EDVOTEK® • The Biotechnology Education Company®

Edvo-Kit #
223/AP08

BIG IDEA AP BIOLOGY

INVESTIGATION #8:

Transformation of *E. coli* with Green Fluorescent Protein

For 10 groups

Experiment Objective:

Students explore the biological process of bacterial transformation using *E. coli* and plasmid DNA. At the end of the activity, students will have experience observing and analyzing acquired traits (ampicillin resistance and fluorescence) as exhibited by transformed bacterial cells.

See page 3 for storage instructions.

Version 223/AP08.231009

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

Experiment Components

COMPONENTS		Storage	Check (✓)
A	BactoBeads™ *E. coli* GFP Host	4°C (with desiccant)	❑
B	Supercoiled pFluoroGreen plasmid DNA	Freezer	❑
C	Ampicillin	Freezer	❑
D	IPTG	Freezer	❑
E	$CaCl_2$	Freezer	❑
•	Growth Additive	Freezer	❑
•	Competent Cell Solution*	Freezer	❑

**This solution is needed only for the alternative Enhanced Transformation Procedure (Appendix A).*

Store all components below at room temperature.

This experiment is designed for 10 lab groups.

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.

REAGENTS & SUPPLIES

- 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 ❑
- 50 mL Conical tube ❑

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
- Long wave UV light (Cat. #969) or Blue Light (Cat. #555 or #557) recommended
- UV safety glasses
- Pipet pumps or bulbs
- Microwave
- Centrifuge (optional, for Enhanced Transformation Procedure)

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.

Background Information

The cell serves as the fundamental building block of life, found in all living organisms, ranging from single-celled bacteria to complex multicellular beings like humans. Inside these cells, there is a special molecule called deoxyribonucleic acid, or DNA for short. Through careful experimentation, scientists have shown that DNA is the master blueprint responsible for the construction and functioning of an organism. The instructions in our DNA controls biological processes, such as growth, development, cell specialization, neural activity, and metabolic pathways (Figure 1).

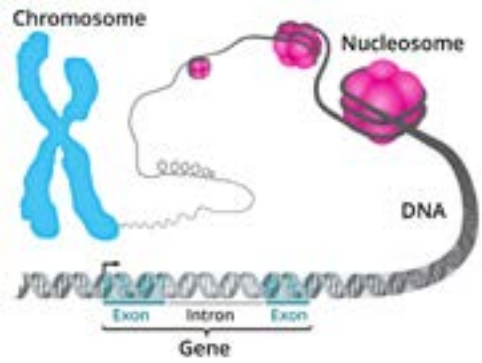


Figure 1:
DNA structure from chromosome to gene*

Within DNA, certain segments are called genes, which consist of discrete sequences of nucleotides that act as the fundamental units of heredity. Genes carry and pass on genetic information from one generation to the next. They do this by providing instructions to cells on how to produce specific proteins or functional RNA molecules. Consequently, an organism's genetic makeup, known as its genotype, manifests as observable traits, or phenotypes, that we can see or experience.

Changes in a gene's sequence, or mutations, can affect their normal function. Some mutations have little impact and don't harm an organism. However, others can be harmful, leading to the creation of faulty proteins or disrupting important processes in the body. These harmful mutations may result in genetic disorders or make someone more vulnerable to certain diseases. On the bright side, some mutations can be beneficial, providing unique traits or improved abilities that help an organism survive and thrive. Studying the effects of mutations on genes is crucial in fields like genetics and medicine, as it helps scientists understand genetic diseases, track how species evolve, and develop treatments to counteract harmful mutations.

After the nature of DNA and genes were realized, scientists worldwide raced to unravel the mysteries hidden within our DNA. However, they encountered challenges in studying individual genes due to the presence of hundreds of genes within a single chromosome. To overcome this obstacle, they developed gene cloning techniques to isolate, combine, and replicate specific DNA sequences. This breakthrough in the form of recombinant DNA technology marked the advent of biotechnology. It enabled researchers to undertake DNA mapping, sequencing, and various genome-wide studies, opening new avenues of exploration in the realm of genetics and biomedicine. In this experiment, we will explore the ways genes can be transferred into bacteria, changing their phenotype.

