EDVOTEK®

The Biotechnology Education Company ®

ATTENTION!
This experiment includes either BactoBeads™ or LyphoCells™. If you have received LyphoCells™, please refer to the addendum posted on the last page of this literature. If you have received the BactoBeads™, refer to the Pre-Lab Preparations on page 16.

EDVO-Kit #
221

Transformation of *E.coli* with pGal™

Storage: See Page 3 for specific storage instructions

EXPERIMENT OBJECTIVE:

The objective of this experiment module is to develop an understanding of bacterial transformation by plasmid DNA. This experiment introduces an opportunity to observe an acquired phenotypic trait of the transformed bacterial cells. The presence of blue bacterial colonies visually demonstrates the expression of a specific gene for the Lac^+ phenotype.

No IPTG used in this experiment.

Table of Contents

Material Safety Data Sheets can be found on our website:
www.edvotek.com

Experiment Components

Experiment # 221 is designed for 10 groups.

ATTENTION!

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Important READ ME!

Transformation experiments contain antibiotics which are used for the selection of transformed bacteria. Students who have allergies to antibiotics such as penicilllin, ampicillin, kanamycin or tetracycine should not participate in this experiment.

All components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

None of the experiment components are derived from human sources.

Component		Storage	Check (✓)
A	BactoBeads™ or LyphoCells™	4° C with desiccant (included)	❑
B	Supercoiled pGal™ (blue colony)	Freezer	❑
C	Control Buffer (no DNA)	Freezer	❑
D	Ampicillin	Freezer	❑
E	X-Gal in solvent (pre-measured)	Freezer	❑
•	$CaCl_2$	Room Temp.	❑

Reagents & Supplies

Store all components below at Room Temp.

Component	Check (✓)
• Bottle ReadyPour™ Luria Broth Agar, sterile (also referred to as "ReadyPour Agar")	❑
• Bottle Luria Broth Medium for Recovery, sterile (also referred to as "Recovery Broth")	❑
• Petri plates, small	❑
• Petri plates, large	❑
• Plastic microtipped transfer pipets	❑
• Wrapped 10 ml pipet (sterile)	❑
• Toothpicks (sterile)	❑
• Inoculating loops (sterile)	❑
• Microcentrifuge tubes	❑

Requirements

- Automatic Micropipet (5-50 µl) and tips
- Two Water baths (37°C and 42°C)
- Thermometer
- Incubation Oven (37°C)
- Pipet pumps or bulbs
- Ice
- Marking pens
- Bunsen burner, hot plate or microwave oven
- Hot gloves

* If a second water bath is not available, water can be heated to 42° C in a beaker. The cells will require this temperature for only a few minutes. Alternatively, 42° C water can be put in a small styrofoam container with a cover. The temperature needs to be held at 42°C.

Background Information

Bacterial Transformation

Bacterial transformation is of central importance in molecular biology. It allows for the introduction of genetically engineered or naturally occurring plasmids in bacterial cells. This makes possible the propagation, genetic expression and isolation of DNA plasmids.

The transformation process involves the uptake of exogenous DNA by cells which results in a newly acquired genetic trait that is stable and heritable. Bacterial cells must be in a particular physiological state before they can be transformed. This state is referred to as competency. Competency can occur naturally in certain species of *Haemophilus* and *Bacillus* when the levels of nutrients and oxygen are low. Competent *Haemophilus* expresses a membrane associated transport complex which binds and transfers certain DNA molecules from the medium into the cell where they are incorporated and their genes are expressed. In nature, the source of external DNA is from other cells.

Most of the current transformation experiments involve *E. coli*. This organism does not enter a stage of competency unless artificially induced. Treatment to achieve competency involves the use of chloride salts, such as calcium chloride, and sudden hot and cold temperature changes. The metal ions and temperature changes affect the structure and permeability of the cell wall and membrane so that DNA molecules can be absorbed by the bacteria. The mechanism of DNA transport in the cell still is not fully understood. Competent *E. coli* cells are fragile and must be treated carefully.

The transformation efficiency is defined by the number of transformants obtained per microgram of DNA. For example, 10 nanograms of DNA were used for a transformation and the cells were allowed to recover in a final volume of 1 ml. One tenth of this volume was plated and produced 100 colonies on a selective agar medium. Therefore, 1000 transformants are present per ml. Keeping in mind that each colony grew from one transformed cell, the efficiency would be 1000/0.01ug = 1 x 10^5. Transformation efficiencies of 10^5 to 10^6 are more than sufficient for most subcloning experiments. When the cloning of single copy genes from genomic DNA is done, the required efficiencies are 10^7 to 10^8.

$$\frac{\text{Number of transformants}}{\mu\text{g of DNA}} \times \frac{\text{final vol at recovery (ml)}}{\text{vol plated (ml)}} = \text{Number of transformants per } \mu\text{g}$$

Specific example:

$$\frac{100 \text{ transformants}}{0.01\ \mu\text{g}} \times \frac{1 \text{ ml}}{0.1 \text{ ml}} = 100{,}000\ (1 \times 10^5) \text{ transformants per } \mu\text{g}$$

Figure 1:
Bacterial Transformation Efficiency Calculation

The determination for transformation efficiency in this case is outlined in Figure 1. Transformation efficiencies generally range from 1 x 10^4 to 1 x 10^7 cells per microgram of DNA. There are special procedures which can produce cells having transformation efficiencies approaching 10^{10}. However, transformation is never 100% efficient. Approximately 1 in every 10,000 cells successfully incorporates plasmid DNA in preparations having average competency. However, there is such a large number of cells in a sample (typically 1 x 10^9) that only a small fraction needs to be transformed to obtain colonies on a plate. The same volume of recovered cells plated on selective (contains antibiotic) and nonselective agar medium will yield vastly different numbers of cells. The nonselective medium will have many more growing cells that form a bcterial lawn.

