the degree of success in your efforts to genetically alter an organism
3. Understand the steps that lead to the expression of GFP

Your Grade is based on the following:

1. Your behavior during the lab
2. Your ability to follow directions: do your plates have the correct outcomes?
3. Your thoughtful and complete answers to all questions on the lab packet.

Predictions:

Will the untransformed bacteria appear neon green under a UV lamp? Will transformed bacteria fluoresce under a UV lamp? List your predictions below. Before beginning the transformation, observe a plate of *E. coli* and a vial of pGLO plasmid under a UV lamp. Then, view your transformed colonies once you complete the protocol below. Explain your results.

3. Complete your predictions here

Item	*Prediction (growth or not)*	View with UV Lamp	Explanation of your Prediction
E. coli growing in petri dish			
Vial of pGLO plasmid			
Transformed *E. coli* in petri dish			

Day One lab Warm Up

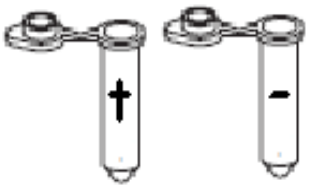
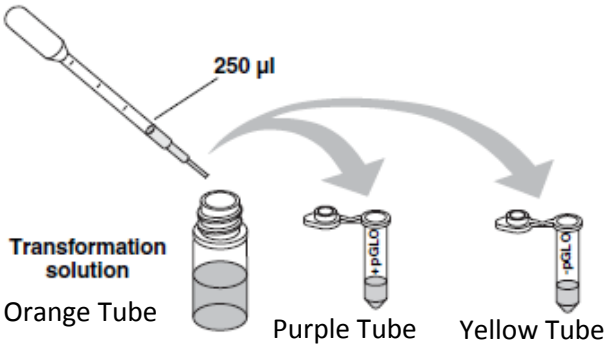
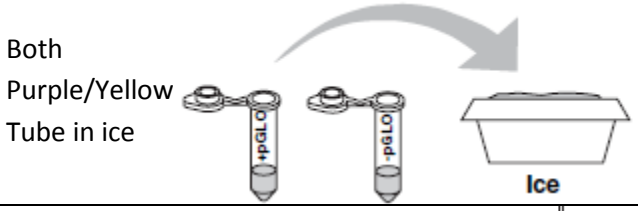
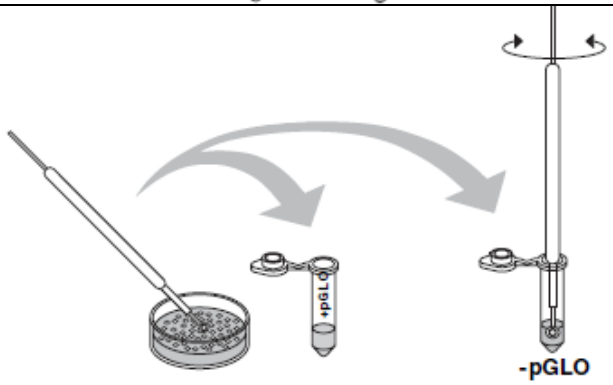
For the Warm Up today, you will "mark-up" your procedures. To do this correctly-follow the directions below.

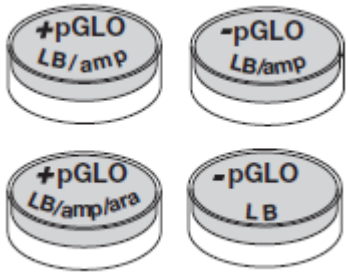
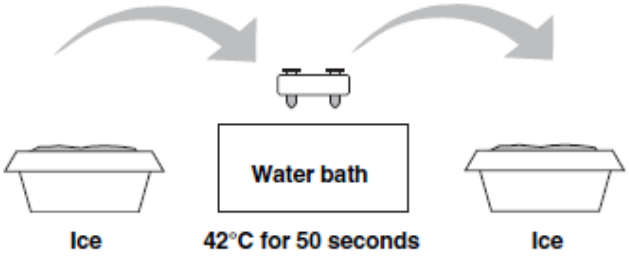
1. Underline the part of the directions anytime you need to LABEL something
2. Circle any part of the directions where specific measurements/amounts are listed (i.e. 1 µl)
3. Box any time constraints.
4. Place a star (★) any time cross contamination is a possibility. When in doubt, throw it out.
5. After "marking up" the lab, you will complete "Day One: Pre-Lab Observations" on page 7.