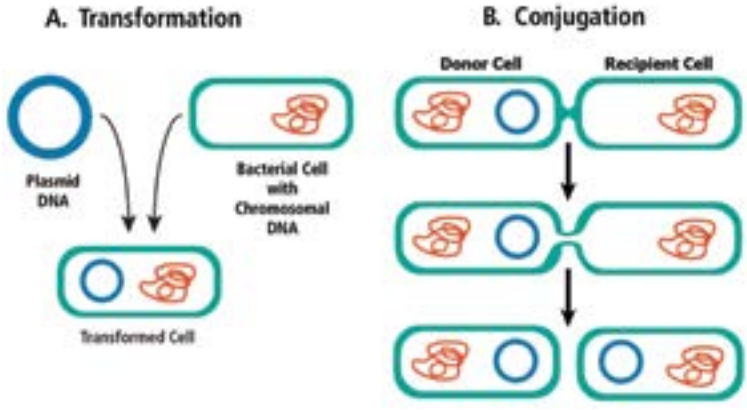


Figure 2: Mechanisms for Bacterial DNA Uptake

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 2A). 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 (Figure 2B). In both cases, the bacteria have acquired new genetic information that is both stable and heritable.

**Attribution, Figure 1: Thomas Splettstoesser (www.scistyle.com), CC BY-SA 4.0 <https://creativecommons.org/licenses/by-sa/4.0>, via Wikimedia Commons*

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. pneumoniae* to determine which was responsible for transformation. Each component was mixed with a non-pathogenic strain of bacteria. Only those recipient cells exposed to DNA became pathogenic. These transformation experiments not only revealed this virulence is transferred but also led to the recognition of DNA as the genetic material.

> ***If we are right, and of course that is not yet proven, then it means that nucleic acids are not merely structurally important but functionally active substances in determining the biochemical activities and specific characteristics of cells and that by means of a known chemical substance it is possible to induce predictable and hereditary changes in cells. This is something that has long been the dreams of geneticists.***
>
> – Oswald T. Avery, 1943

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 like *Escherichia coli* —to take up DNA and become transformed. This can be done with electricity, in a process called electroporation, or through physical means in a heat shock.

In a heat shock transformation, bacterial cells are treated with specific chemicals, like calcium, rubidium, or magnesium chloride, that make them "competent" for transformation. DNA is added to the competent cells and the suspension is "heat shocked," or moved quickly between widely different temperatures. It is believed that a 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.

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 ampicillin 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.

> ***With genetic engineering, a bacterium can acquire a human gene--and treat it as one of its own. In some ways there is nothing very new about this; every time you get a cold you acquire unwelcome viral genes, but the point about genetic engineering is having some control over the transfer process.***
>
> – Susan Aldrich, The Thread of Life: The Story of Genes and Genetic Engineering, 1996.

In the laboratory, we can engineer plasmids to contain genes that are not normally found in bacteria using recombinant DNA technology, also known as molecular cloning. Once transformed into the cells, the plasmids turn the bacteria into living factories to create medications, vitamins, and other useful products. For example, scientists first used bacteria to make insulin, the medication used to treat diabetes. This technique is commonly known as recombinant DNA technology or molecular cloning, and it has been revolutionary for biotechnology, medicine, and research. The specialized plasmids designed for molecular cloning are called vectors (Figure 3a), and they 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. Examples of promoters for expression in bacteria include T4 and T7.

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 3a:
Plasmid with Multiple Cloning Site

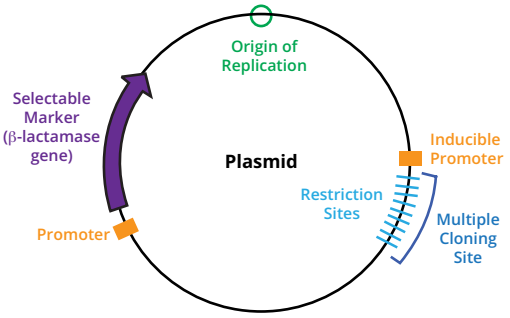
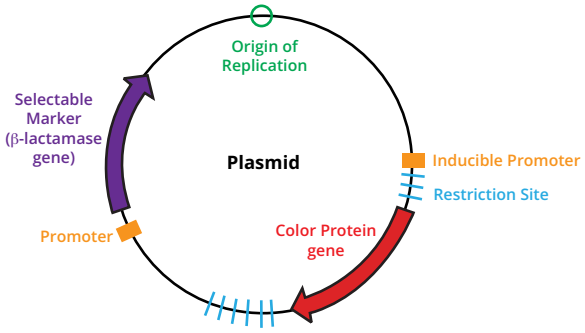


