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!**

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.

**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.**



| | 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.

## 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 $10^8$.

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

***Specific example:***

$$\frac{100 \text{ transformants}}{0.01\ \text{µg}} \times \frac{1\ \text{ml}}{0.1\ \text{ml}} = \begin{array}{c}100{,}000 \\ (1 \times 10^5) \\ \text{transformants per µg}\end{array}$$

**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.

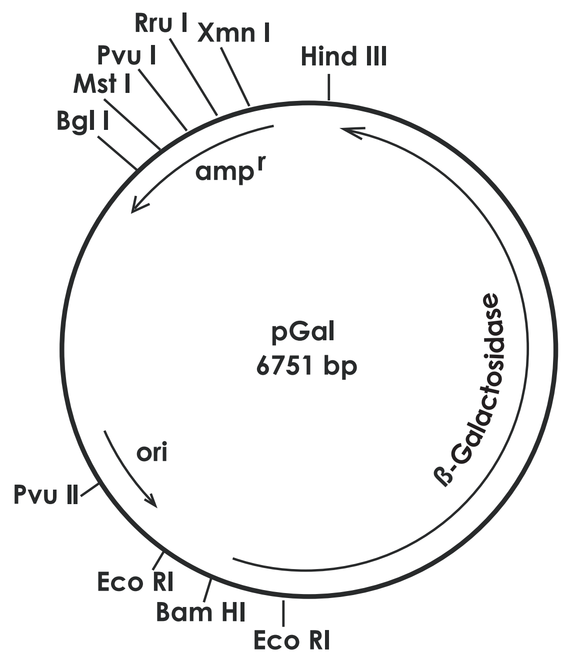



**Figure 2:**
DNA map of pGal.
Not all restriction enzymes are shown.

## 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.

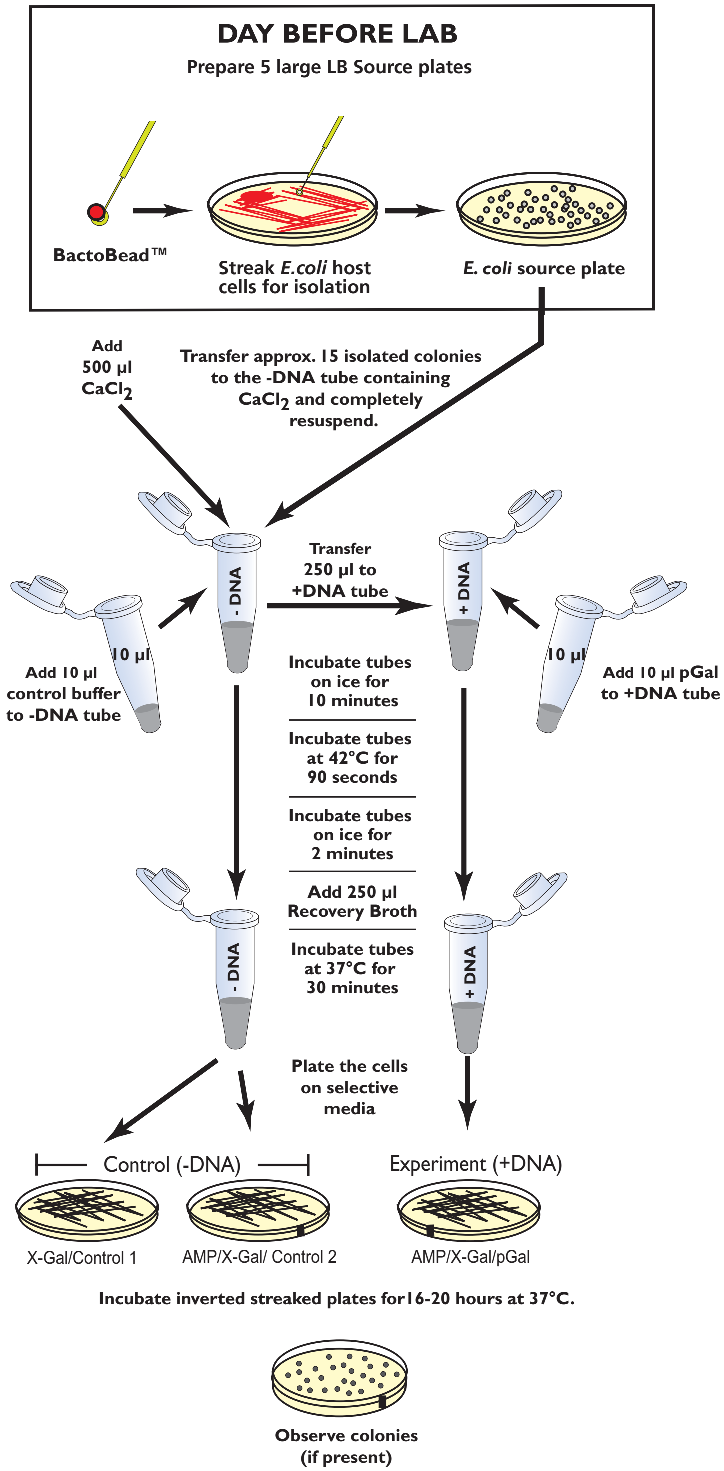
### EXPERIMENT OBJECTIVE:

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.