Bacterial Transformation

Many different plasmids serve as useful tools in molecular biology. One example is the pGal plasmid, present in multiple copies in specified host *E. coli* host cells. It contains 6751 base pairs and has been cleverly modified by genetic engineering. In the cell, it **does not** integrate into the bacterial chromosome, but replicates autonomously. The pGal plasmid contains the *E. coli* gene which codes for β-galactosidase. In the presence of artificial galactosides such as 5-Bromo-4 Chloro 3-indolyl-β-D-galactoside (X-Gal), pGal colonies appear blue when X-Gal is cleaved by β-galactosidase and forms a colored product.

This experiment has been designed to utilize EDVOTEK BactoBeads™ or LyphoCells™. It also contains the proprietary plasmid, pGal (Blue Colony), which was engineered by EDVOTEK. Plasmid pGal carries the complete gene for β-galactosidase. Since the host *E. coli* does not contain a β-galactosidase gene, only cells transformed by the pGal plasmid will produce the functional β-galactosidase enzyme. Cells that express β-galactosidase will cleave X-Gal and the pGal transformed colonies will be blue.

In addition to the expression and cleavage of X-Gal by β-galactosidase, transformation by pGal is also demonstrated by resistance to ampicillin. *E. coli* host cells used in this experiment are **not** naturally resistant to ampicillin. The plasmid pGal contains the gene which encodes for β-lactamase that inactivates ampicillin. *E. coli* cells transformed by pGal will express the resistance gene product β-lactamase as an extracellular enzyme excreted from *E. coli* cells. Once outside the cell, the enzyme diffuses into the surrounding medium and inactivates ampicillin.

With time, small "satellite" colonies may appear around a large blue colony. Cells in the small "satellite" or "feeder" colonies are not resistant to ampicillin and have not been transformed with the pGal plasmid. They are simply growing in a region of agar where β-lactamase has diffused and inactivated the antibiotic ampicillin. The number of satellite colonies increases if the concentration of ampicillin is low or the plates have incubated for longer times.

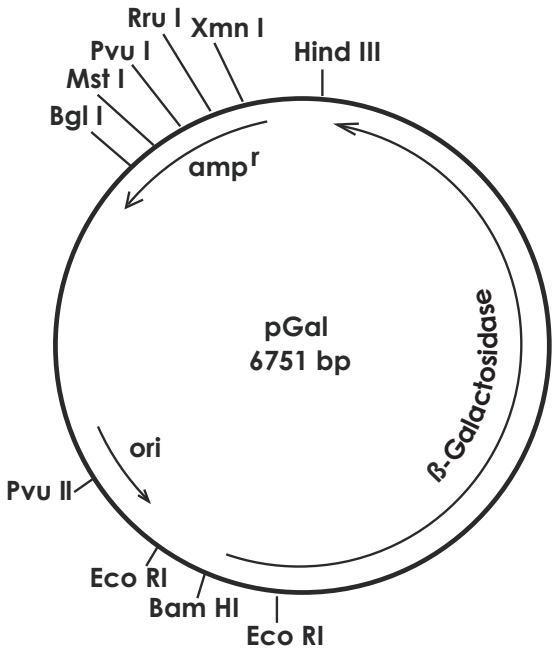


Figure 2:
DNA map of pGal.
Not all restriction enzymes are shown.

Background Information

Experiment Overview

BEFORE YOU START THE EXPERIMENT

1. Read all instructions before starting the experiment.
2. Write a hypothesis that reflects the experiment and predict experimental outcomes.

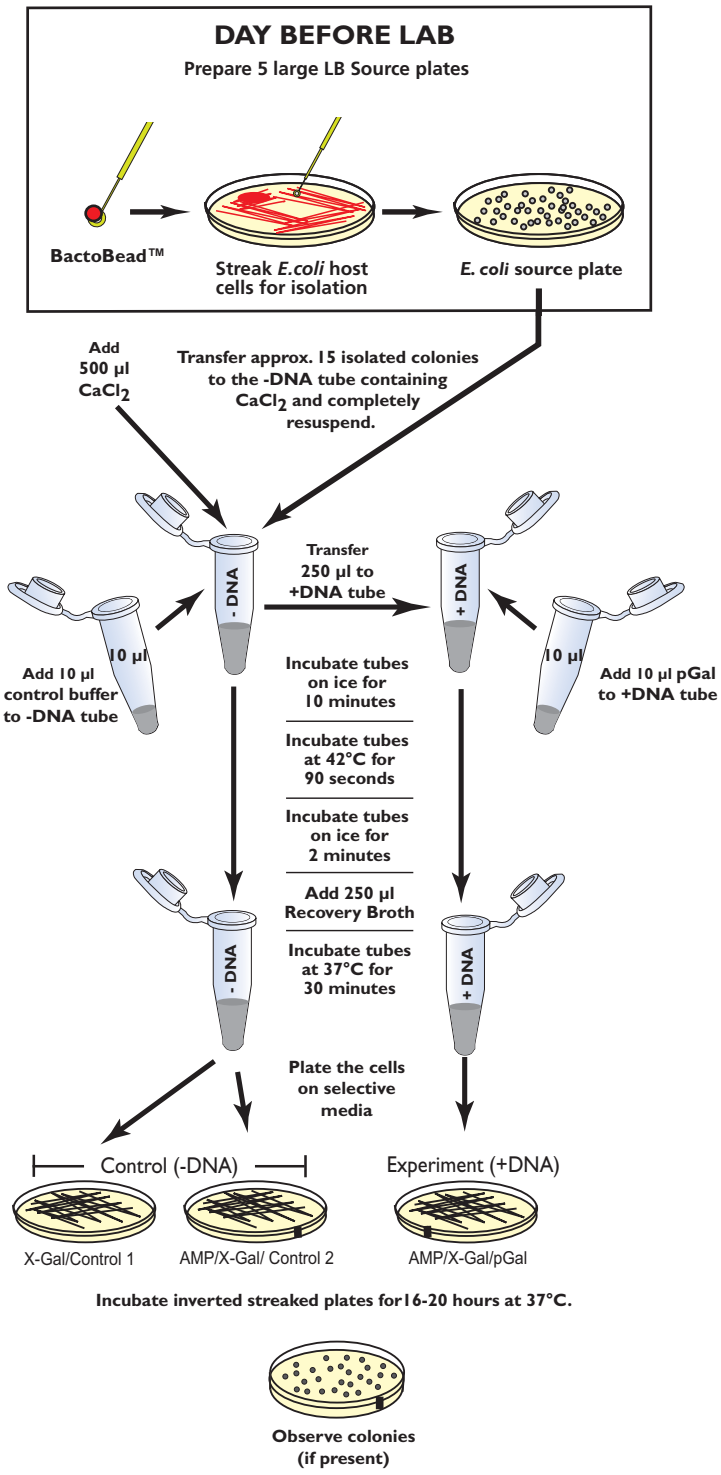
EXPERIMENT OBJECTIVE:

The objective of this experiment module is to develop an understanding of the biologic process of bacterial transformation by plasmid DNA. This experiment demonstrates the acquired Lac^+ phenotypic trait of the transformed bacterial cells as shown by the presence of blue bacterial colonies.

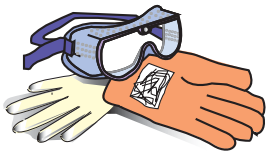
BRIEF DESCRIPTION OF EXPERIMENT:

In this experiment, students will transform host bacterial cells with a plasmid DNA. The transformants acquire antibiotic resistance and exhibit a blue color due to the incorporation and expression of β-galactosidase and ampicillin resistance genes. IPTG is not required since pGal™ contains the intact β-galactosidase gene. The number of transformants will be counted and the transformation efficiency will be determined.

Experiment Overview



Laboratory Safety



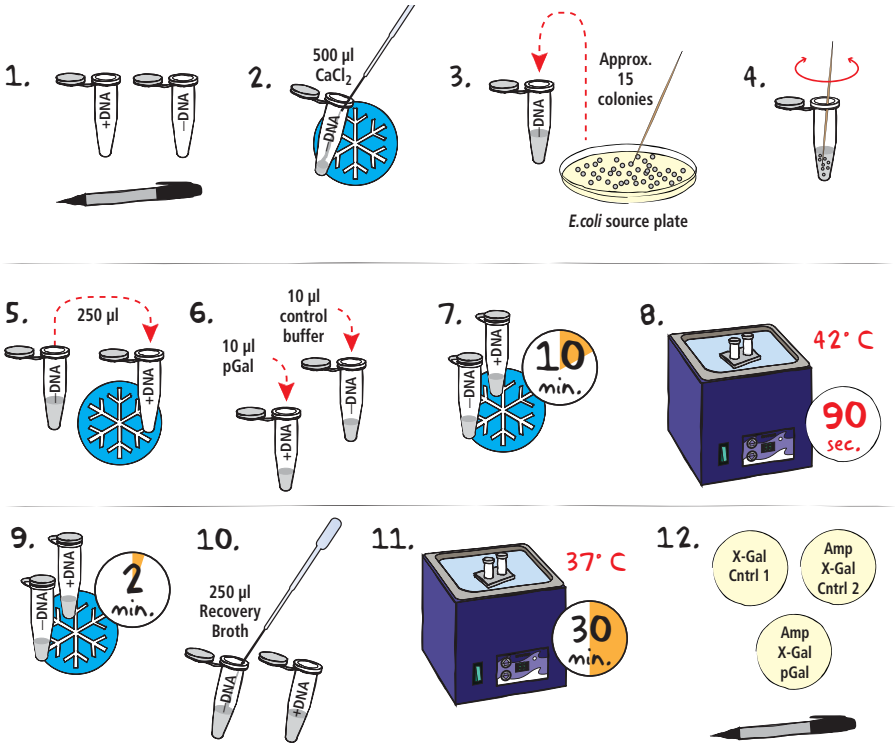
IMPORTANT READ ME!

Transformation experiments contain antibiotics to select for transformed bacteria. Students who have allergies to antibiotics such as penicilllin, ampicillin, kanamycin or tetracycine should not participate in this experiment.

1. Wear gloves and goggles while working in the laboratory.
2. Exercise extreme caution when working in the laboratory - you will be heating and melting agar, which could be dangerous if performed incorrectly.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS OR BULBS.
4. The *E. coli* bacteria used in this experiment is not considered pathogenic. Regardless, it is good practice to follow simple safety guidelines in handling and disposal of materials contaminated with bacteria.
   A. Wipe down the lab bench with a 10% bleach solution or a laboratory disinfectant.
   B. All materials, including petri plates, pipets, transfer pipets, loops and tubes, that come in contact with bacteria should be disinfected before disposal in the garbage. Disinfect materials as soon as possible after use in one of the following ways:
   - Autoclave at 121° C for 20 minutes.
     Tape several petri plates together and close tube caps before disposal. Collect all contaminated materials in an autoclavable, disposable bag. Seal the bag and place it in a metal tray to prevent any possibility of liquid medium or agar from spilling into the sterilizer chamber.
   - Soak in 10% bleach solution.
     Immerse petri plates, open tubes and other contaminated materials into a tub containing a 10% bleach solution. Soak the materials overnight and then discard. Wear gloves and goggles when working with bleach.
5. Always wash hands thoroughly with soap and water after working in the laboratory.
6. If you are unsure of something, ASK YOUR INSTRUCTOR!

