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RECENTLY UPDATED!

Edvo-Kit # 221

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Transformation of *E. coli* with pGAL™

Experiment Objective:

In this experiment, students will explore the biological process of bacterial transformation using *E. coli* and a plasmid DNA containing β-galactosidase and β-lactamase genes. The resulting bacteria will be examined for the presence of blue pigment and ampicillin resistance.

See page 3 for storage instructions.

Version 221.140331

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Table of Contents

Safety Data Sheets can be found on our website: www.edvotek.com/safety-data-sheets

Experiment Components

Components		Storage	Check (✓)
A	BactoBeads™ *E. coli*	Freezer, with desiccant	❑
B	Supercoiled pGAL™ (blue colony)	Freezer	❑
C	Control Buffer (no DNA)	Freezer	❑
D	Ampicillin	Freezer	❑
E	X-Gal in solvent	Freezer	❑
F	$CaCl_2$	Freezer	❑

This experiment is designed for 10 lab groups.

All experiment 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.

REAGENTS & SUPPLIES

Store all components below at room temperature.

- Bottle of ReadyPour™ Luria Broth Agar, sterile (also referred to as "ReadyPour Agar") ❑
- Bottle of Recovery Broth, sterile ❑
- Petri plates, small ❑
- Petri plates, large ❑
- Plastic microtipped transfer pipets ❑
- Wrapped 10 mL pipet (sterile) ❑
- Inoculating loops (sterile) ❑
- Microcentrifuge tubes ❑

IMPORTANT READ ME!

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

Experiment Requirements

- Adjustable Volume Micropipette (5-50 µL and 50 - 200 µL) and tips
- Two Water baths (37°C and 42°C)
- Floating racks or foam tube holders
- Thermometer
- Incubation Oven (37°C)
- Ice Buckets and Ice
- Marking pens
- Tape
- Pipet pumps or bulbs
- Microwave
- Hot gloves

Background Information

DNA CAN BE TRANSFERRED BETWEEN BACTERIA

In nature, DNA is transferred between bacteria using two main methods—transformation and conjugation. In transformation, a bacterium takes up exogenous DNA from the surrounding environment (Figure 1). In contrast, conjugation relies upon direct contact between two bacterial cells. A piece of DNA is copied in one cell (the donor) and then is transferred into the other (recipient) cell. In both cases, the bacteria have acquired new genetic information that is both stable and heritable.

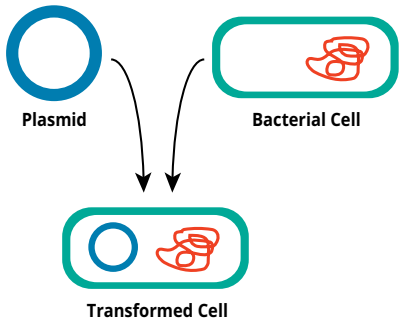


Figure 1: Bacterial Transformation

Frederick Griffith first discovered transformation in 1928 when he observed that living cultures of a normally non-pathogenic strain of *Streptococcus pneumonia* were able to kill mice, but only after being mixed with a heat-killed pathogenic strain. Because the non-pathogenic strain had been "transformed" into a pathogenic strain, he named this transfer of virulence "transformation". In 1944, Oswald Avery and his colleagues purified DNA, RNA and protein from a virulent strain of S. pneumonia to determine which was responsible for transformation. Each component was mixed each with a non-pathogenic strain of bacteria. Only those recipient cells exposed to DNA became pathogenic. These transformation experiments not only revealed how this virulence is transferred but also led to the recognition of DNA as the genetic material.

The exact mode of transformation can differ between bacteria species. For example, *Haemophilus influenzae* uses membrane-bound vesicles to capture double-stranded DNA from the environment. In contrast, *S. pneumoniae* expresses competency factors that allow the cells to take in single-stranded DNA molecules. In the laboratory, scientists can induce cells—even those that are not naturally competent—to take up DNA and become transformed. To accomplish this, DNA is added to the cells in the presence of specific chemicals (like calcium, rubidium, or magnesium chloride), and the suspension is "heat shocked"— moved quickly between widely different temperatures. It is believed that the combination of chemical ions and the rapid change in temperature alters the permeability of the cell wall and membrane, allowing the DNA molecules to enter the cell. Today, many molecular biologists use transformation of Escherichia coli in their experiments, even though it is not normally capable of transforming in nature.

GENETIC ENGINEERING USING RECOMBINANT DNA TECHNOLOGY

Many bacteria possess extra, non-essential genes on small circular pieces of double-stranded DNA in addition to their chromosomal DNA. These pieces of DNA, called plasmids, allow bacteria to exchange beneficial genes. For example, the gene that codes for ß-lactamase, an enzyme that provides antibiotic resistance, can be carried between bacteria on plasmids. Transformed cells secrete ß-lactamase into the surrounding medium, where it degrades the antibiotic ampicillin, which inhibits cell growth by interfering with cell wall synthesis. Thus, bacteria expressing this gene can grow in the presence of ampicillin. Furthermore, small "satellite" colonies of untransformed cells may also grow around transformed colonies because they are indirectly protected by ß-lactamase activity.

