GENES R US PRE-EMPLOYMENT INTERVIEW
JOB TITLE:
TRANSFORMATION SPECIALIST

Name: _____ Applicant Group: _____

Please answer the following questions. If you feel you can't answer a question, do your best and move on to the next.

1. What, in simple terms, is DNA? _____

   _____

   _____

2. Do you have any lab experience with transformation of organisms?
   ❏ YES   ❏   No

3. Explain what you think genetic transformation of an organism is:_____

   _____

   _____

   _____

4. Explain what an experimental variable is:_____

   _____

   _____

5. Explain what an experimental control is:_____

   _____

   _____

6. How can we give organisms new phenotypes, or characteristics?_____

   _____

   _____

   _____

7. Is it possible to insert a new gene into an organism and have that gene function correctly? If so, how do you know the gene is working? _____

_____

_____

_____

_____

8. What do antibiotics do to bacteria? _____

_____

_____

_____

_____

_____

9. Can bacteria be used as factories to produce products?

❏ Yes ❏ No

10. What is *E. coli*? _____

_____

_____

11. What is a plasmid? _____

_____

_____

Confidential

Some genes and their protein products have enormous commercial value. Examples include: insulin used by diabetics for lowering blood sugar, enzymes added to laundry detergent for removing stains, and restriction enzymes for cutting DNA. Scientists in the Research and Development Division of Genes-R-Us Inc. have developed a process in which enzymes (used to produce a blue dye, Indo-Blu) are produced by genetically transformed bacteria.

You have been hired to work on an extension of this Indo-Blu project. The members of your team will first learn some of the steps used to create Indo-Blu producing bacteria (using our Standard Operating Procedure). Then your team will be asked to suggest possible improvements to the bacterial transformation process, that is, successfully getting genes of interest into bacteria.

Background information:
Scientists can insert genes into bacteria. The genes inserted in the Indo-Blu (SOP #T-1) process are on a circular piece of DNA called a **plasmid**. (The plasmid we use is called **pBLU®.**) The bacteria with the inserted genes are used as factories that produce large quantities of the gene and its product. The process of giving bacteria (or another organism) a new gene is called **transformation**.

Our transformation process uses the bacterium *Escherichia coli* (or *E. coli* for short) as the "factory"to produce the Indo-Blu dye. We use a highly specialized, harmless strain of *E. coli*. We insert the pBLU® plasmid, which contains a gene that produces the enzyme beta-galactosidase (**β-gal** for short).

Indo-Blu is produced by the reaction between beta-galactosidase (the enzyme) and **X-gal** (the substrate). β-gal normally modifies lactose, the sugar in milk. X-gal chemically looks like lactose. β-gal splits a sugar group from X-gal, producing the blue dye, Indo-Blu.

Transformation is a rare event, so to make it easier to find transformants (transformed bacteria), a gene for ampicillin resistance was included on the pBLU® plasmid. Expression of this gene, which produces the enzyme beta-lactamase, allows transformed *E. coli* to grow in the presence of **ampicillin**, an antibiotic that usually prevents the growth of bacteria. The beta-lactamase enzyme dismantles ampicillin, rendering it non-functional. Hence, transformed bacteria will live in the presence of ampicillin, untransformed bacteria will not live.

Proper controls are essential in any experiment. In this experiment we will grow *E. coli* on agar plates which contain nutritional requirements for the bacteria. In the pBLU® transformation, our control is another sample of bacteria which is treated exactly the same as the bacteria we're transforming, except the control does not receive the pBLU® plasmid.

Controls help you understand what happened if things go wrong and to know things work out as expected. For example, if there are no bacteria on your pBLU®-transformed plate, is this because none of them were transformed (a definite possibility) or because all the bacteria were flat-out dead already? A control plate helps answer the question. If the bacteria on the control plate are alive, you can rule out that the procedure killed the bacteria.

Controls also validate that the protocol was performed correctly when the experiment appears to have been successful. Experiments can utilize positive and negative controls.

Overview of the Transformation Process:

1. Grow the bacteria on a stock plate (the head of the Molecular Biology group may have done this for you already).

2. Make the bacteria "competent." This makes them able to accept new DNA. The method we use involves coating the bacteria and the DNA with positively charged molecules, to minimize charge repulsion of the DNA by the bacteria.

