



Genes
R
Us

Genes-R-Us Pre-Employment Interview

1

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 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? _____

12. What is your desired salary? Final salary offered will be dependent on experience, educational background, and a probationary period.
\$ _____

Confidential



Genes-R-Us Briefing and Assignment

Some genes and their products have enormous commercial value. Insulin is a good example, as are the enzymes added to laundry detergent for removing stains and restriction enzymes for DNA cutting, to name just a few examples. Scientists in the R & D Division of your biotechnology company, Genes-R-Us, Inc., have developed a new dye for blue jeans, code-named Indo-Blu. Jeans treated with this fabulous blue dye fade to just the way consumers like them. Large quantities of this dye can be made by a chemical lab, but the process uses chemicals toxic to the environment. However, when produced by bacteria, this process is environmentally safe. Indo-Blu clearly has incredible value, and it needs to be produced in large quantities, fast. Based on your interview and the recommendation of your science teacher, you've been hired! You'll start your job by being trained with a team in one step of the process now in use. Then, your next assignment will be to improve this step in the process of making Indo-Blu.

How is this dye produced? Scientists can insert genes into bacteria — using a circular piece of DNA called a plasmid — and use them as factories to produce large quantities of that gene or its product. As in most biotech companies, we usually use highly specialized, harmless strains of the bacterium Escherichia coli, or E. coli for short. The process of giving bacteria (or another organism) a new gene is called transformation.

Here's where your team comes in. We plan many new products using the approach of transforming E. coli with genetically engineered plasmids. Your job is to improve this step of the transformation process, making a gene product in larger quantities. The gene responsible for making Indo-Blu codes for an enzyme called beta-galactosidase, or β -gal for short. β -gal normally modifies lactose, the sugar in milk. However, instead of lactose, we give the bacteria X-gal, which chemically looks like lactose. β -gal splits the sugar group off the X-gal, producing the blue dye.

How do you know if a plasmid carrying the β -gal gene was taken in by the bacteria? The colony will be blue, whereas ordinary bacterial colonies will be white. Transformation is a rare event, though, so to make it easier to find transformants (a transformed bacterium), another gene was included on this plasmid. Presence of this gene (for ampicillin resistance) allows E. coli which have been transformed to grow in the presence of ampicillin, an antibiotic that otherwise kills bacteria. How well your transformation went will be measured by how many bacterial colonies able to grow in the presence of ampicillin you obtain.

What does the process of transformation involve? First, you must grow the bacteria which you're going to use for transformation (the head of the Molecular Biology group may have done this for you already). In this case, we're using E. coli strain JM101. Then, you must make them "competent," or able, to accept new DNA. The method you're going to use involves coating the bacteria and the DNA with positively charged molecules, to

minimize charge repulsion of the DNA by the bacteria. Then, you'll "shock" the bacteria with heat to push the DNA into pores in the bacteria. To see the results of the transformation, you plate the bacteria onto a nutrient, solid support (LB-agar). If you put the bacteria onto an agar plate containing ampicillin, they will only grow if they've received the plasmid. If the agar plate contains X-gal, the β -galactosidase gene product (an enzyme) will convert X-gal into Indo-Blu, our dye. The bacteria can't move, so wherever they landed they keep dividing in the same area. Small numbers of bacteria can't 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 bacteria, each of which arose from a single bacterial cell.