Experiment Procedure

Transformation of *E. coli* with pGal™ (blue colony)



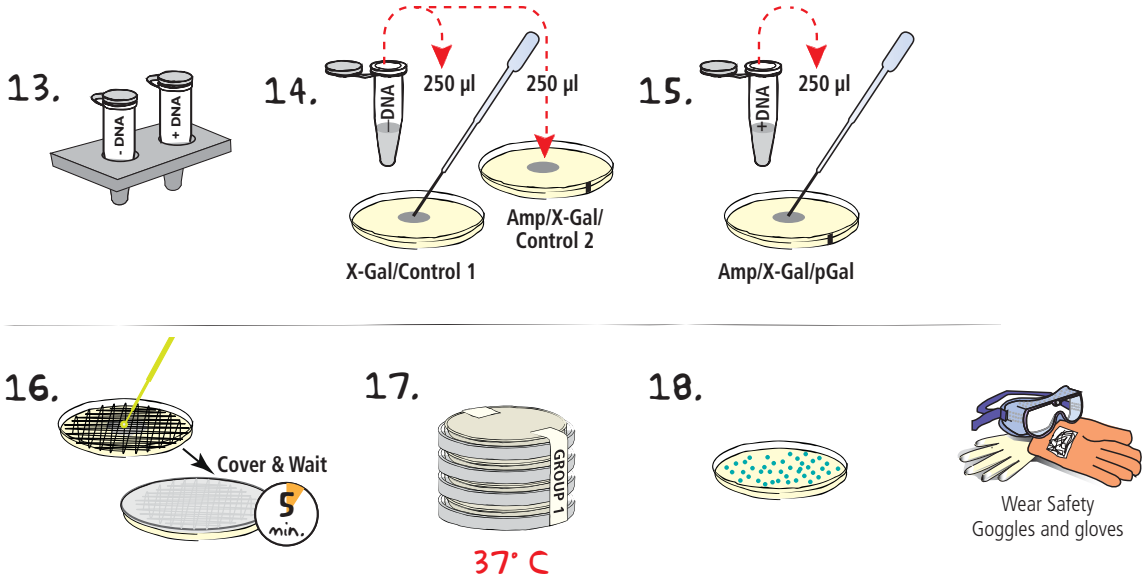
For best results, make sure that the cells are completely resuspended.

Make sure to keep the actual labels small!

1. **LABEL** one microcentrifuge tube with "+DNA" and a second microcentrifuge tube with "-DNA".
2. **TRANSFER** 500 µL ice-cold $CaCl_2$ solution into the "– DNA" tube using a sterile 1 mL pipet.
3. Using a toothpick, **TRANSFER** approx. 15 well-isolated colonies (each colony should be approx. 1-1.5 mm in size) from the *E. coli* source plate to the "-DNA" tube.
4. **TWIST** the toothpick between your fingers to free the cells. **RESUSPEND** the bacterial cells in the $CaCl_2$ solution by vortexing vigorously until no clumps of cells are visible and the cell suspension looks cloudy.
5. **TRANSFER** 250 µl of the cell suspension to the tube labeled "+ DNA". **PLACE** tubes on ice.
6. **ADD** 10 µl of pGal™ to the tube labeled "+ DNA". **ADD** 10 µl control buffer to the tube labeled "- DNA".
7. **INCUBATE** the tubes on ice for 10 minutes.
8. **PLACE** the transformation tubes in a 42° C water bath for 90 seconds.
9. Immediately **RETURN** the tubes to the ice bucket and **INCUBATE** for two minutes.
10. **TRANSFER** 250 µL of Recovery Broth to each tube using a sterile 1 mL pipet. Gently **MIX** by flicking the tube.
11. **INCUBATE** the cells for 30 minutes in a 37° C water bath.
12. While the cells are recovering, **LABEL** the bottom of three agar plates as indicated bellow:
    X-Gal/Control 1 (plate no stripe)
    Amp/ X-Gal/ Control 2 (plate with one stripe)
    Amp/X-Gal/pGal (plate with one stripe)

Experiment Procedure

Transformation of *E. coli* with pGal™



13. After the recovery period, **REMOVE** the tubes from the water bath and place them on the lab bench.
14. Using a sterile 1 ml pipet, **TRANSFER** 250 µL recovered cells from the tube labeled " –DNA " to the middle of the X-Gal/Control 1 plate and the Amp/X-Gal/Control 2 plate.
15. Using a new sterile 1 ml pipet, **TRANSFER** 250 µL recovered cells from the tube labeled " +DNA " to the middle of the Amp/X-Gal/pGal plate.
16. **SPREAD** the cells over the entire plate using an inoculating loop. Use one sterile loop to spread both -DNA samples. Change to a fresh loop before spreading the +DNA samples. Make sure the cells have been spread over the entire surface of the plates. **COVER** the plates and **WAIT** five minutes for the cell suspension to be absorbed by the agar.
17. **STACK** the plates on top of one another and **TAPE** them together. **LABEL** the plates with your initials or group number. After cells have been absorbed, **PLACE** the plates in the inverted position (agar side on top) in a 37° C bacterial incubation oven for overnight incubation (16-20 hours). If you do not have an incubator, colonies will form at room temperature in approximately 24 - 48 hours.
18. **OBSERVE** the transformation and control plates. For each of the plates, **RECORD** the following:
    - The number of colonies on the plate.
    - Color of the bacteria.