Recombinant DNA technology has allowed scientists to link genes from different sources to bacterial plasmids (Figure 2). These specialized plasmids, called vectors, contain the following features:

1. Origin of Replication: a DNA sequence from which bacteria can initiate the copying of the plasmid.
2. Multiple Cloning Site: a short DNA sequence that contains many unique restriction enzyme sites and allows scientists to control the introduction of specific genes into the plasmid.
3. Promoter: a DNA sequence that is typically located just before ("upstream" of) the coding sequence of a gene. The promoter recruits RNA polymerase to the beginning of the gene sequence, where it can begin transcription.
4. Selectable marker: a gene that codes for resistance to a specific antibiotic (usually ampicillin, kanamycin or tetracycline). When using selective media, only cells containing the marker should grow into colonies, which allows researchers to easily identify cells that have been successfully transformed

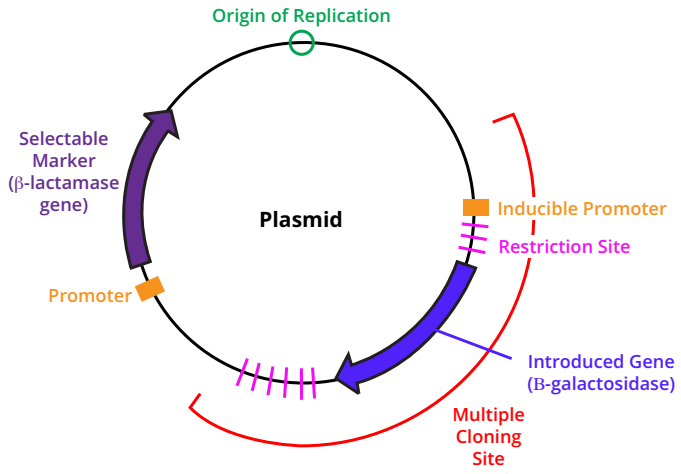


Figure 2: Plasmid Features

TRANSFORMATION EFFICIENCY

In practice, transformation is highly inefficient—only one in every 10,000 cells successfully incorporates the plasmid DNA. However, because many cells are used in a transformation experiment (about 1 x 10^9 cells), only a small number of cells must be transformed to achieve a positive outcome. If bacteria are transformed with a plasmid containing a selectable marker and plated on both selective and nonselective agar medium, we will observe very different results. Nonselective agar plates will allow both transformed and untransformed bacteria to grow, forming a bacterial "lawn". In contrast, on the selective agar plate, only transformed cells expressing the marker will grow, resulting in recovery of isolated colonies.

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

Specific example:

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

Figure 3: Bacterial Transformation Efficiency Calculation.

Because each colony originates from a single transformed cell, we can calculate the transformation efficiency, or the number of cells transformed per microgram (µg) of plasmid DNA (outlined in Figure 3). For example, if 10 nanograms (0.01 µg) of plasmid were used to transform one milliliter (mL) of cells, and plating 0.1 mL of this mixture (100 microliters, or 100 µl) gives rise to 100 colonies, then there must have been 1,000 bacteria in the one milliliter mixture. Dividing 1,000 transformants by 0.01 µg DNA means that the transformation efficiency would be 1 X 10^5 cells transformed per µg plasmid DNA. Transformation efficiency generally ranges from 1 x 10^5 to 1 x 10^8 cells transformed per µg plasmid.

THE pGAL™ PLASMID

The plasmid that we will be using to transform our *E. coli* has been engineered to contain the DNA sequence for the enzyme ß-galactosidase. ß-galactosidase, also known as beta-gal or sometimes lactase, is a digestive enzyme that helps break down the complex sugars ß- galactosides. Beta-gal is a large enzyme. The molecule is made of 1024 amino acids and its molecular weight is 464 kilodaltons. It's also a common and often essential enzyme. For example, in humans, it plays a key role in the breakdown of lactose. Consequently, people who lack this enzyme have trouble digesting dairy products. Most *E. coli* also contain the gene that codes for beta-gal. However, the *E. coli* strain that you will be transforming in this experiment does not.

In addition to the gene for beta-gal, the p-Gal plasmid also contains a gene that codes for ß-lactamase. This allows scientists to easily isolate and grow bacteria that have the pGAL plasmid because only transformed cells can survive and reproduce in an ampicillin environment. The plasmid also has a site of origin, several promoter regions, and numerous restriction enzyme cut sites (figure 4).