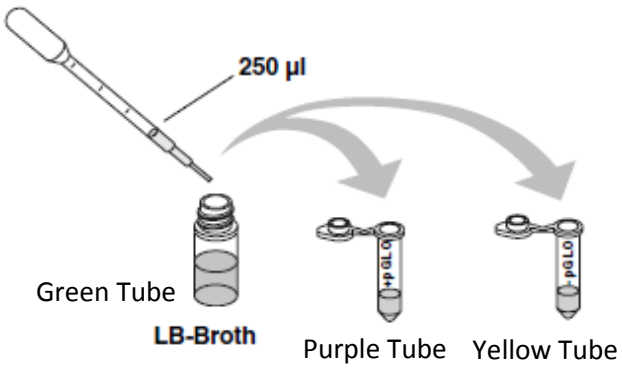
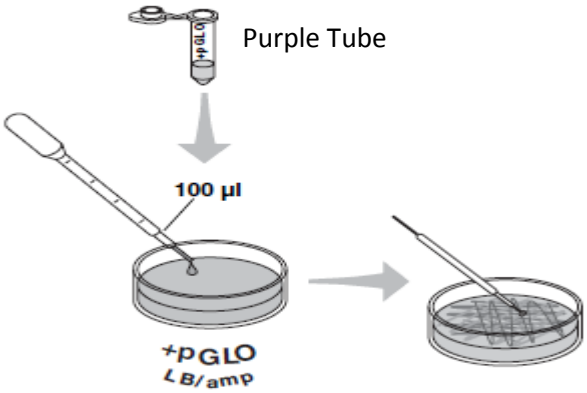
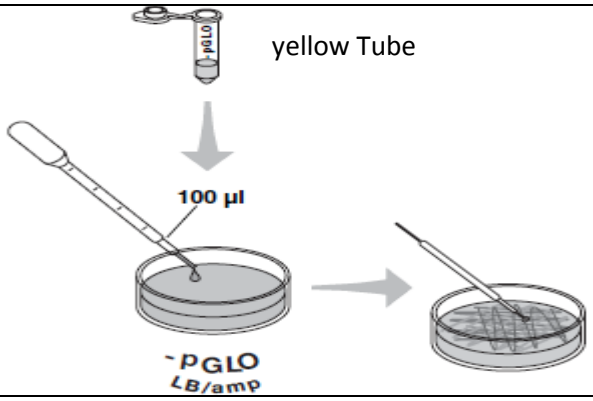
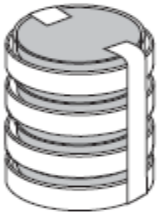
Day One lab Procedures

1. As you complete the lab, each group member must keep track of what step you are on by placing a check mark in the appropriate column to indicate the step is complete.
2. Before you begin, investigate the supplies on your tray and around the room.

Supply	Amount	(✓)
E. Coli starte plate	One per class in front	
Agar Plates 1 red: LB/-pGLO 2 blue: LB/amp/-pGLO 2 blue: LB/amp/+pGLO 3 black: LB/amp/ara/+pGLO	4	
Transformation Solution (orange)	1	
LB nutrient broth (gerrn)	1	
Inoculation loops	7 (1 pk of 10)	
Pipets (wrapped up)	5	
Foam microtube holder/float	1	
Container of crushed ice	1	
Sharpie	1	
Your lab manual	1	
pGLO plasmid	1 class vial in the front	
42º Water Bath	1 for class in back	
37º incubator	1 for class in front	

Check	Procedures	Diagram	Special Notes
1. ☐	On your tray you will see 4 centrifuge tubes. Labeled as follows: - **Purple: +** (for transformed bacteria with plasmid) - **Yellow: –** (for non-transformed bacteria without plasmid) - **Orange: TS** (Transformation Solution) - **Green: LB** (Luria Broth)	 **Purple:** + (for transformed bacteria with plasmid) **Yellow:** – (for non-transformed bacteria without plasmid)	What is your group number? _____ **Using a sharpie ADD YOUR GROUP # to each TUBE**
2. ☐	Open one sterile transfer pipet. Open the orange tube and pipet 250 µl of transformation solution ($CaCl_2$) into the purple (+pGLO) tube and the yellow (-pGLO) tube.	 	Make sure you know where 250 µl is indicated
3. ☐	Place tubes on ice.	 	DO NOT PUNCTURE THE STYRAFOAM IN ANY WAY
4. ☐	Using a sterile loop, pick up a single colony of bacteria from the starter plate. Pick up the purple (+pGLO) tube and immerse the loop into the tube. Spin the loop between you index finger and thumb until the entire colony is dispersed in the transformation solution (with no floating chunks). Place the tube back in the tube rack in the ice.	 Make sure to use a new sterile loop and add bacteria to both the purple and the yellow tube	
5. ☐	Repeat step 4 to add bacteria to the yellow (-pGLO) tube. Be sure to get colonies with a NEW sterile loop		Make sure you use a NEW one.