Experiment Summary:

E. coli from the source plate are resuspended in an ice-cold $CaCl_2$ solution. Plasmid DNA is added to half of the cells before they are "heat shocked" in a 42°C water bath. The heat shock step facilitates the entry of DNA into the bacterial cells. Recovery Broth is added to the cell suspension, and the bacteria are allowed to recover for 30 minutes at 37°C. This recovery period allows the bacteria to repair their cell walls and to express the antibiotic resistance gene. Lastly, the transformed *E. coli* are plated on LB plates and allowed to grow at 37°C overnight.

NOTE for Step 17:

It may take longer for the cells to absorb into the medium. Do not invert plates if cells have not completely been absorbed.

Experiment Results and Analysis

DATA COLLECTION

1. Observe the results you obtained on your transformation and control plates.

   Control Plates: (-) DNA
   - X-Gal/Control 1
   - Amp/X-Gal/Control 2

   Transformation Plate: (+) DNA
   - Amp/X-Gal/pGal

2 Draw and describe what you observe. For each of the plates, record the following:

   - How much bacterial growth do you observe? Determine a count.
   - What color are the bacteria?
   - Why do different members of your class have different transformation efficiencies?
   - If you did not get any results, what factors could be attributed to this fact?

DETERMINATION OF TRANSFORMATION EFFICIENCY

Transformation efficiency is a quantitative determination of the number of cells transformed per 1 µg of plasmid DNA. In essence, it is an indicator of the success of the transformation experiment.

You will calculate the transformation efficiency using the data collected from your experiment.

1. Count the number of colonies on the plate that is labeled: Amp/X-Gal/pGal

   A convenient method to keep track of counted colonies is to mark each colony with a lab marking pen on the outside of the plate.

2. Determine the transformation efficiency using the following formula:

$$\frac{\text{Number of transformants}}{\mu\text{g of DNA}} \times \frac{\text{final vol at recovery (ml)}}{\text{vol plated (ml)}} = \text{Number of transformants per µg}$$

Example:
Assume you observed 40 colonies:

$$\frac{40 \text{ transformants}}{0.05\ \mu\text{g}} \times \frac{0.5\text{ ml}}{0.25\text{ ml}} = 1600\ (1.6 \times 10^3) \text{ transformants per µg}$$

Quick Reference for Expt. 221:

50 ng (0.05 µg) of DNA is used.
The final volume at recovery is 0.50 ml
The volume plated is 0.25 ml

Study Questions

Answer the following study questions in your laboratory notebook or on a separate worksheet.

1. Did you observe any satellite colonies? Why are the satellite, feeder colonies white?
2. Why did the competent cells which did not receive DNA (control) fail to grow on the plates containing ampicillin?
3. Why are there so many cells growing on the X-Gal plate? What color are they?
4. What evidence do you have that transformation was successful?
5. What are some reasons why transformation may be unsuccessful?

Instructor's Guide

IMPORTANT READ ME!

Transformation experiments contain antibiotics which are used for the selection of transformed bacteria. Students who have allergies to antibiotics such as penicilllin, ampicillin, kanamycin or tetracycine should not participate in this experiment.

ADVANCE PREPARATION:

What to do:	Time Required:	When?	Page
Prepare LB Agar Plates	One hour	2-7 days before use	14
Prepare *E. coli* Source plates	20 minutes to streak plates; 16-20 hours to incubate plates	The day before performing the experiment	16
Dispense, control buffer, plasmid DNA,$CaCl_2$, and recovery broth	30 minutes	One day to 30 min. before performing the experiment	17

DAY OF THE EXPERIMENT:

What to do:	Time Required:	When?	Page
Equilibrate waterbaths at 37° C and 42° C; incubator at 37°C	10 minutes	One to two hours before performing the experiment	17
Perform laboratory experiment	50 minutes	The class period	9
Incubate cells at 37° C	16-20 hours	Overnight after the class period	10

RESULTS AND CLEAN UP:

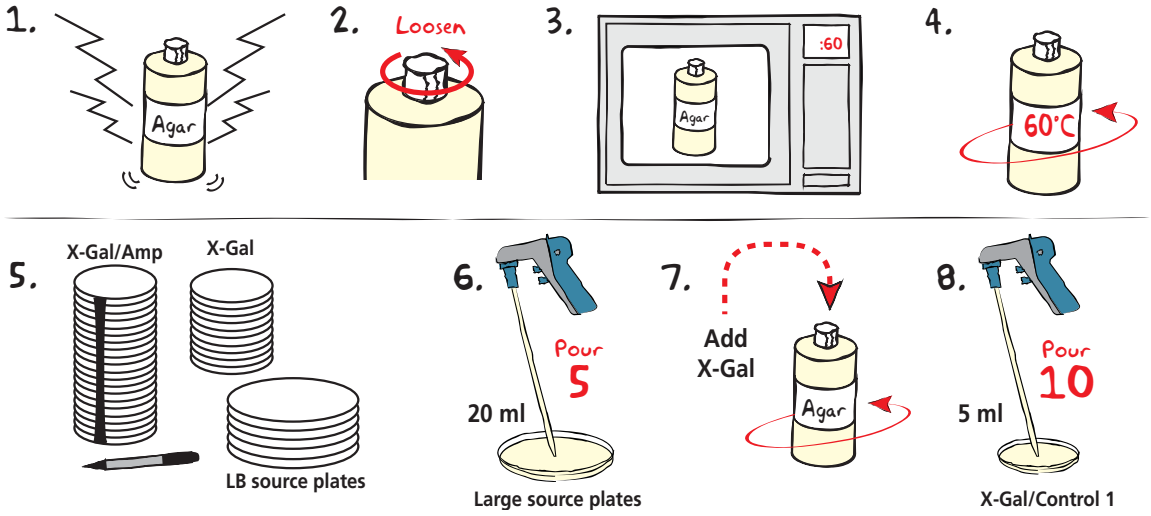
What to do:	Time Required:	When?	Page
Students observe the results of their experiment and calculate transformation efficiency	50 minutes	The following class period	11
Discard any contaminated materials	45 minutes - overnight	After the students have analyzed their results	8

Wear Hot Gloves and Goggles during all steps involving heating.