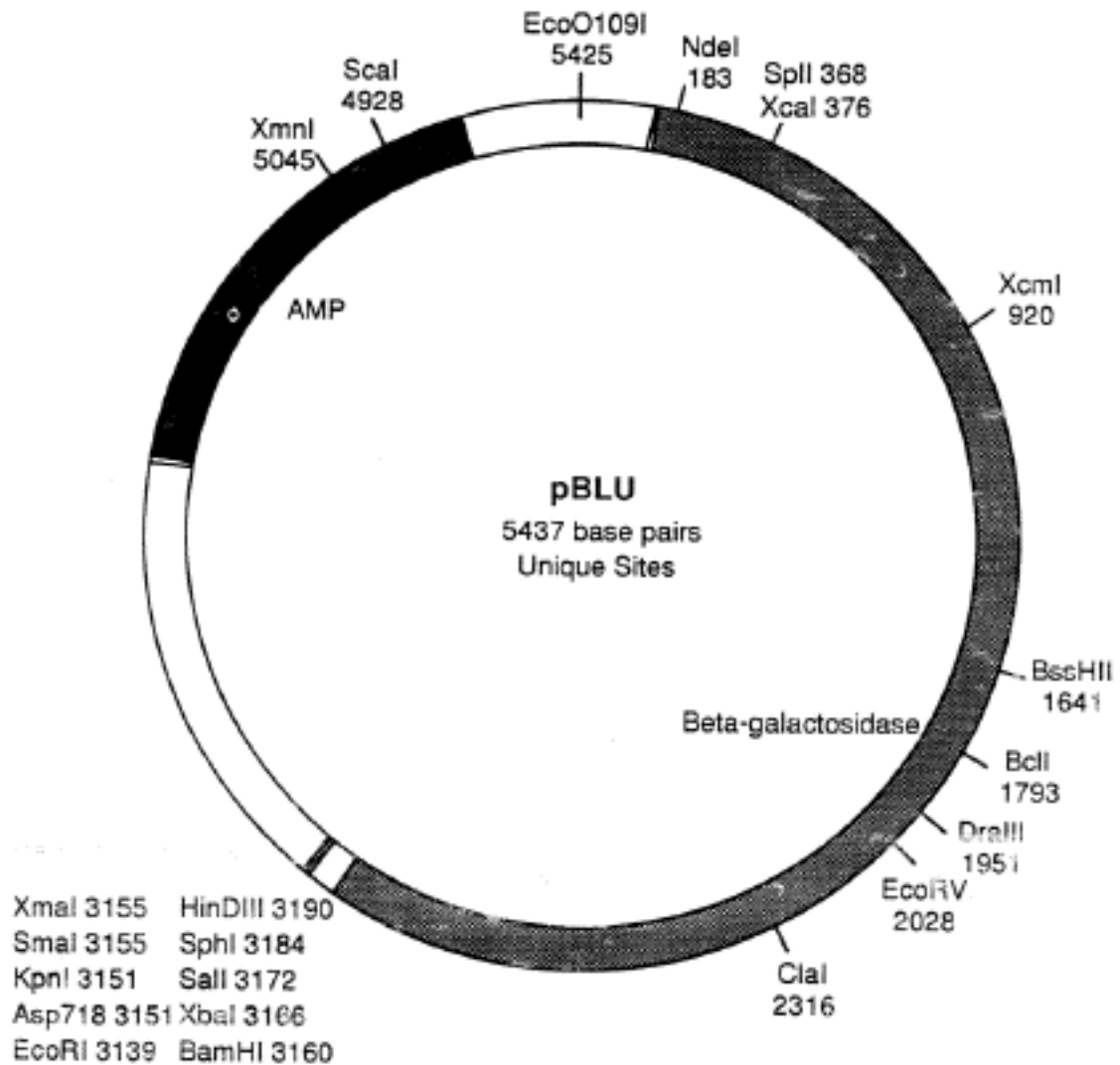
It's important, in any experiment, to use proper controls. To know if something has worked, or changed, you have to have something to compare to. A control is an experimental sample which is treated exactly the same, except for the one factor you're looking at. In the case of transformation, we treat another sample of bacteria (the control) exactly the same as the one we're transforming, except the control won't get any pBLU[®] DNA. In all other ways, the control sample is treated the same as the experimental sample. Another use for controls is to help you understand what happened if things go wrong. For example, say 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 your control, untransformed plate are alive, you can rule out that the procedure killed the bacteria (unless there's something in your DNA that killed the bacteria). If they're dead too, there was something major wrong. From lots of experience, the head of the Molecular Biology Division recommends using controls in all our work.

As part of your job assignment at Genes-R-Us, your team has been given the urgent task of increasing the efficiency of transformation. The higher the efficiency of transformation, the more bacteria that contain the gene you'll get, and the more likely that certain rare genes will be contained in the bacteria. Scientists will save a lot of time by using your product, and use fewer materials which might harm the environment. Therefore, achieving the maximum transformation efficiency will mean your company will be selling its product to more scientists, profits will soar, and your team will get a big bonus. This is part of our company's long range plans to increase the efficiency of all new product development.