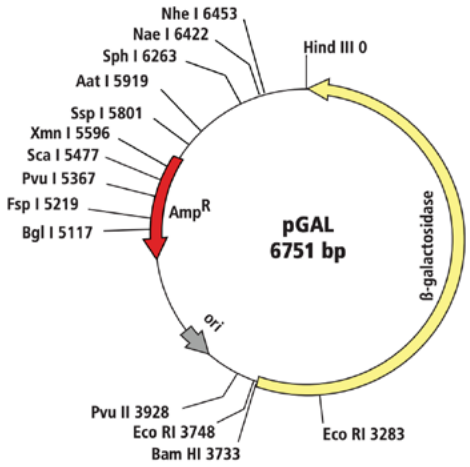


Figure 4: The pGAL™ Plasmid

When the pGal plasmid enters an *E. coli* cell it does not directly integrate into the chromosomes of the cell but rather remains independent within the cell. However, it can still undergo transcription and translation which means that the cell can still express the genes contained in the plasmid and produce the proteins that they code for. The plasmid can also replicate autonomously which means it can copy itself, regardless of whether the bacterial host is replicating its own DNA. As a result, *E. coli* cell that has been transformed with pGal will often have several copies of this plasmid.

In this experiment, you will transform host bacterial cells with plasmid DNA. The transformants will acquire antibiotic resistance and the enzyme ß-galactosidase. The latter will allow cells to cleave the artificial galactosides 5-Bromo-4 Chloro 3-indolyl-ß-D-galactoside (X-Gal). This creates a colored pigment within the cells that can turn bacterial colonies blue. Transformed cells will be identified based on color and on their ability to survive on agar plates that contain ampicillin. The number of transformants will be counted and used to calculate transformation efficiencies.

Experiment Overview

LABORATORY SAFETY

IMPORTANT: Be sure to READ and UNDERSTAND the instructions completely BEFORE starting the experiment. If you are unsure of something, ASK YOUR INSTRUCTOR!

Wear gloves and safety goggles

- *This experiment contains antibiotics to select for transformed colonies. Students who have allergies to antibiotics such as penicillin, ampicillin, kanamycin, or tetracycline should not participate in this experiment.*
- Wear gloves and goggles at all times.
- The *E. coli* bacteria used in this experiment is not considered pathogenic, but it is still important to follow simple safety guidelines. Wipe down the lab bench with a 10% bleach solution or a laboratory disinfectant before and after the experiment, wash hands thoroughly with soap and water after working in the laboratory, and disinfect material that has come in contact with bacteria before disposing them. To disinfect used materials: autoclave at 121°C for 20 minutes (make sure to first package agar plates etc. in an autoclavable, disposable bag to prevent liquid spilling into the sterilization chamber) *OR* soak materials overnight in a 10% bleach solution.
- Always wash hands thoroughly with soap and water after working in the laboratory.

LABORATORY NOTEBOOKS

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

During the Experiment:

- Record your observations in your lab notebook or in the Student Handout in Appendix B.

After the Experiment:

- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

TIMING REQUIREMENTS:

TRANSFORMATION
30 - 40 minutes

↓

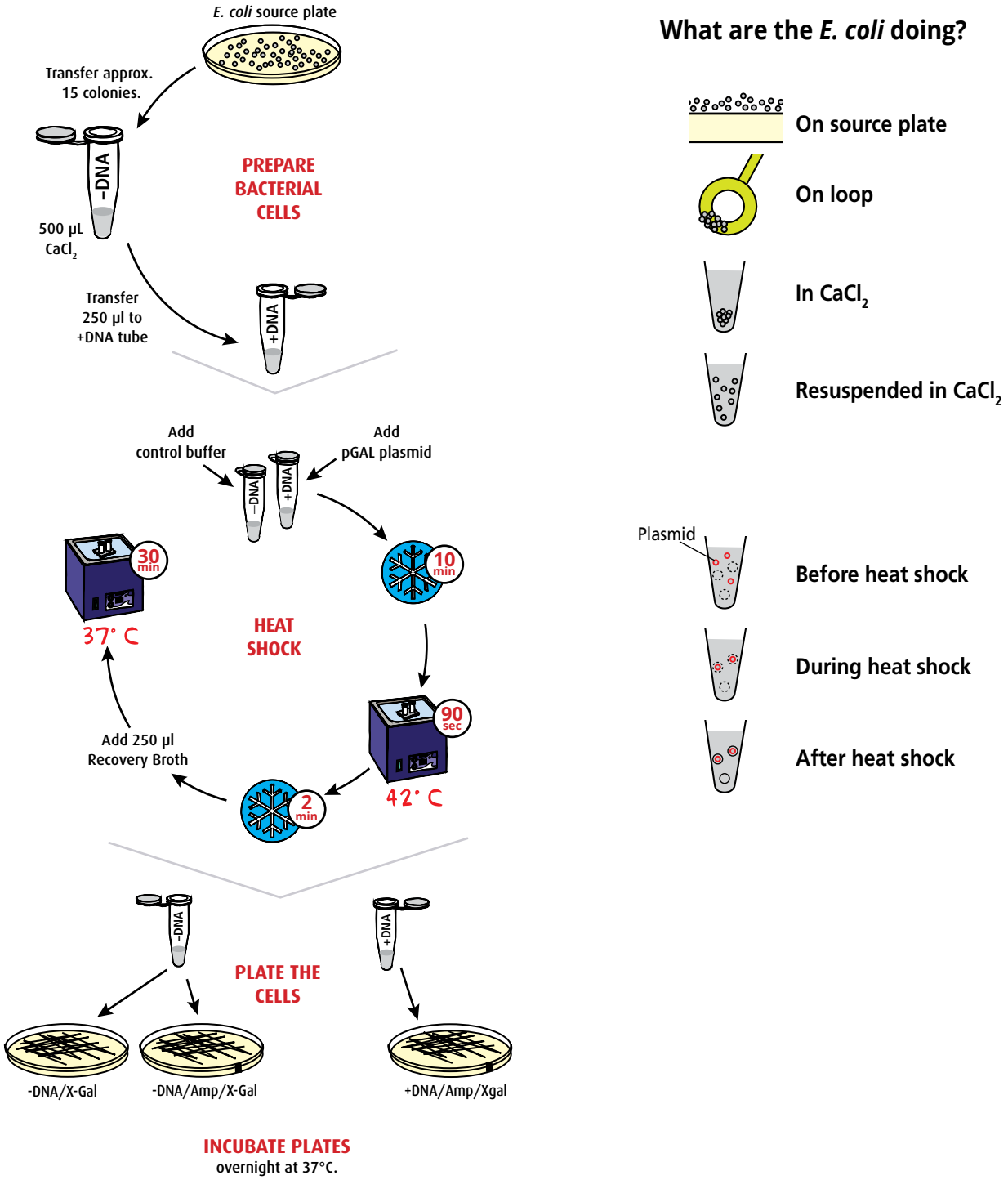
INCUBATION
Overnight at 37°C

↓

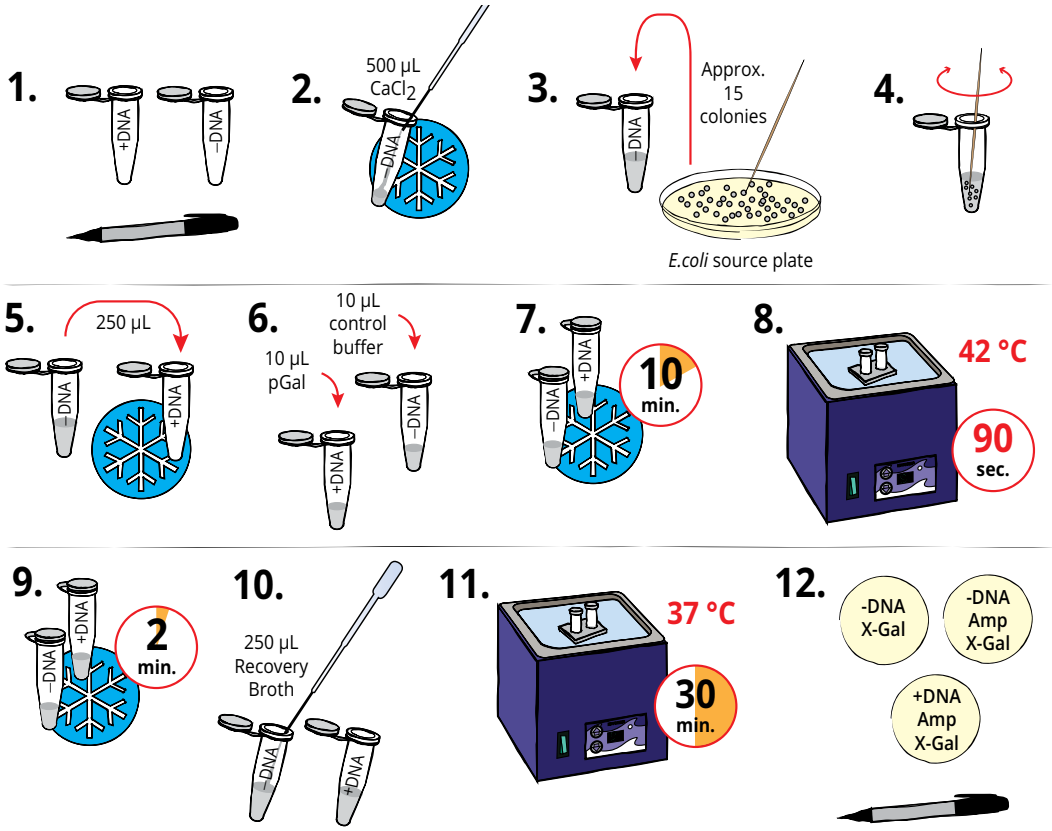
STOPPING POINTS
Transformed plates can be stored for up to 1 week at 4°C