Pre-Lab Preparations

POUR LB AGAR PLATES

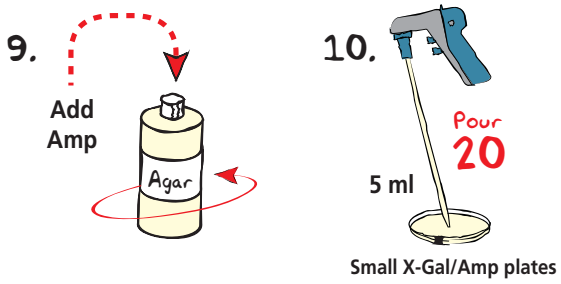
One bottle of ReadyPour™ Luria Broth Agar will make five large LB source plates, ten X-Gal plates, twenty X-Gal/Amp plates.



1. **BREAK** solid ReadyPour™ LB Agar into small chunks by vigorously squeezing and shaking the plastic bottle.
2. **LOOSEN**, but **DO NOT REMOVE**, the cap on the ReadyPour™ Agar bottle. This allows the steam to vent during heating. **CAUTION:** Failure to loosen the cap prior to heating may cause the bottle to break or explode.
3. **MICROWAVE** the ReadyPour™ Agar on high for 60 seconds to melt the agar. Carefully **REMOVE** the bottle from the microwave and **MIX** by swirling the bottle. Continue to **HEAT** the solution in 30-second intervals until the agar is completely dissolved (the amber-colored solution should be clear and free of small particles).
4. **COOL** the ReadyPour™ Agar to 60°C with careful swirling to promote even dissipation of heat.
5. While the medium is cooling, **LABEL** 20 small (60 x 15 mm) petri dishes with a permanent marker. These will be the X-Gal/Amp plates. DO NOT label the remaining 10 plates. These will be the X-Gal/Control plates. (You should also have 5 large petri dishes for the LB source plates).
6. **ADD** 20 mL of the cooled ReadyPour™ Agar into each of the five large petri dishes (source plates) by pipetting twice with a 10-ml pipet and pipet pump.
7. **THAW** and **ADD** all of the X-Gal solution to the cooled ReadyPour™ Agar. **RECAP** the bottle and **SWIRL** to mix. ONLY ADD REAGENTS TO COOLED AGAR. Reagents like X-Gal and Amp degrade at high temperature.
8. Using a fresh 10 mL pipet, **POUR** 5 mL of the agar into the ten X-Gal/Control 1 labeled plates.

NOTE for Step 3:
Use extra care and make sure the agar does not boil out of the bottle. Pay close attention and stop the heating if it starts to bubble up.

Pre-Lab Preparations



Small X-Gal/Amp plates

9. **ADD** the entire amount of the Ampicillin to the remaining ReadyPour™ Agar bottle. **RE-CAP** the bottle and **SWIRL** to mix the reagents.
10. Using a fresh 10 mL pipet, **POUR** 5 mL of the X-Gal/Amp medium into the twenty small petri plates labeled X-Gal/Amp.
11. **COVER** and **WAIT** at least twenty minutes for the LB-agar plates to solidify. For optimal results, leave plates at room temperature overnight.
12. **STORE** plates at room temperature for no more than two days. Plates should be inverted and placed in a sealable plastic bag to ensure that they do not dry out.

NOTE: If plates are prepared more than two days before use, they should be stored inverted in a plastic bag in the refrigerator (4°C). Remove the plates from the refrigerator and warm in a 37°C incubator for 30 minutes before use.

REMINDER:
Only add reagents to cooled agar (60° C)!

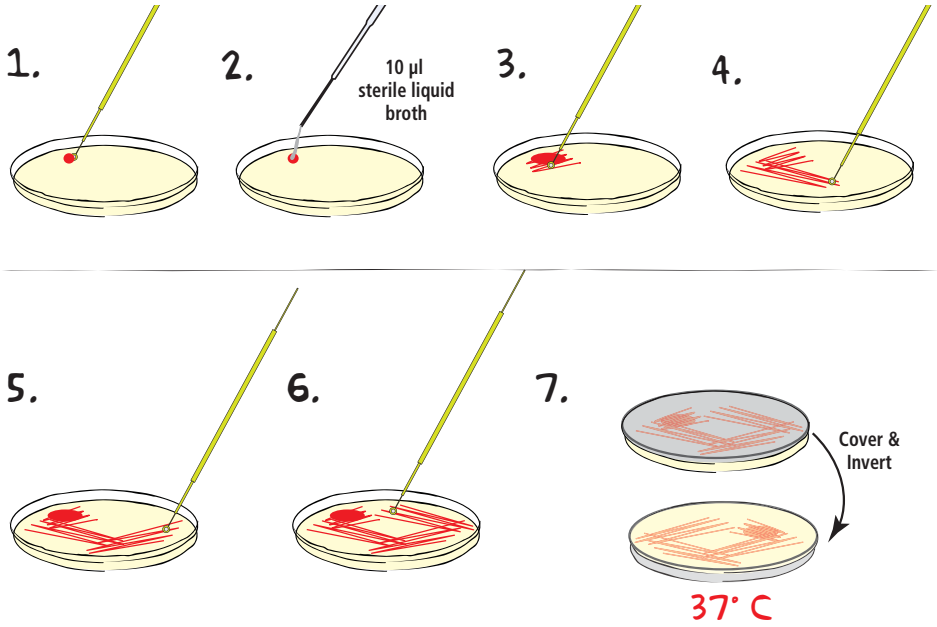
Quick Reference: Pouring LB Agar Plates

- Use a sterile 10 ml pipet with a pipet pump to transfer the designated volume of medium to each petri plate. Pipet carefully to avoid forming bubbles.
- Rock the petri plate back and forth to obtain full coverage.
- If the molten medium contains bubbles, they can be removed by passing a flame across the surface of the medium.
- Cover the petri plate and allow the medium to solidify.