However, other companies and other teams within your own company are working on this question too, so there's no time to waste. Watch the training video to get an introduction to the techniques you need. Then you will become familiar with our standard operating procedure (SOP), practicing it once using a basic protocol. Using appropriate controls (remember your boss), your team will make a proposal to modify one aspect of the transformation protocol. If your boss thinks there is enough time, your team will experimentally determine if your proposed modification improves the transformation efficiency.



pBLU[®] Plasmid Map



From DNA Science, Micklos & Freyer, 1995



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S.O.P #T-1: Transformation & Expression of pBLU[®]

Plan

- become familiar with the basic transformation method by transforming *E. coli* with a plasmid carrying genes for beta-galactosidase production and ampicillin resistance
- assess the success of transformation by plating on agar containing X-gal and ampicillin
- use proper controls
- prepare a research proposal in which your team identifies one variable to modify in the S.O.P. to increase the number of transformant colonies producing Indo-Blu. If R & D approves, you will test your proposed modification.

Materials Needed per Team (available in stockroom or from head of Molecular Biology)

pBLU [®] plasmid [0.01 µg/µl]	container for waste
<i>E. coli</i> stock plate (strain JM 101)	crushed ice, in containers
2 microcentrifuge tubes of 250µl cold CaCl ₂	disinfectant (10% bleach or 70% alcohol)
(calcium chloride) solution [50mM]	permanent marker pen
sterile graduated transfer pipets	bacterial spreading rod
metal container of sterile toothpicks	alcohol lamp — optional
sterile disposable or metal inoculating loops	dish of alcohol — optional
sterile distilled water	rack for microcentrifuge tubes
sterile Luria broth (LB)	37°C incubator
2 LB plates (2-3 days old)	42°C water bath
2 LB/X-gal/ampicillin plates (2-3 days old)	blue floating rack for waterbath

- S.O.P. #T-1 is our standard protocol 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 label plates with your team name. Make sure the head of Molecular Biology knows who the members of each 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. See the training video or ask the head of Molecular Biology.
- If you use alcohol lamps: they are fire hazards. Keep long hair and shirt sleeves safely away from flames. Wear goggles. Know where the fire extinguisher is.

- If you spill bacterial culture or alcohol, notify the head of Molecular Biology immediately.

Procedure:

Day 1

1. Remove existing bacteria from your table top by cleaning with disinfectant. Wash your hands! Obtain two sterile 1.5 ml microfuge tubes containing 250 μ l cold calcium chloride each. Label one tube "pBLU" and the other "C" (for control). Label both tubes with your team name.



2. Place both tubes on ice.

3. Add bacteria to both tubes:

- a) Carefully remove a sterile toothpick from the metal tube. Pick or gently scrape up a large, single colony from the stock plate.

- b) Insert the toothpick into the tube labeled "pBLU" and vigorously tap or twirl the toothpick against the side of the tube. Look closely to make sure the cell clump has come off.

- c) Suspend cells by pipeting 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.

- d) Return the "pBLU" tube to ice.

- e) Using a new toothpick, transfer a large cell mass to the tube labeled "C" just as you did for the "pBLU" tube.

- f) Return the "C" tube to ice. Both tubes should now be on ice.

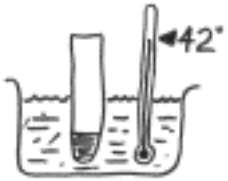
4. Using a disposable plastic inoculating loop, or a just-flamed metal loop, add pBLU[®] plasmid to the "pBLU" tube ONLY. Hold the loop handle by the pointed end and dip it into the tube of pBLU[®] plasmid. Withdraw the loop and check that there is liquid in the loop (like when you're going to blow soap bubbles). This amount of liquid is 10 μ l. Dip the loop containing the plasmid into the cell suspension, swish it around, then remove the loop and discard it (if disposable) or flame it (if metal). Mix the tube contents by tapping or flicking the tube with your fingers.