3. "Shock" the bacteria with heat, then chill to push the DNA into pores in the bacteria.

4. Grow the bacteria on LB agar.
   Bacteria on an agar plate containing the antibiotic ampicillin will only grow if they have received the plasmid.
   If the agar plate also contains X-gal, the β-galactosidase gene product will convert X-gal into Indo-Blu, our dye.
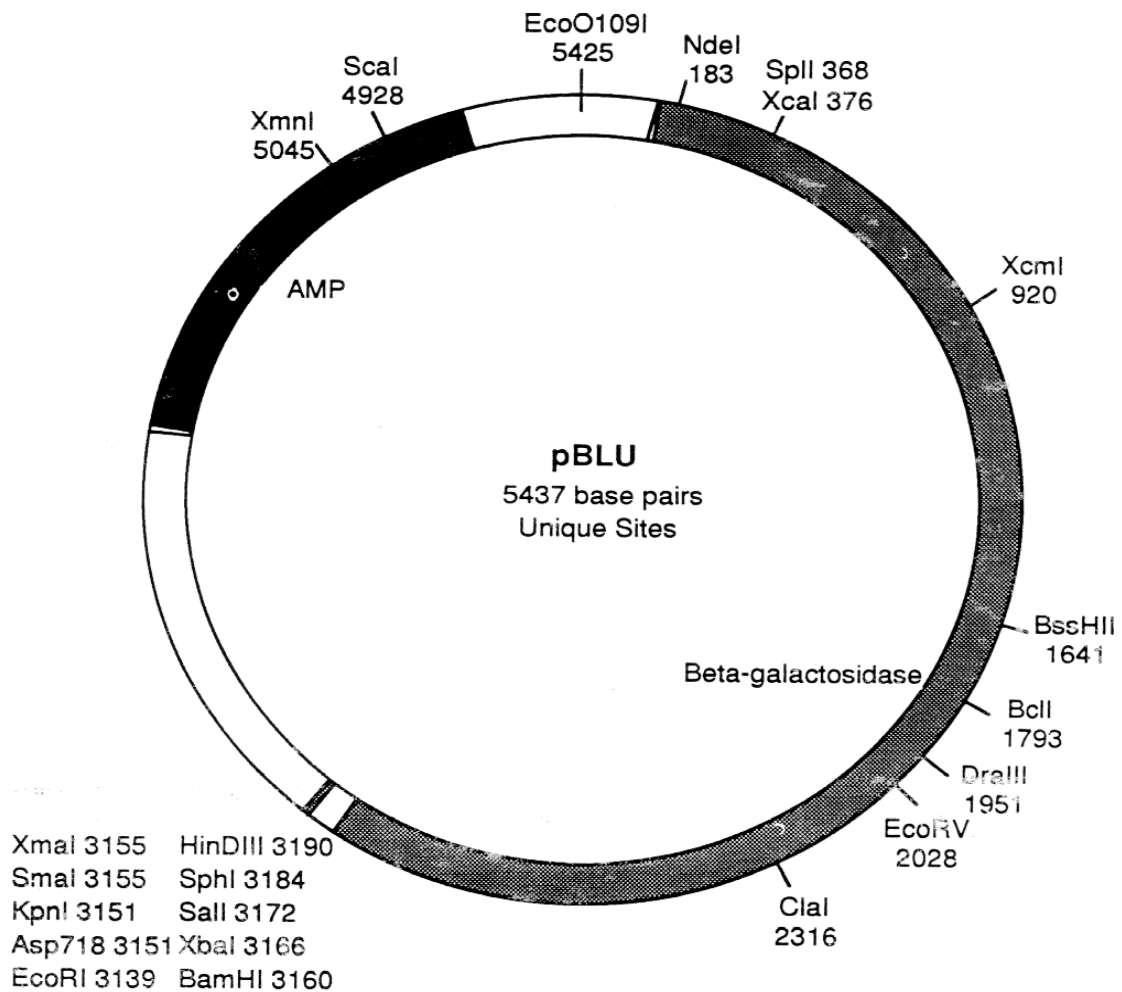
   ***E. coli* colonies are usually white. Those producing Indo-Blu will be blue.**

The bacteria cannot move, so they will grow and divide in the same spot on the plate. Small numbers of bacteria cannot be seen without a microscope, but colonies (millions of daughter cells from one bacterium) can be seen. This allows you to identify, count, and isolate bacterial colonies, each of which arose from a single bacterial cell.