Experiment Overview, continued

In this experiment, host *E. coli* bacteria is transformed with a pGAL™ plasmid. The bacteria will be grown for 18-22 hours on LB-agar "source plates", collected using a sterile loop, and made competent in $CaCl_2$. Next, the plasmid will be added to half of the cells before they are briefly heat shocked. Finally, the bacteria will be allowed to briefly recover before they are plated on LB-Agar plates and incubated at 37°C overnight.



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



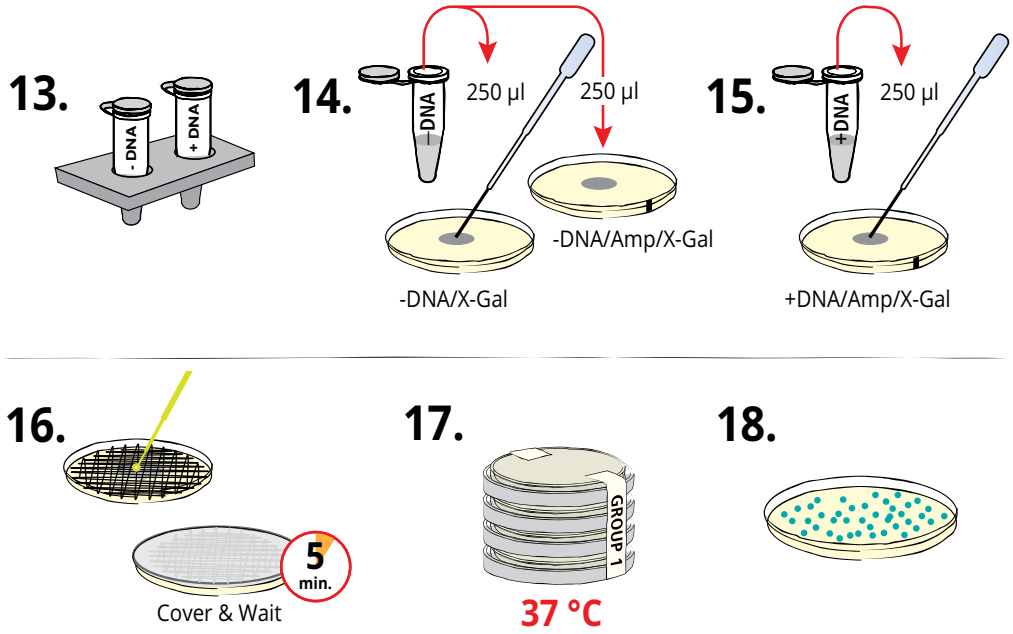
IMPORTANT:
For best results, make sure that the cells are completely resuspended.

IMPORTANT:
For best results, ensure that the $CaCl_2$ is ice cold and that the cells are rapidly moved from ice to 42°C and then immediately back to ice. Follow all times exactly.

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 below:
    -DNA/X-Gal (plate no stripe)
    -DNA/Amp/ X-Gal (plate with one stripe)
    +DNA/Amp/X-Gal (plate with one stripes)

Transformation of *E. coli* with pGAL™, continued



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 -DNA/X-Gal plate and the -DNA/Amp/X-Gal.
15. Using a new sterile 1 mL pipet, **TRANSFER** 250 µL recovered cells from the tube labeled " +DNA " to the middle of the +DNA/Amp/X-Gal 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.

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
   - -DNA/X-Gal
   - -DNA/Amp/X-Gal

   Transformation Plates: (+) DNA
   - +DNA/Amp/X-Gal

2. **DRAW** and **DESCRIBE** what you observe. For each of the plates, **RECORD** the following:
   - How much bacterial growth do you observe? If possible, determine the total number of colonies.
   - 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: +DNA/Amp/X-Gal. 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:

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

Example:
Assume you observed 40 colonies.

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

Quick Reference for Experiment #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

3. **COMPARE** your transformation efficiency to the other groups in your class.
   What factors could have contributed to differences in efficiency between groups?

Study Questions

ANSWER THESE QUESTIONS IN YOUR NOTEBOOK ***BEFORE*** PERFORMING THE EXPERIMENT

1. On which plate(s) would you find only genetically transformed bacterial cells? Explain.

2. What is the purpose of the control plates? Explain the difference between each and why it is necessary to run each.

3. Why would one compare plates -DNA/AMP/X-Gal and +DNA/AMP/X-Gal?

ANSWER THESE QUESTIONS IN YOUR NOTEBOOK ***AFTER*** PERFORMING THE EXPERIMENT

1. Exogenous DNA does not passively enter *E. coli* cells that are not competent. What treatment do cells require to be competent?