5. Return the "pBLU" tube to ice.

6. Add 10 μ l of sterile distilled water to the "C" tube ONLY. Use a loop as above. Mix by tapping the tube with your fingers.

7. Return the "C" tube to ice.

8. Wait 15 minutes (this gives time for the pBLU[®] 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.



9. When the 15 min. wait period is up, pick up your ice bucket containing the "pBLU[®]" and "C" tubes and move next to the 42°C water bath (check the temperature). Transfer both tubes from the ice to the water bath for a heat shock of exactly 90 seconds.

IT IS ESSENTIAL THAT CELLS BE GIVEN A SHARP AND DISTINCT HEAT SHOCK

10. Immediately return tubes to ice for at least 2 minutes.



11. Add 250 µl of sterile Luria broth (LB) to each tube, using a new sterile transfer pipet for each one, and tap each tube with your finger to mix its contents. See the illustration below to understand the volume graduations of the transfer pipet.

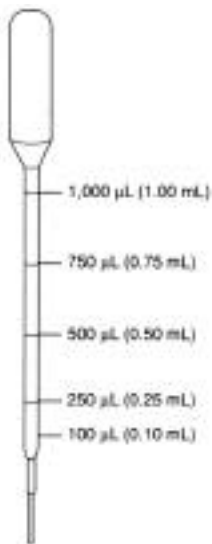


Optional: incubate the tubes for 15-30 min. at 37°C before plating (see Day 2). This allows the bacteria to recover and will increase the number of transformed bacteria.

OR



If stopping at this point, set the tubes in a rack and store in the refrigerator until the next day.



Transfer pipet. Keep the tip sterile. Squeeze the bulb to empty it of air, insert into the solution, and slowly let the bulb expand, sucking up solution to the graduation mark you need. Hold that position, remove from the solution, and add to your new container. Hint: if you just want a small volume, try squeezing the shaft of the pipet instead of the bulb.

Upon completion:

- Dispose of designated materials in the appropriate places.
- Leave equipment as you found it.
- Check that your work station is in order.
- Wash your hands.

Day 2: Plating Cells

Purpose: to spread individual cells evenly over the surface of the plate for overnight growth into visible colonies. We'll use the number of colonies to calculate the transformation efficiency.

- Now you need to plate the cells. Remove existing bacteria from your table top by cleaning with disinfectant and wash your hands.
- Obtain and label your plates. 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 bottom of
 - an LB plate
 - an LB/ X-gal/ampicillin plate.
 - Put a "C" and your team name and class period on the bottom of
 - an LB plate
 - an LB/ X-gal/ampicillin plate.
- Start with the "C" tube. Carefully remove a sterile transfer pipet from the container, resuspend the bacteria by pipetting up and down gently, and draw up 100 μ l (refer to the drawing for step 11). Drip this onto the center of one of your two plates labeled "C." Repeat for the other plate.
- Spread cells over the surfaces of each plate as follows:

**If you're using an alcohol lamp:**

- Dip your spreading rod into a dish of alcohol, allow excess to drip off, then pass through flame to ignite the alcohol.
- Allow alcohol to burn off the spreading rod, away from the dish of alcohol.
- Cool the spreading rod by touching it to the agar in an area off to the side, away from your pool of cells.
- Use the rod to evenly spread the cell suspension over the surface of the plate. (This will remind you of frosting a cake, or spreading peanut butter on an sandwich.)

- e) Flame the rod after each plate.

OR If you're using reusable plastic spreaders:

- a) Carefully remove a sterilized plastic spreader from the bag.
 - b) Use the rod to evenly spread the cell suspension over the surface of the plate. (This will remind you of frosting a cake, or spreading peanut butter on a sandwich.)
 - c) Place the used spreader in a waste container with 10% bleach in it. Use a new spreader for each plate. The Genes-R-Us Dishwashing staff will wash and re-sterilize the spreaders, so don't throw them away.
16. Resuspend, then transfer 100 μ l of cells from the "pBLU" tube to the center of each of your "pBLU" plates.
 17. Spread the cell suspension over the plates' surfaces as described in step # 15.
 18. Allow several minutes for the plates' surfaces to absorb the cell suspensions. Then, place your four plates, **upside-down**, in the 37°C incubator.
 19. Conclude by disinfecting your table top and washing your hands.