As part of your job assignment at Genes-R-Us, your team has been given the task of increasing the efficiency of transformation. The higher the efficiency of transformation, the more Indo-Blu producing bacteria colonies will grow.

A prominent wildlife protection organization recently requested our collaboration of a project to preserve the genomes (genetic information) of endangered species. The project uses the transformation process to create "libraries" of bacteria. Each transformed bacterium carries a different small piece of the genetic material being preserved. The challenge for Genes R Us is to increase the efficiency of the transformation process so that these libraries are complete. Our scientific advisory board is keen to help with this project. Achieving higher transformation efficiency will reduce the costs and increase the success to the project.

Your first assignment is to familiarize yourself with our standard operating procedure (SOP #T-1), practicing it once using a basic protocol. You will then make a proposal to modify one aspect of the transformation protocol (using appropriate controls). If there is enough time, your team will experimentally determine whether your proposed modification improves the transformation efficiency.

pBLU
5437 base pairs
Unique Sites

EcoO109I 5425
NdeI 183
SpII 368
XcaI 376
ScaI 4928
XmnI 5045
XcmI 920
AMP
BssHII 1641
Beta-galactosidase
BclI 1793
DraIII 1951
EcoRV 2028
ClaI 2316

Xmal 3155   HinDIII 3190
Smal 3155   SphI 3184
Kpnl 3151   Sall 3172
Asp718 3151 Xbal 3166
EcoRI 3139   BamHI 3160

Key: *AMP* = ampicillin resistance gene
Beta-galactosidase = gene coding for the β-galactosidase enzyme
Unique sites = the restriction enzymes shown cut the plasmid in only one place

EcoRV 2028= the restriction enzyme EcoRV cuts at nucleotide number 2028

From <u>DNA</u> <u>Science</u>, Micklos & Freyer, 1995

<u>**PLAN**</u>

- Practice our basic transformation method by transforming *E. coli* with the pBLU® plasmid (which carries genes for beta-galactosidase production *and* ampicillin resistance).
- Assess the success of transformation by plating the bacteria on LB agar plates containing ampicillin and X-gal (LB/amp/x-gal).
- Prepare a research proposal, identifying one variable to modify in the S.O.P. to increase the number of transformant colonies producing Indo-Blu and verify with the appropriate controls.
- If R & D approves, test your proposed modification.

Materials Needed Per Team

Equipment	*Reagents*
microcentrifuge tubes	disinfectant (10% bleach or 70% alcohol)
rack for microcentrifuge tubes	sterile distilled water
permanent marker pen	sterile Luria Broth (LB)
sterile toothpicks	2 LB agar plates
sterile graduated transfer pipets	2 LB agar/amp/X-gal plates
2-20 µl micropipet + tips	pBLU® plasmid (0.01 μg/μl)
floating tube rack for waterbath	50mM $CaCl_2$ (cold) 250 µl per sample
100-1000 µl micropipet + tips (shared)	container for crushed ice
2 sterile plate spreaders	
(These are not disposable)	

<u>Materials Needed Per Class</u>

42°C waterbath
37°C incubator
container for waste
E. coli stock plate
crushed ice

S.O.P. #T-1 is our standard protocol for bacterial transformation at Genes-R-Us. Take careful notes as you work through it. Write down any observations and deviations from this procedure.

- Give your team a name so that you can be identified as a group and use this name to label your samples and plates. Make sure the head of Molecular Biology knows who the members of your team are.
- As you go through the protocol, think about the procedure and what it does to the bacteria. Try imagining yourself as a bacterium, taking in new genes.

CAUTIONS

- Because we are adding DNA to living cells, all transfers should be made with sterile technique and sterile instruments. Talk with the head of Molecular Biology if you need help.
- If you spill bacterial culture, notify the head of Molecular Biology immediately.