2. Why are there so many cells growing on the X-Gal plate? What color are they?

3. Did you observe any satellite colonies? Why are the satellite, feeder colonies white?

4. What evidence do you have that transformation was successful?

5. What are some reasons why transformation may not be successful?

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 penicillin, ampicillin, kanamycin or tetracycline should not participate in this experiment.

NOTES TO THE INSTRUCTOR

To maximize your classroom's transformation efficiency, we have provided four additional resources:

1. A transformation tips and tricks section (Appendix A) that complements the student protocol on pages 9 and 10. This list describes best practices in greater detail, offers suggestion to make the experiment more inquiry based, and links specific steps back to key biology concepts.

2. A transformation troubleshooting guide (Appendix B) that identifies and explains common experimental problems. As many of these are best addressed proactively, we suggest reading this *before* starting the experiment as well as using it afterwards to identify potential errors.

3. A short (and delicious) microbiology practice activity (Appendix C) to prepare students to harvest bacteria colonies.

Notes to the Instructor, continued

ADVANCE PREPARATION:

What to do:	Time Required:	When?	Page
Prepare LB Agar Plates	One hour	2-7 days before use	15
Prepare *E. coli* Source plates	20 minutes to streak plates; 16-18 hours to incubate plates	The day before performing the experiment	16
Dispense plasmid DNA, $CaCl_2$, and recovery broth	30 minutes	One day to 30 min. before performing the experiment	17

DAY OF THE EXPERIMENT:

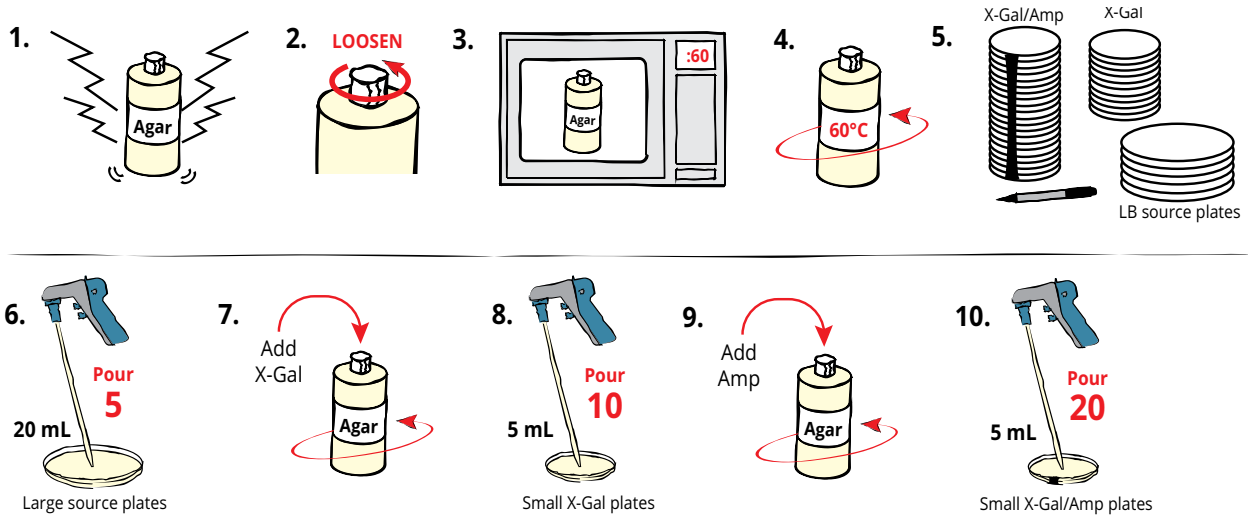
What to do:	Time Required:	When?	Page
Equilibrate waterbaths at 37°C and 42°C; incubator at 37°C	10 minutes	One to two hours before performing the experiment	17
Perform laboratory experiment	50 minutes	The class period	10
Incubate cells at 37°C	16-18 hours	Overnight after the class period	11

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	12
Discard any contaminated materials	45 minutes - overnight	After the students have analyzed their results	12

Pouring 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.
9. **ADD** the entire amount of the Ampicillin to the remaining ReadyPour™ Agar bottle. **RECAP** 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.