Upon completion:

- Dispose of designated materials in the appropriate places.
- Leave equipment as you found it.
- Check that your work station is in order.
- Wash your hands.

Day 3

20. Examine the plates and analyze them for bacterial growth and colony color. Make sure your controls have performed as expected. Fill out your report and discuss your team's results in class.
21. As a team, discuss the experiment you've done and which variable you want to try changing. Individually or as a team (check with the head of Molecular Biology) fill out your report. Calculate the transformation efficiency for the experiment you've just done. This way you'll know when you've improved the transformation efficiency (you'll have something to compare to). Then, write your proposal on the "Proposal" sheet.
22. The head of Molecular Biology will review your work and let you know if you are to perform the experiment your team has proposed.

Name _____ Team Name _____

Date _____ Period _____

pBLU[®] TRANSFORMATION (S.O.P. #T-1) Report

Observations during Procedure: the head of Molecular Biology will tell you what format to use.

Results

1. Having examined your four plates, record the number of colonies on each. (If there are too many colonies to count, record "TMTC".)

	# colonies on LB agar plate	# colonies on LB agar/X-gal/amp
Control cells		
pBLU-transformed cells		

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

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

4. Were the results as you expected? Explain.

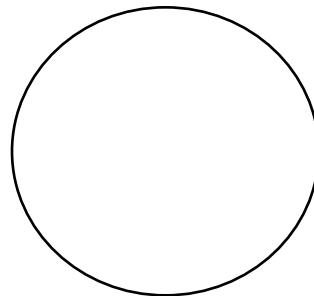
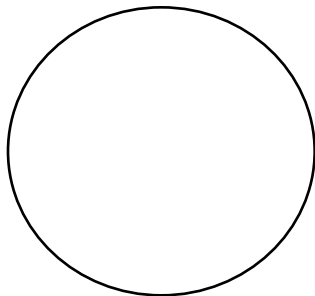
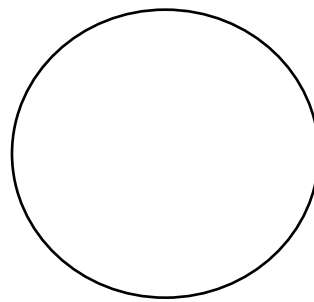
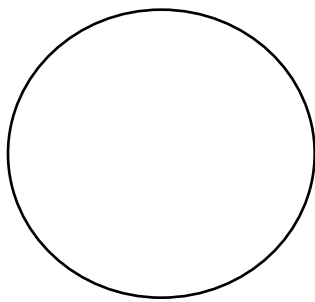
5. Suppose a new employee conducts this lab and the C tube cells grow on an LB/amp/X-gal agar plate. Suggest a testable hypothesis to explain this result.

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

7. From what you've learned about the possible mechanism of transformation, do you think it's essential that CaCl_2 be used, or could another cation (positively charged molecule) be used?

8. Why would it be useful 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?

9. Supplement your report by drawing your results on the plates below. Make sure you label what kind of plate it was and how you treated the bacteria you put on it.



Transformation Efficiency = # of antibiotic-resistant colonies per microgram (μg) of plasmid

$$= \left(\frac{\text{\# of ampicillin-resistant colonies that grow on the LB/Amp/X-gal plate}}{\text{Mass of pBLU plasmid DNA that was used to transform these cells (in } \mu\text{g)}} \right)$$

You count this from your plates

This must be calculated and it will be the same for all teams using the same protocol

$$\text{Mass of pBLU plasmid DNA} = \left(\text{Mass of pBLU added to the reaction tube in } \mu\text{g} \right) \left(\text{Fraction of the reaction tube's volume that was spread on the plate, in } \mu\text{l} \right)$$

How to calculate this:

$$\left(\text{Concentration of pBLU, in } \mu\text{g}/\mu\text{l} \right) \left(\text{Volume of pBLU used, in } \mu\text{l} \right)$$

How to calculate this:

$$\left(\frac{\text{Volume of cell suspension spread at step 14, in } \mu\text{l}}{\text{Total volume of cell suspension in tube, in } \mu\text{l}} \right)$$

After Sue Black, '96
Inglemoor HS, Bothell WA

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Research
Proposal

Team name _____ Date _____

Team members _____

Now that you've gotten familiar with S.O.P. #T-1, it's 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 are 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 it. Discuss your proposal with the Head of Molecular Biology.