STEP 1: TRANSFORMATION

Purpose: To transform bacteria with pBLU$^®$ plasmid.

Prepare tubes:
1. Remove existing bacteria from your table top and from your micropipets by cleaning with disinfectant. Wash your hands!
2. Obtain two sterile 1.5 ml microfuge tubes containing cold calcium chloride solution (CaCl$_2$), 250 μl each.
   - Label one tube "**C**" (for control) and the other "**pBLU**."
   - Label both tubes with your team name.
   - Place both tubes on ice.

Add bacteria to tubes:
3. Carefully remove a sterile toothpick from the aluminum can.
4. Using the toothpick, pick or gently scrape up a large, single bacteria colony from the stock plate.
5. Insert the toothpick into the tube labeled **C** and vigorously tap or twirl the toothpick against the side of the tube. Look closely to make sure the cell clump has come off.
   - Suspend the cells by pipetting repeatedly with a sterile transfer pipet. After a few moments, hold the tube up to the light and check that no clumps of cells are visible.
   - Return the **C** tube to ice.
   - Place used toothpicks and pipets in waste container.
   6. Using a new toothpick, transfer another colony to the tube labeled **pBLU** just as you did for the **C** tube.
   - Suspend the cells in the **pBLU** tube just as you did for the **C** tube.
   - Return the **pBLU** tube to ice. Both tubes should now be on ice.
   - Place used toothpicks and pipets in waste container.
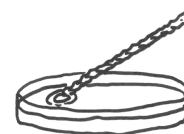
Add plasmid to bacteria:
7. Using a 2-20 μl micropipet and a new sterile tip, add 10 μl pBLU$^®$ plasmid to the **pBLU** tube ONLY.
   - Mix the tube contents by tapping or flicking the tube with your fingers.
   - Return the **pBLU** tube to ice.
8. Using a new tip, add 10 μl of sterile distilled water to the **C** tube ONLY.
   - Mix by tapping the tube with your fingers.
   - Return the **C** tube to ice.
9. Wait 15 minutes.
   - This gives time for the pBLU$^®$ plasmid to settle onto the surfaces of the bacteria. While you're waiting, think ahead about the next two steps. The timing of these steps is thought to be important in the success of the procedure.

Heat shock:
10. When the 15 minute waiting period is over, take the ice container with the **pBLU** and **C** tubes to the 42°C water bath (check the temperature).
11. Transfer the tubes into a floating tube rack.
12. Place the rack with the tubes in the water bath for a heat shock of *exactly* 90 seconds.
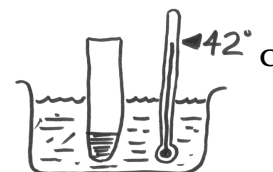
  IT IS ESSENTIAL THAT CELLS BE GIVEN A SHARP AND DISTINCT HEAT SHOCK
13. Immediately return tubes to ice for at least 2 minutes.

14. Add 250 *µ*l of sterile Luria broth (LB) to *each* tube, using a new sterile tip on a 100-1000 *µ*l micropipet for each one. Tap each tube with your finger to mix its contents.

If continuing on to next step this day:
Optional: incubate the tubes for 15-30 min. at 37°C before plating. This allows the bacteria to recover and express the ampicillin resistance gene.
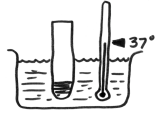
If stopping at this point:
Set the tubes in a rack and store in the refrigerator until the next day (optional incubating step can be performed after removal from refrigerator).

Upon completion:
- Dispose of designated materials in the appropriate places.
- Leave equipment as you found it.
- Check that your work station is in order.
- Wipe down the table and your micropipets with disinfectant.
- Wash your hands.

STEP 2: PLATING CELLS
Purpose: To spread individual cells evenly over the surface of the plate for overnight growth into visible colonies.

Preparing Plates:
1. Eliminate existing bacteria from your work area by cleaning with disinfectant. Wash your hands.
2. Label plates:
   - Obtain your plates (Petri dishes).
   - Handle the plates carefully so that they remain sterile while you label them.
   - Using a permanent marker pen, put a **pBLU** and your team name and class period on the <u>bottom</u> of:
   - an LB plate
   - an LB/amp/X-gal plate.
   - Put a **C** and your team name and class period on the <u>bottom</u> of:
   - an LB plate
   - an LB/amp/X-gal plate.