6. ☐	**Ask your teacher for help:** a. Have your teacher help you use a micropipette and alloquate 10 µl of the pGLO plasmid solution you're your purple (+pGLO) tube. **Return to your group:** b. Close both the purple (+pGLO) and yellow (-pGLO) tubes c. Examine the +pGLO solution and the –pGLO with the UV lamp. d. Put both on ice	 **DO NOT add plasmid DNA to the yellow tube.**	What the does both solutions look like?
7. ☐	Incubate the tubes on ice for 10 minutes. Make sure to push the tubes all the way down in the rack so the bottom of the tubes stick out and make contact with the ice.		**During the 10 minutes, complete step 8 and then answer questions on page 8/9**
8. ☐	While the tubes are sitting on ice, label your four LB nutrient agar plates **on the bottom** (*not the lid*) as shown in the diagram. Indicators: 1 red: LB/-pGLO 2 blue: LB/amp/-pGLO 2 blue: LB/amp/+pGLO 3 black: LB/amp/ara/+pGLO	 Label the correct dish with the label above **ALSO INCLUDE YOUR GROUP # AND PERIOD**	IT IS CRUCIAL TO LABEL THESE CORRECTLY (answer the questions on pg. 8/9)
9. ☐	When the 10 minutes has passed: **HEAT SHOCK!** Using the foam rack as a holder, transfer both the + and – pGLO (purple and yellow) tubes into the water bath, set at 42º for EXACTLY 50 seconds. IMMEDIATELY after the 50 seconds, place both tubes back on ice. For the best transformation results, transfer from ice to water to ice, must be as rapid as possible. Incubate tubes on ice for 2 minutes.		Make sure to the bottom of the tubes are touching the water. Ice makes plasmids stick to the way. Heating allows plasmids in through weak cell wall. Ice again seals plasmids in.

10. ☐	After two minutes, remove the rack containing the purple and yellow tubes from the ice. One at a time, open the purple and yellow tube and, using a NEW sterile pipet, add 250µl of LB nutrient broth to the tube and reclose it. Repeat with a NEW sterile pipet for the other tube. Let tubes incubate (rest) for 10 minutes at room temperature.		During the ten minutes, continue to work on pages 8 to 9. What you do not finish will be homework
11. ☐	Tap the closed tubes with your finger to mix.		
12. ☐	Using a new sterile pipet transfer 100µl +pGLO solution (purple tube) to the following plate: • LB/amp/+pGLO plate Then, using a sterile loop, spread the small drop of solution around the surface of the agar by *quickly and lightly skating* the flat surface of the loop back and forth across the plate *as the teacher demonstrated.*		When spreading the solution, DO NOT press down into the agar.
13. ☐	Follow step 12 for the following plate (using +pGLO solution): • LB/amp/ara/+pGLO plate		**use a new innoculation loop!**
14. ☐	Following the procedures in step 12, you will add 100µl of the -pGLO (yellow) solution to the following plate. • LB/amp/-pGLO plate		**Make sure to use a new pipet and a new loop!**
15. ☐	Following step 14, you will add 100µl of the -pGLO (yellow) solution to the following plate. • LB/-pGLO plate		**Be sure to use a new inoculation loop!**
16. ☐	Stack up your plates and tape them together as the picture shows. Place the stack upside down in the 37ºC incubator until the next day.		They **must** be **UPSIDE DOWN** or your plates will get soggy from condensation.

Day One: Pre-lab Observations

How can I tell if cells have been genetically Transformed?

Recall that the goal of genetic transformation is to change an organism's traits (phenotype). Before any change in the phenotype of an organism can be detected, a thorough examination of its natural (pre-transformation) phenotype must be made. Look at the colonies of E. coli on your starter plates.

List all observable traits or characteristics that can be observed. This will be our baseline data to reference to see if transformation occurred.

a) Number of colonies (estimate)

b) Size of the largest colony: _____

c) Size of the smallest colony: _____

d) Size of the majority of colonies: _____

e) Color of the colonies

f) Distribution of the colonies on the plate

g) Visible appearance when viewed with ultra violet (UV) light

h) Describe how you would test the whether the E. Coli had the ability of the cells to live and reproduce in the presence of an ampicillin. What do you think the result of your test would be?

When your entire group is finished with the Warm Up and pre-lab questions. Go back to the procedures page, and begin the lab.

Day One: lab Questions

Answer the following questions while your samples sit on ice (10 minutes), and while your samples incubate (10 minutes). Make sure you are keeping track of time.