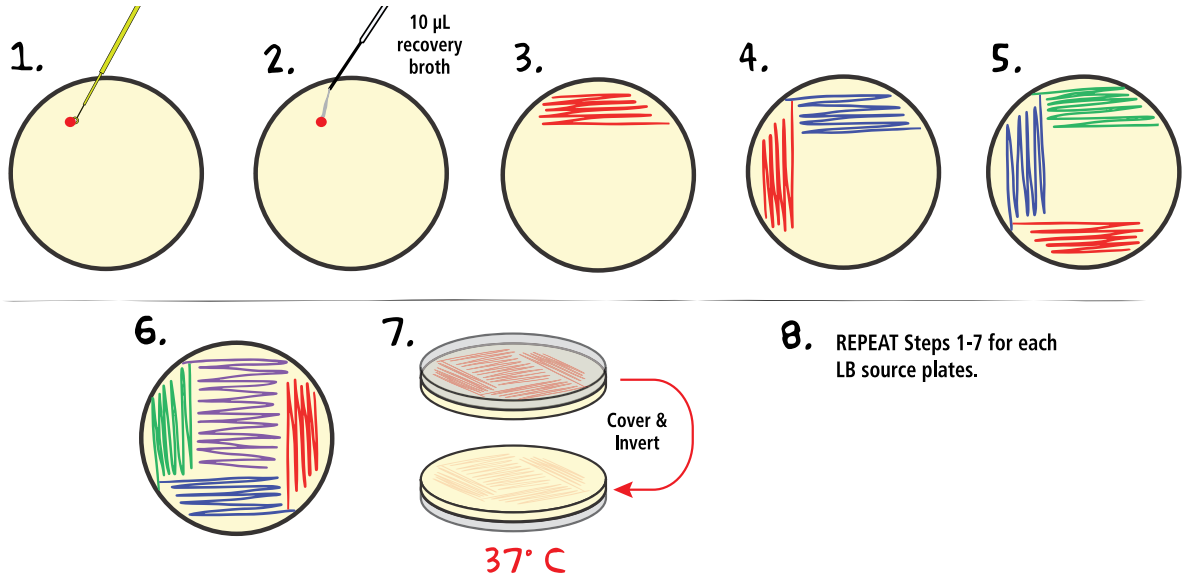
NOTE for Step 3:
Use extra care and make sure the agar does not boil out of the bottle. Pay close attention and stop the heating if it starts to bubble up.

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.

Preparation of *E. coli* Source Plates

For best results, the *E. coli* source plates should be streaked **18-22** 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.



1. **REMOVE** a single BactoBead™ from the *E. coli* GFP Host 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. **DISSOLVE** the bead by adding 10 µL of recovery broth.

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. **ROTATE** the plate 90°. **STREAK** the loop through primary streak once, then zig-zag across a clean part of the agar several times to create a secondary streak.

5. **ROTATE** the plate. **STREAK** the loop through the secondary streak once and then across a clean part of the agar several times.

6. **ROTATE** the plate once more. **STREAK** the loop through the third streak and then zig-zag across the remaining clean agar. This should produce isolated colonies.

7. **COVER** the plate and **INCUBATE INVERTED** at 37°C for 18-22 hours. If you do not have an incubator, colonies will form at room temp. in approximately 24 - 48 hours, although transformation efficiency will decrease.

8. **REPEAT** the above steps for each of the five large LB source plates using a new loop for each plate.

NOTE: Ideal colonies will be 1-1.5 mm in diameter. If growth on plates is heavy (i.e. lawn of colonies), instruct students to transfer a small loopful of cells into the $CaCl_2$ solution.

Pre-Lab Preparations

Each Group Requires:

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

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

Classroom Equipment:

- Waterbath(s)
- Incubation Oven

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 micropipette, **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.

Experiment Results and Analysis

TRANSFORMATION

-DNA/X-Gal
plated with
non-transformed cells
(no DNA)

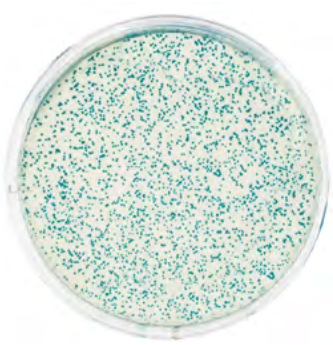
Result: Plate covered with white colonies.May look like a smeared layer or lawn of cells.

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.

-DNA/AMP/X-Gal
plated with
non-transformed cells
(no DNA)

Result: No growth

Demonstrates:
Demonstrates: Host cells are sensitive to ampicillin. Without pGal DNA, they are not ampicillin-resistant. They do not make β-lactamase.



+DNA/AMP/X-Gal
plated with
transformed cells
(pGal)

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

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

Appendices

A Transformation Tips and Tricks
B Troubleshooting Guide
C Pre-Transformation Practice - Bacteria Colony Collection

Safety Data Sheets:

Now available for your convenient download on **www.edvotek.com/safety-data-sheets**

Appendix A
Transformation Tips and Tricks

Want a classroom of glowing GFP colonies and excited students? Here's how to optimize the student's experiment to maximize transformation efficiency (✔), student involvement (☆), and understanding (❑).