   - **Plate the cells:**

3. Control sample:
   - Set a 100-1000 µl micropipet to 100 µl.
   - Resuspend the bacteria in the "**C**"tube by pipetting up and down *gently*.
   - Draw up 100 µl and carefully drip it onto the center of the **control LB plate**.
   - Repeat the process (use a new micropipet tip) to spread 100 µl of the control suspension on the **control LB/amp/X-gal plate.**
   - Carefully remove a sterilized plastic spreader from the sterilization bag.
   - Touch only the handle.
   - Use the spreader to evenly spread the cell suspension over the surface of the **control LB plate**. (This may remind you of frosting a cake or spreading peanut butter.)
   - Use the same spreader to spread the suspension on the **control LB/amp/X-gal** plate.
   - Place the used spreader in a container of disinfectant.

A spreader
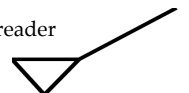
4. pBLU sample:
   • Use the above procedure to spread 100 μl of the suspension from the **pBLU** tube on the **pBLU LB plate** and the **pBLU LB/amp/x-gal plate.**
   • Place the used spreader in a container of disinfectant.

   ### *Save the spreaders. They are not disposable!*

Incubation:
5. Allow **5 minutes** for the plates' surfaces to absorb the cell suspensions.
6. Place your four plates, **upside-down**, in the 37°C incubator.
7. Make predictions about what you expect to happen to each plate after overnight incubation. (See Transformation Report Form, Step 2.)

Upon completion:
   • Dispose of designated materials in the appropriate places.
   • Leave equipment as you found it.
   • Check that your work station is in order.
   • Wipe down the table and your micropipets with disinfectant.
   • Wash your hands.

STEP 3: REPORTING
Purpose: To report results and calculate transformation efficiency.

   • Examine the plates and analyze them for bacterial growth and colony color.
   • **Make sure your controls have performed as expected.**

   • Fill out a Transformation Report, including a calculation of your transformation efficiency. See Transformation Efficiency page.

   • As a team, discuss the experiment you've done and which variable you want to try changing in order to optimize the transformation efficiency for the genome preservation project. Individually or as a team (check with the head of Molecular Biology) write your proposal on the Optimization Proposal form.

   • The head of Molecular Biology will review your work and let you know whether you are to perform the experiment your team has proposed.
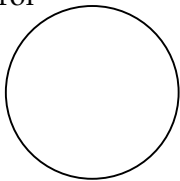
Name _____   Team Name _____
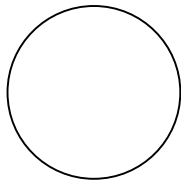
Date _____   Period _____

MAKE PREDICTIONS (STEP 2):
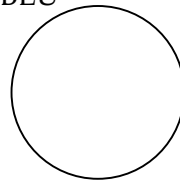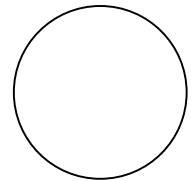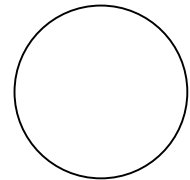- Use the diagrams below to predict the results you expect.
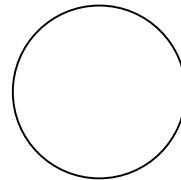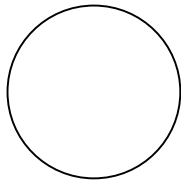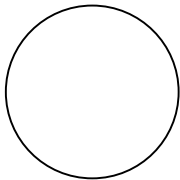
Control

pBLU

LB   LB/amp/X-gal   LB   LB/amp/X-gal

RECORD RESULTS (STEP 3):
1. Use the diagrams below to record your actual results:
   - Label each circle using the same label as the plate it represents.
   - Draw your observations in the circle.