There are many considerations that need to be thought through in the process of planning a scientific laboratory investigation. Below are a few for you to ponder as you take on the challenge of doing genetic transformation. Since scientific laboratory investigations are designed to get information about a question, our first step might be to form late a question for this investigation.

Can I Genetically Transform an organism? Which Organism?

1. To genetically transform an entire organism, you must insert the new gene into every cell in the organism. Which organism is better suited for total genetic transformation—one composed of many cells, or one composed of a single cell? **Why?**

2. Scientists often want to know if the genetically transformed organism can pass its new traits on to its offspring and future generations. To get this information, which would be a better candidate for your investigation, an organism in which each new generation develops and reproduces quickly, or one which does this more slowly? **Why?**

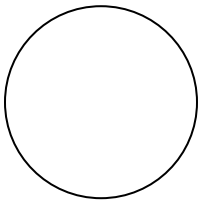
3. Safety is another important consideration in choosing an experimental organism. What traits or characteristics should the organism have (or not have to be sure it will not harm you or the environment? **Why?**

4. Based on the above consideration, which would be the best choice for a genetic transformation: a bacterium, earthworm, fish, or mouse? Describe your reasoning.

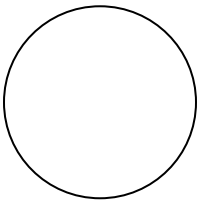
Petri-Dish Roster: make sure you know your plates!

Read each of the descriptions of the plates below. Make sure to fill in all blanks. Additionally, you should write grow/die/glow on each circle/plate.

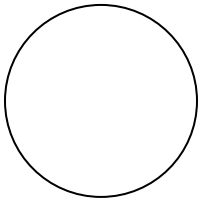
1. ***E. coli* starter plate**: This plate has the bacteria that will be used in our lab. These one-celled organisms are not _____ (have not had plasmids placed into them). They are growing on an agar plate with _____ (LB) a nutrient rich mixture. The agar acts as a matrix (structure) on which the bacteria may grow.



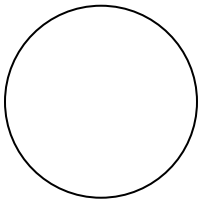
2. **LB/amp/+pGLO:** This plate will have *E. coli* bacteria on LB agar to which _____ has been added. The +DNA means that the bacteria will possibly have been _____ (by its acceptance of a recombined plasmid with the gene from a _____ which codes the Green Fluorescent Proteins [GFPs]). The plasmid has a _____ site (a gene for beta-lactamase protein) which allows it to resist, or not be _____ by the antibiotic (ampicillin) we have added to the agar.



3. **LB/amp/ara/+pGLO:** This plate is also being given the genetically altered bacteria with a gene for _____. However, the agar has an additional ingredient, _____, which is a sugar needed to _____.

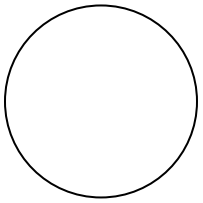


4. **LB/amp/-pGLO:** These bacteria _____ received the plasmid with the gene for GFP (thus ="-DNA") nor have they received the resistance site on the plasmid for ampicillin. What do you predict will happen to the bacteria placed on this plate?
_____



5. **LB/-pGLO:** This plate has bacteria without the plasmid. Do you expect it to be able to make GFP? _____. The agar in this dish does not have ampicillin. Do you expect bacteria to be able to grow in this dish? _____. This dish is acting as a _____ to be used in comparison to the LB/amp plates. Explain why this is important.

_____



Answer the following questions.

1. **Fill in the blanks to explain the steps needed to create a recombinant plasmid.**

First, use _____ enzymes to cut out the desired gene from the source DNA (in this case a jellyfish gene for making Green Flourescent Protein [GFP]). We also cut the _____ with the same enzyme so that matching _____ _____ are created that will allow the two sets of DNA to re-combine into one mixed plasmid. In our lab, this plasmid will also have a _____ site (= a gene) to make proteins to allow it to protect itself against ampicillin.

2. **Fill in the blanks to explain how a bacteria is transformed.**

The bacteria cells will have their cell walls altered by calcium chloride (Transformation Solution). This makes the cell "_____" meaning that it now will allow plasmids to enter it. The heat shock (of cold to hot to cold) increases the permeability of the cell membrane to DNA, and must be timed accurately. The _____ time afterward with the rich LB broth added, is to allow the transformed bacteria cells to _____ and to express the amp resistance protein. This should allow them to not be _____ when they are placed on the LB/amp plates. Arabinose is a _____ needed for the GFP gene to be _____ (= turned on).