1. Prepare a healthy and receptive cell culture. (Steps 1 - 4)

✔ Ensure the $CaCl_2$ is ice cold throughout the experiment by: (1) incubating it in the fridge or freezer the night before, (2) storing tubes on finely crushed ice, and (3) having students hold tubes only by the upper rim.
✔ Be "picky" when picking colonies. The "best" bacteria come from middle sized colonies (1 - 1.5 mm) and fresh source plates (16 -20 hours old).
✔ Agar can inhibit transformation. Make sure students know how to gently collect bacteria colonies without gouging the agar. If in doubt, practice beforehand (Appendix D).
✔ Factor in cell stickiness! Visually confirm that cells make it onto the loop (step 2) and then off of the loop and into the solution (step 3). To dislodge cells from the loop in step 3, move the loop up and down while twisting in order to take advantage of the $CaCl_2$'s surface tension.
✔ Allow as many bacteria cells as possible to come in contact with the ice cold $CaCl_2$ and with the extracellular plasmids by taking the time to fully break up clumps in step 4.

2. Introduce just the right amount of foreign DNA. (Steps 5 & 6)

✔ Adding too little or too much plasmid can reduce transformation efficiency. If your class is unfamiliar with pipetting small volumes practice the technique before hand.
☆ This experiment can be turned into an inquiry investigation by having students vary key features like plasmid amount, colony numbers, colony age, incubation times etc. to determine how these effect transformation efficiency.
❑ The tube without DNA (-DNA) is used as a conceptual control to demonstrate that untransformed cells are sensitive to ampicillin and as an experimental control to confirm host cell viability and proper incubation conditions.

3. Execute a fantastic heat shock step. (Step 7 - 9)

✔ Maximize the temperature contrast between the ice and 42°C water bath. Have students place their tubes into individual floating racks at the beginning of the 10 minute ice incubation and then carry these tubes on ice to the water bath. Following the 45 second heat shock have students immediately transfer the tubes back to the ice (i.e. before taking off the floating rack or returning to their lab bench). If individual floating racks are unavailable, have students place tubes into the classroom's floating rack during the 10 minute ice incubation and as a group perform the heat shock steps.

4. Give cells the tools they need to recover and grow. (Steps 10 - 16)

❑ The recovery broth does not contain ampicillin because transformed bacteria have not yet begun to produce the protein β-lactamase that gives them ampicillin resistance. This will occur in the next step.
☆ While the cells incubate (step 11) engage students in experimental planning by asking them to brainstorm what control plates they need. (You will need to black out the list in step 12.)
✔ Transformed colonies do not grow well on broken agar. Remind students to gently manipulate the loop during step 16.
✔ It may take longer than five minutes for recently prepared agar plates to absorb the cell solution. If there is still liquid on the surface of a plate wait up to 30 minutes before inverting.

Appendix B
Troubleshooting Guides

TRANSFORMATION TROUBLESHOOTING GUIDE

PROBLEM:	CAUSE:	ANSWER:
Poor cell growth on source plate	Incubation time too short	Continue to incubate source plate at 37ºC for a total of 18-22 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 37ºC (18-22 hours).
Colonies appeared smeary on transformation plate	Plates containing transformants were inverted too soon	Allow cells to fully absorb 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 individual colonies seen on source plates	Cells were not properly quadrant streaked.	Have students transfer a small loopful of bacteria to the $CaCl_2$.
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 and are properly calibrated.
	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 42ºC & heat shock step took place for exactly 45 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 pipet up and down to fully resuspend cells). Cell suspension should be cloudy.
Low transformation efficiency	Not enough cells used for transformation	Pick more colonies from source plate (5 colonies @ 1-1.5 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 pipet up and down 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 45 seconds.
	Antibiotics were degraded prior to pouring plates	Make sure ReadyPour is cooled to 60°C before adding antibiotic.
	Incorrect concentration of antibiotics	Ensure that the correct concentration of antibiotic was used in plates.

Appendix C
Pre-Transformation Practice - Bacteria Colony Collection

For this activity you will need a pack of Jell-O®, a small tube of icing or similarly viscous liquid, water, a beaker and ten additional petri plates (or the lids from any wide mouth jar or container). You will also need ten toothpicks or inoculating loops and ten small test tubes.

1. Make 10 Jell-O® practice petri plates.
   - In a large breaker mix Jell-O® powder and water according the package directions.
   - Quickly pour the Jell-O® into petri plates, filling about half way.
   - Allow plates to solidify in the fridge for 30-60 minutes.
   - Add 10 dots using whatever liquid you choose. Dots may be larger than true bacterial colonies.

2. Give each student group a plate, a toothpick or loop, and a microcentrifuge tube containing water.

3. Challenge students to transfer all the "bacteria" colonies into the tube without breaking the Jell-O®.

4. Students can also practice steps 15&16 of the transformation experiment by mixing the icing and water mixture, pipetting the solution back onto their Jell-O® plates, and then gently spreading the solution over the entire plate.