2. Record the number of colonies on each plate.
   - Label each circle using the label on the plate it represents.
   - Record the number of colonies in the correct circle.
   - If there are too many colonies to count, record "TMTC".
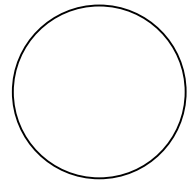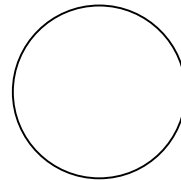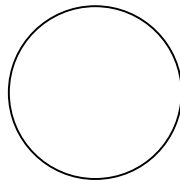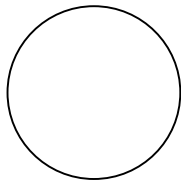
Calculate the Transformation Efficiency (see Transformation Efficiency page).
   - Record the Transformation Efficiency in the correct circle above.

1. What is the evidence that cells are resistant (or not) to ampicillin?

2. What is the evidence that cells are producing the enzyme β-galactosidase?

3. Were the results as you expected? Explain.

4. Suppose a new employee conducts this lab and the C tube cells grow on an LB/amp/X-gal plate.

    What could have gone wrong? Suggest a testable hypothesis to explain this result.

5. What are three factors that might influence the success of this transformation procedure?

6. Why would it be good for a bacterium (and bad for us) to maintain a plasmid that carries resistance to an antibiotic? What if the antibiotic is not in that bacterium's environment?

TRANSFORMATION EFFICIENCY (USE EITHER METHOD)

CALCULATION OF TRANSFORMATION EFFICIENCY METHOD #1

Calculating the transformation efficiency to quantify the success of your transformation experiment can be likened to calculating the fuel efficiency of your car. Transformation efficiency is expressed as the number of antibiotic-resistant colonies per microgram of DNA used to transform the cells, while fuel efficiency is reported as miles/gallon of gasoline consumed. If you put 1 quart of gasoline (0.25 gallon) in your vehicle and measured the distance in miles you drove before running out of gas you could use that information to estimate the number of miles you could have driven if you had put 1 gallon of gas in your vehicle. For example, if you were able to go 5 miles with 0.25 gallons of gas you would have been able to drive approximately 20 miles if you would have put 1 gallon of gas in the car [1 gallon/ 0.25 gallon = 4; (4)(5 miles) = 20 miles].

In the case of transformation efficiency the number of colonies can be equated to the miles the car travels and the plasmid used to transform the bacteria can be equated to the gasoline. As in the fuel efficiency calculation given above, you did not use 1 μg of pBLU DNA (1 gallon of gas) in your experiment, you used a fraction of 1 μg. You will calculate the amount of pBLU DNA you actually added to the bacterial in step 1 below. In addition, you did not plate all of the transformed bacteria, you plated a fraction of the bacteria that was mixed with the pBLU DNA. You will calculate the faction of the total volume of bacteria that was spread on the plate in step 2 below. Once you have determined the mass of plasmid DNA used to transform the bacteria and the faction of transformed bacteria you spread on the plate, you can calculate the mass of the plasmid DNA that was used to transform the bacteria that were actually spread on the plate (step 3). In step 4 you divide the number of blue colonies on your plate by the amount of plasmid DNA used to transform the bacteria spread on the plate (calculated value from step 3). For example, if you count 100 colonies and you calculate that you used 0.04 μg (40 ng) of plasmid DNA to transform the bacteria that were actually spread on the plate you know that you obtained 100 colonies/0.04 μg of DNA (5 miles/0.25 gallons). But you want to know how many blue colonies you would get if you used 1 μg of pBLU not 0.04 μg of plasmid. To get your answer you need to extrapolate from the data you have. 1 μg/0.04 μg = 25, so if you used 1 μg of DNA to transform bacteria you would expect 25 x more colonies or 2,500 colonies (100 colonies x 25 = 2,500) or as demonstrated in step 4, 100 colonies/0.04 μg = 2,500 colonies.

Note: symbols connect the places where the answer for one calculation is used in the next calculation.