Day Two: Warm UP

Before collecting and analyzing your results, answer the following questions.

1. On which of the plates would you expect to find bacteria most like the original non-transformed E. Coli colonies you initially observed on the starter plate? **Explain**.

2. If there are any genetically transformed bacteria cells, on which plate(s) would they most likely be located? **Explain.**

3. Which plates should be compared to determine if any genetic transformation has occurred? **Why?**

4. What is meant by a control plate? What purpose does a control serve? Which plates were our control?

5. Explain the purpose of these processes or substances during transformation.

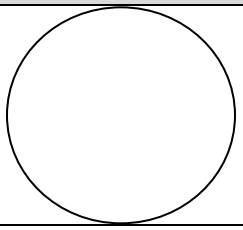
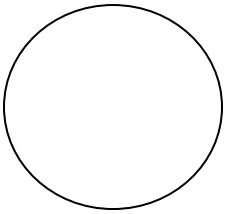
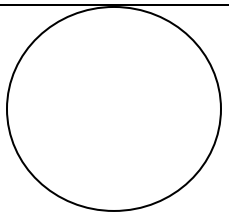
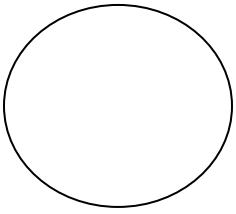
	Process or Substance	**Purpose**
a.	LB agar	
b.		Prevents growth of untransformed bacteria on LB/amp and LB/amp/ara plates
c.	Calcium chloride	
d.	Heat shock	
e.	Arabinose	

6. Describe 2 differences and 2 similarities between these Bacteria.

Condition	- pGLO DNA bacteria	+ pGLO DNA bacteria
Difference		
Similarity		

Day Two: Observations

1. Carefully observe and draw what you see on each of the four plates. Put your drawings in the data table in the column on the right. Record your data to allow you to compare observations of the "+pGLO" cells with your observations for the non-transformed E. Coli. Write down the following observations for each plate.

Plate	Drawing	Description
LB/-pGLO		
LB/amp/-pGLO		
LB/amp/+pGLO		
LB/amp/ara/+pGLO		

Day Two: Analysis

The goal of data analysis for this investigation is to determine if genetic transformation has occurred.

1. Which of the traits that you originally observed for E. coli did not seem to be altered?

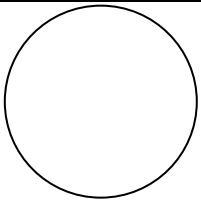
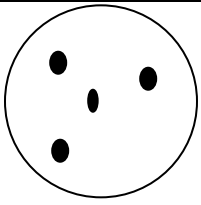
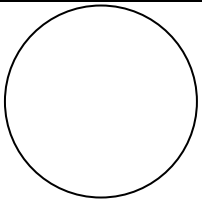
2. Of the E. coli traits you originally noted, which seems now to be significantly different after performing the transformation procedure?

3. If the genetically transformed cells have acquired the ability to live in the presence of the antibiotic ampicillin, then what might be inferred about the other genes on the plasmid that you used in your transformation procedure?

4. From the results that you obtained, how could you prove that the changes that occurred were due to the procedure that you performed?

5. Compare your predictions with your actual lab results. Describe how close your predictions were to your actual results and explain possible reasons for any differences.

6. These results were obtained by a lab group who *did not have the correct outcome*. Explain what may have occurred to produce these results. (• = colony)

Contents	**LB -DNA**	**LB/amp/-pGLO**	**LB/amp/ara/+pGLO**
Illustration of Results	 (no colonies)		 (no colonies)
Description of Results			
Possible explanation for results			

Day Two: Review

1. If growth appeared on the LB/amp/+pGLO plate, would these bacteria…
   - Show successful transformed? Explain.

   - fluoresce under UV light? Why or why not?

2. Provide an example of how transformation can be beneficial and an example of how it can be potentially harmful to humans.

	Condition	**Transformation example**
a.	Beneficial	
b.	Harmful	

3. Although transformed cells appear white, with the same phenotypic expression of the wild-type bacteria when the growth media lacks arabinose, they will fluoresce green with a long-wave UV lamp when arabinose is present. Explain why this color change occurs.

4. Provide a rational or benefit of adding DNA sequences coding for fluorescent proteins such as GFP, to tag genes of interest in plasmids used for transformation. See the following site if you are unsure http://news.nationalgeographic.com/news/2009/05/photogalleries/glowing-animal-pictures/#/crystal-jelly-gfp-glowing-animals_11833_600x450.jpg

5. In your own words, explain the process of transformation.