Pre-Lab Preparations

Preparation of *E. coli* Source Plates

For best results, the *E. coli* source plates should be streaked 16-20 hours before the experiment is performed. Preparing the source plates more than 24 hours before the laboratory may compromise the success of the transformation experiment. If you do not have an incubator, colonies will form at room temperature in approximately 24 - 48 hours.



1. **REMOVE** a single BactoBead™ from the vial using a sterile inoculating loop. Using aseptic technique, **TRANSFER** the bead to the edge of a large petri plate (LB source plate) and replace lid. **CAP** the vial immediately after using to limit exposure to moisture in the air.
2. Instantly **DISSOLVE** the bead by adding 10 µl of sterile liquid broth or sterile water.
3. **STREAK** the loop back and forth through the dissolved BactoBead™ to make a primary streak at the top of the plate. Try not to gouge the loop into the medium.
4. **STREAK** the loop through primary streak to a clean part of the agar several times to create a secondary streak.
5. **ROTATE** the plate. **STREAK** the loop through the secondary streak to a clean part of the agar several times.
6. **ROTATE** the plate once more. **STREAK** the loop through the third streak to a clean part of the agar. This should produce isolated colonies.
7. **COVER** the plate and **INCUBATE INVERTED** at 37°C for 16 to 20 hours. If you do not have an incubator, colonies will form at room temperature in approximately 24 - 48 hours.
8. **REPEAT** the above steps for each of the LB source plates.

NOTE: If growth on plates is heavy (i.e. lawn of colonies), instruct students to transfer a loopful of cells into the $CaCl_2$ solution.

Pre-Lab Preparations

DAY OF THE LAB:

1. Equilibrate water baths at 37° C and 42° C; incubator at 37°C.
2. Dispense 1 ml of $CaCl_2$ into microcentrifuge tubes for each of the 10 groups and place on ice.
3. Dispense 1 ml of Recovery Broth into tubes for each of the 10 groups and keep at room temperature.

   Alternatively, the Recovery Broth bottle can be placed at a classroom pipeting station for students to share.

Preparation of Control Buffer and pGal™ Plasmid DNA

Aliquots of Control Buffer and plasmid DNA can be prepared the day before the lab and stored at 4°C.

4. Place the tube of Control Buffer and pGal™ Plasmid DNA on ice to thaw.
5. Label 10 microcentrifuge tubes "Control" and 10 microcentrifuge tubes "pGal".
6. Before dispensing, tap the tube of samples until all the sample is at the tapered bottom of the tube.
7. Using an automatic micropipet, dispense 12 µl of Control buffer to each of the microcentrifuge tubes labeled "Control". Then, dispense 12 µl of the plasmid DNA to each of the microcentrifuge tubes labeled "pGal".

NOTE: Students will use 10 µl for the transformation experiment.

8. Cap the tubes and place them on ice.

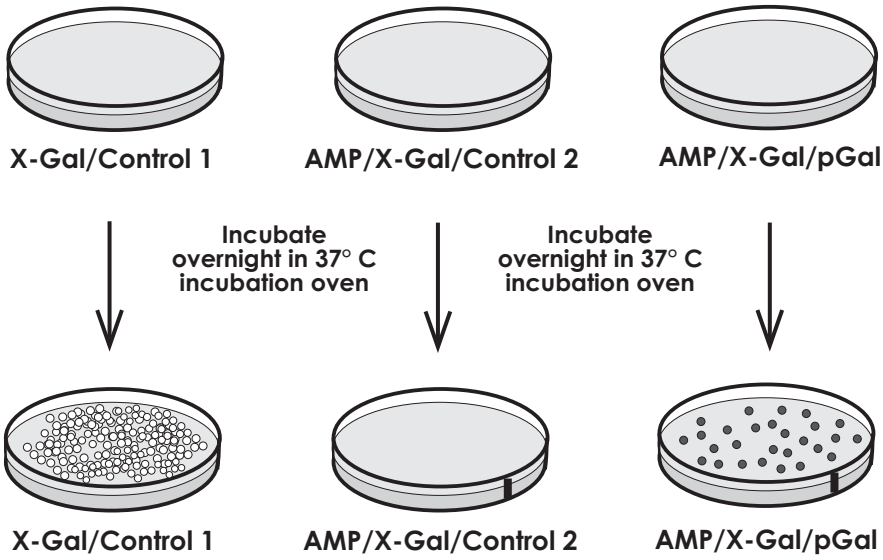
Each Group Requires:

- Sharing - one of five *E. coli* source plates
- 1 tube (1 ml) $CaCl_2$
- 1 tube Control Buffer
- 1 tube pGal™ plasmid DNA
- 1 tube (1 ml) of Recovery Broth
- 2 one-striped plates
- 1 unstriped plate
- 4 sterile 1ml pipets
- 2 sterile inoculating loops
- Toothpicks

Classroom Equipment:

- Water bath(s)
- Incubation Oven

Experiment Results and Analysis



White colonies (Lawn of bacteria)	No colonies	Blue colonies
X-Gal plated with control cells (no DNA)	**AMP/X-Gal plated with control cells (no DNA)**	**AMP/X-Gal plated with cells + DNA (pGal)**
Result: Plate covered with white colonies. May look like a smeared layer or lawn of cells. Demonstrates: Colonies are white because the cells do not utilize X-Gal. They do not contain pGal DNA which contains a gene that will allow the cell to have a functional β-galactosidase.	Result: No growth Demonstrates: Host cells are sensitive to ampicillin. Without pGal DNA, they are not ampicillin-resistant. They do not make β-lactamase.	Result: Blue colonies that may have white satellite colonies Demonstrates: 1) A small portion of the cells are transformed; 2) Cells are transformed with pGal and therefore can utilize X-Gal to give a blue color; 3) Cells acquire pGal DNA and therefore ampicillin resistance.