What do you propose to change? _____

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

What controls will you use to make sure that any effect you see is truly because of changing one variable? _____

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

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

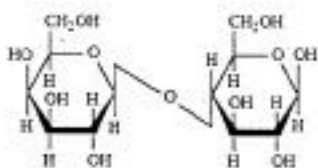


Genes
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Genes-R-Us
pBLU[®] Technical Information Sheet

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. Its structure and some information are 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?

Product	Cat. No.	Size	Qty.	Price
X-gal	15520-034	100 mg	each	\$ 42
	15520-018	1 g	each	197
	15520-026	5 g	each	750

X-gal (5-bromo-4-chloro-3-indolyl- β -D-galactoside), a histochemical substrate for β -galactosidase, yields a blue precipitate upon hydrolysis, making it suitable for use in immunoblotting or immunocytochemical assays. Bacteria containing active β -galactosidase produce blue colonies when grown on plates containing X-gal. The GIBCO BRL X-gal is supplied as white crystals and is soluble in dimethylformamide.

Analytical specifications:

molecular weight 408.64
appearance white crystals
purity $\geq 95\%$
melting point (decomposition) 228°C-232°C
dioxane none detected

Performance and quality testing: Performance as a substrate for β -galactosidase is evaluated in an enzymatic assay.

Recommended storage condition: -20°C.

See also:
Histo-gal (Cat. Nos. 15519-030-028), page 12-36.
LIBRARY EFFICIENCY DH5 α MCR™ Competent Cells (Cat. No. 18289-017), page 9-11.
LIBRARY EFFICIENCY DH11S™ Competent Cells (Cat. No. 18307-017), page 9-7.
MAX EFFICIENCY DH5 α F10™ Competent Cells (Cat. No. 18288-019), page 9-6.
MAX EFFICIENCY DH10Bip3™ Competent Cells (Cat. No. 18309-013), page 9-4.

From the Gibco BRL/Life Technologies Catalog; 1-800-828-6686.

Plasmid Information

Plasmids such as the one from which pBLU[®] was derived (pUC19) are used by laboratories to identify transformants which not only have received the plasmid, but which bear, by inference, a piece of DNA which has been added to the plasmid sequence. If you want to make more of a particular sequence of DNA, you must insert it into a plasmid so that you can use bacteria to generate large quantities of your sequence of interest. However, the process of insertion happens at relatively low efficiency. This “insertion” is more properly called recombination: cutting the plasmid and your DNA with the same enzymes, adding them together and ligating the pieces together. Therefore, these plasmids have been designed with the cloning site within the β -galactosidase gene. A successful insertion will interrupt the transcription of β -gal and result in bacteria which are white (but still ampicillin resistant; you haven't disturbed that gene). Bacteria which were successfully transformed, but not with a plasmid containing your insert, will be blue. Therefore, you are selecting not only for bacteria which got the plasmid (successfully transformed) but for those which have the piece of DNA you're after. Therefore, you would pick a colony which was white on an X-gal and ampicillin plate.

Bacteria Information

- The strain of *E. coli* we're using is called JM101, after Joachim Messing and his lab colleagues, the people who originally developed it. Maybe they tried 100 transformations with this strain before it worked!
- The genotype (a listing of particular genes it has we're interested in) is:

F' traD36 proA⁺ proB⁺ lacIq lacZ Δ M15/ supE thi Δ (lac-proAB)

All of this is a kind of shorthand, or code, to explain what form of a gene this strain has, or whether it has that gene at all. See the attached sheet for an explanation of some of these markers. For us, the key one is Δ (*lac-proAB*). This means the bacteria's original β -galactosidase gene is gone. The Δ symbol, Greek for the letter delta, stands for “deleted” (get it, starts with d!). So, when we add a β -galactosidase gene through transformation with pBLU, we restore the bacteria's original genotype.