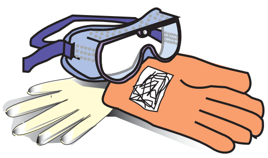
### BRIEF DESCRIPTION OF EXPERIMENT:

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:

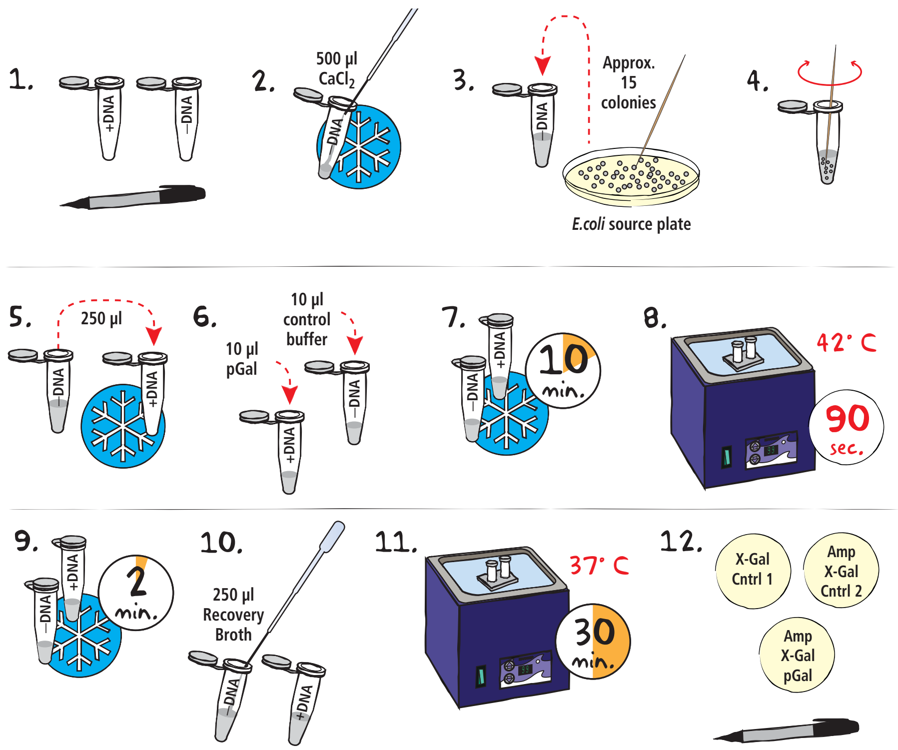
   - Autoclave at 121° C for 20 minutes.
     Tape several petri plates together and close tube caps before disposal. Collect all contaminated materials in an autoclavable, disposable bag. Seal the bag and place it in a metal tray to prevent any possibility of liquid medium or agar from spilling into the 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!

## 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!



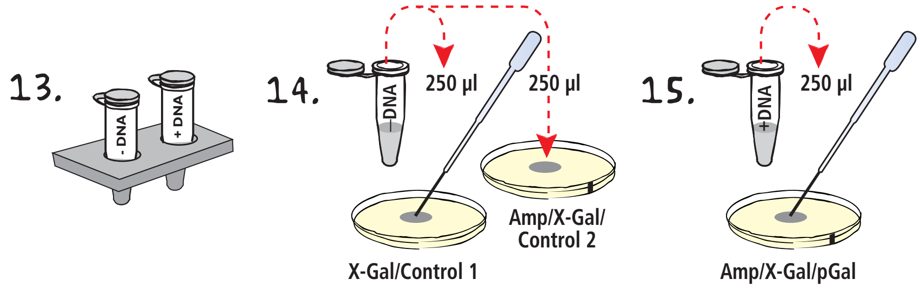
1. **LABEL** one microcentrifuge tube with "+DNA" and a second microcentrifuge tube with "-DNA".
2. **TRANSFER** 500 µL ice-cold $CaCl_2$ solution into the "– DNA" tube using a sterile 1 mL pipet.
3. Using a toothpick, **TRANSFER** approx. 15 well-isolated colonies (each colony should be approx. 1-1.5 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:

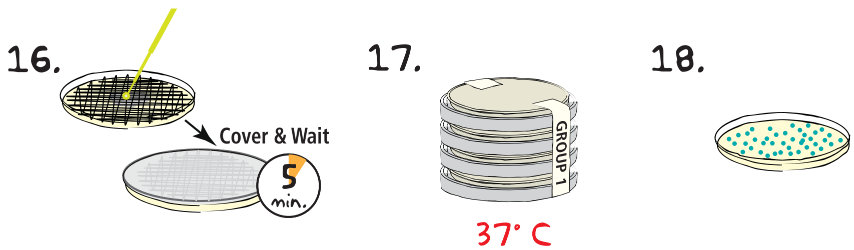
    X-Gal/Control 1 ( plate no stripe)

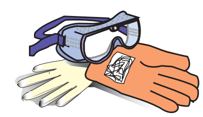
    Amp/ X-Gal/ Control 2 (plate with one stripe)

    Amp/X-Gal/pGal (plate with one stripe)

## Transformation of *E. coli* with pGal™





Wear Safety
Goggles and gloves

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.

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## 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 experi-ment.

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:

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

**Example:**
Assume you observed 40 colonies:

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

**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

Experiment Procedure

## 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?