Determine the following:
1. Mass of pBLU DNA in tube (Step 1 #7):
   ♥Mass of p BLU = [concentration of pBLU ($μg/μl$)] [volume put in tube ($μl$)]
   ♥Mass of p BLU = _____

2. Portion (fraction) of bacterial suspension from the tube that actually went on to the plate (Step 2 #3 & #4):
   ♣Fraction spread on plate = volume ($μl$) put on plate/ volume ($μl$) in tube.
   ♣Fraction spread on plate = _____

3. Mass of pBLU in the portion spread on plate:
   ♦Fractional mass of pBLU on plate = (♥Mass of p BLU) (♣Fraction spread on plate)
   ♦Fractional mass of pBLU on plate = _____

4. Transformation efficiency, or the number of colonies produced from each microgram of pBLU
   ♠Transformation Efficiency = # of colonies counted on your plate/ ♦Fractional mass of pBLU
   ♠Transformation Efficiency = _____

CALCULATION OF TRANSFORMATION EFFICIENCY METHOD #2

= # of colonies you count on LB/amp/X-gal plate

Transformation Efficiency = $\dfrac{\text{\# of antibiotic-resistant colonies}}{\textit{microgram (µg) of plasmid}}$

microgram (µg) of plasmid = mass of pBLU added (µg) **X** *portion of the volume actually spread on plate*
to the reaction tube

mass of pBLU added (µg) = concentration of pBLU (µg/µl) **X** volume of pBLU (ul)
to the reaction tube

portion of the volume actually spread on plate = $\dfrac{\textit{volume of cell suspension spread on plate (µl)}}{\textit{volume of suspension in tube (µl)}}$

Genes R Us

Confidential
Optimization Proposal

Name _____   Team Name_____

Date_____   Period _____

Now that you are familiar with S.O.P. #T-1, it is time to fine-tune, or optimize, the transformation so that you increase the number of transformants obtained.

As a team, choose a variable (something you can change) by looking at the basic protocol. Possibilities include using a different positively charged salt solution, changing its concentration, varying the amount of plasmid, changing the heat shock temperature, or changing the time of heat shock, to name just a few. (Think of the questions you had while you were working through the basic procedure.) Discuss your proposal with the Head of Molecular Biology.

1. What variable do you propose to change? _____

    _____
    _____
    _____
    _____

2. Why do you think changing this variable will improve the transformation efficiency? _____

    _____
    _____
    _____

3. What controls will you use to make sure that any effect you see is truly due to changing one variable? _____

    _____
    _____
    _____
    _____

4. How will you know if your experiment has improved the transformation efficiency? _____

    _____
    _____
    _____
    _____

5. Discuss any potential problems: (continue on back if necessary) _____

    _____

*PBLU® **TECHNICAL INFORMATION SHEET***

## X-GAL/β-GALACTOSIDASE INFORMATION

- Some strains of *E. coli*, a common bacterium, are incapable of hydrolyzing (splitting the molecule using water) the sugar lactose, found in milk. This is, in part, because they lack the gene for the enzyme beta-galactosidase (β-gal). The pBLU® plasmid contains the β-gal gene. If *E. coli* acquires this plasmid, it acquires the ability to digest lactose. However, if we feed the bacteria not lactose, but a lactose analog -- a substance that is chemically similar to lactose called X-gal, we'll make Indo-Blu.
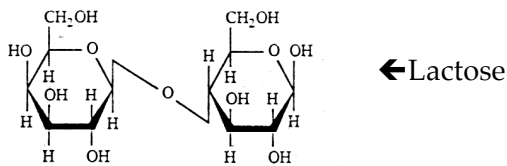
←Lactose

- X-gal is just a convenient way to say a complicated chemical name. X-gal's real name is 5-bromo-4-chloro-3-indolyl-β-D-galactoside. The structure is shown below. Galactose is the large ring-shaped structure; it's the sugar group. The dye color is generated when the sugar is clipped off from the 5-bromo-4-chloro-3-indolyl group. The β indicates where on the sugar the two groups are connected. Do you think the galactose or the rest is responsible for the color?
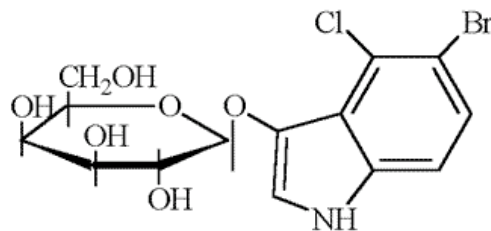
X-gal

Reference:
Sambrook, et al. **Molecular Cloning: A Laboratory Manual**, Cold Spring Harbor Laboratory, B. 14, p186 (**1989**).