Please refer to the kit insert for the Answers to Study Questions

TRANSFORMATION TROUBLESHOOTING GUIDE

PROBLEM:	CAUSE:	ANSWER:
Poor cell growth on source plate	Incubation time too short	Continue to incubate source plate at 37°C for a total of 16-20 hours.
	Antibiotic added to source plate	When pouring plates, be sure to add antibiotics & additives at the correct step.
	Incorrect incubation temperature	Use a thermometer to check incubator temperature. Adjust temp. to 37°C if necessary.
Satellite colonies seen on transformation plate	Incorrect concentration of antibiotics in plates	Ensure the correct concentration of antibiotic was added to plates - Make sure ReadyPour is cooled to 60°C before adding antibiotic.
	Antibiotic is degraded	Make sure ReadyPour is cooled to 60°C before adding antibiotic.
	Plates were incubated too long	Incubate the plates overnight at 37°C (16-20 hours).
Colonies appeared smeary on transformation plate	Plates containing transformants were inverted too soon	Allow cell suspension to fully absorbed into the medium before inverting plates.
	Experimental plates too moist	After pouring plates, allow them dry overnight at room temp. Alternatively, warm plates at 37°C for 30 min. before plating cells
No colonies seen on transformation plates	Plasmid DNA not added to transformation mix	Ensure plasmid DNA was added to transformation tube.
		Make sure that pipets are used properly. If using micropipets, make sure students practice using pipets
	Incorrect host cells used for transformation	Confirm that correct bacterial strain was used for transformation
	Cells were not properly heat shocked	Ensure that temp. was 42°C & heat shock step took place for no more than 90 seconds.
	Incorrect antibiotics	Be certain that the correct antibiotic was used.
	Cells not well resuspended in $CaCl_2$	Completely resuspend the cells in the $CaCl_2$, leaving no cell clumps (vortex or mix vigorously to fully resuspend cells). Cell suspension should be cloudy.
Low transformation efficiency	Not enough cells used for transformation	Pick more colonies from source plate (15 colonies @ 1-2 mm width per 500µl $CaCl_2$)
	Source plates were incubated for more than 20 hours	Important that source cells grow no longer than 20 hrs. Refrigerate plates after 20 hrs if necessary. Do not use source plates that have been incubated longer than 24 hours, refrigerated or not).
	Experimental plates too old	Prepare transformation plate and use shortly after preparation
	Cells not well resuspended in $CaCl_2$	Completely resuspend the cells in the $CaCl_2$, leaving no cell clumps (vortex or mix vigorously to fully resuspend cells). Cell suspension should be cloudy.
	$CaCl_2$ solution not cold enough	Pre-chill $CaCl_2$ before adding cells to the $CaCl_2$
	Cell solution not cold enough	Extend incubation of celll suspension on ice 10-15 min. (should not exceed 30 min. total). This increases the transformation efficiency.
	Too much or too little plasmid DNA added to cell suspension	Ensure that correct volume of plasmid was added to the transformation tube. If using micropipets, make sure students practice using pipets.
	Cells were not properly heat shocked	Ensure that temperature was 42°C and that heat shock step took place for no more than 90 seconds.
	Antibiotics were degraded prior to pouring plates	Make sure ReadyPour is cooled to 60°C before adding antibiotic.
	Incorrect concentration of antibiotics in plates	Ensure that the correct concentration of antibiotic was used

NEW

Add 2 ml recovery broth to LyphoCell™ vial

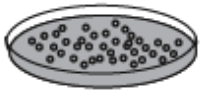
Incubate cells
30 - 60 minutes at 37°C

Transfer 50-75 µl cells to each source plate and streak

LB Agar

Incubate source plates 16-20 hours overnight @ 37°C

Source Plate
E. coli Cells



Students begin Experiment

Students transfer 15 colonies to 500 µl $CaCl_2$ and split between two tubes

LyphoCells™ for Transformation

Substitute for BactoBeads™

Day before the experiment

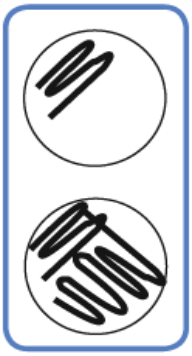
This experiment requires preparation of isolated *E.coli* host transformation colonies 16-20 hours before the laboratory experiment, so plan accordingly.

Important: Do not prepare source plates more than 20 hours before the experiment. Older source plates will compromise the success of the transformation experiment.

Preparation of *E. coli* Cells

1. Use a sterile pipet to aseptically add 2 ml of recovery broth to the vial of LyphoCells™.
2. Replace the rubber stopper of the LyphoCell™ vial and cap. Mix by gently inverting until the freeze dried plug is dissolved.
3. Incubate the vial of cells for 30 - 60 minutes in a 37°C incubation oven.

   Growth should be evident (Broth should be slightly turbid or cloudy). If growth is not evident, incubate for a longer period of time.
4. Transfer 50 - 75 µl of cells to each source plate and streak the cells on one quadrant of each plate with a sterile loop. (figure top right).
5. With the same loop, streak through the cells once or twice into another clean section of the plate (figure bottom right) to obtain isolated colonies.



6. Label the plates "*E. coli*", invert and incubate the plates overnight (16-20 hours) at 37°C in an incubation oven.

   If growth on plates is heavy (i.e. few or no isolated colonies), instruct students to touch the toothpick to a small amount of cells.