Figure 3b:
Fluorescent protein cloned into MCS



FLUORESCENT PROTEINS

In the laboratory, we can engineer a plasmid that contains the DNA sequence for the Green Fluorescent Protein (GFP) (Figure 3b). Once transformed into *E. coli*, this protein makes the bacteria glow bright green! GFP is a small protein, approximately 27 kilodaltons in size. GFP possesses the ability to absorb blue light and emit green light in response. This activity, known as fluorescence, does not require any additional special substrates, gene products or cofactors to produce visible light.

Quick Reference Abbreviations

GFP	Green fluorescent protein
pGFP	Plasmid for GFP expression
gfp	Gene for green fluorescent protein

GFP was first isolated from the jellyfish *Aequorea victoria* in the 1970's. Once scientists identified its DNA sequence, they were able to use genetic engineering to introduce fluorescent proteins into other organisms, such as *E. coli* and the nematode *Caenorhabditis elegans*. Scientists also identified specific amino acid substitutions in GFP that altered the behavior of its 'chromophore', a special structure within the protein that is responsible for light production (Figure 4). Different changes bring about different patterns of light absorption and emission, allowing scientists to develop a rainbow of fluorescent proteins (Figure 5). For example, GFP can be converted to BFP by making two amino acid substitutions, one of which is in the chromophore (His-Tyr). For their discovery and development of GFP and other fluorescent proteins, Osamu Shimomura, Martin Chalfie and Roger Tsien were awarded the Nobel Prize in Chemistry in 2008.

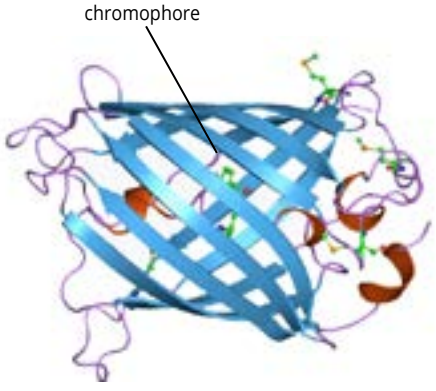


Figure 4:
Green Fluorescent Protein.

Fluorescent proteins have become an essential tool in cell and molecular biology. Using DNA cloning strategies, proteins can be "tagged" with fluorescent proteins and then expressed in cells. These tags simplify purification

because fluorescently labeled proteins can be tracked using UV light. The most useful application of fluorescent proteins is as a visualization tool during fluorescent microscopy studies. By tagging other proteins with GFP, researchers can determine where those proteins are normally found in the cell. Similarly, using a fluorescent protein as a reporter, scientists can observe biological processes as they occur within living cells. For example, in the model organism zebrafish (*Danio rerio*), scientists use GFP to fluorescently label blood vessel proteins so they can track blood vessel growth patterns and networks. Scientists also tag regulatory DNA sequences with the GFP coding sequence so they can observe patterns of when and where the gene is expressed. In this way, GFP can reveal the role these regulatory sequences might normally play in a cell. In summary, fluorescent proteins, including GFP and BFP, and fluorescent microscopy have enhanced our understanding of many biological processes by allowing scientists to watch biological processes in real-time.

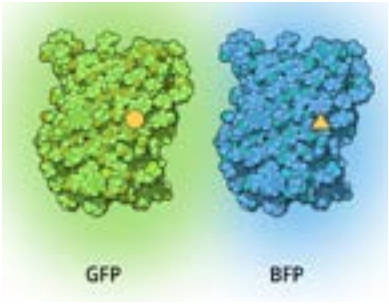


Figure 5:
Structural changes in the chromophore (yellow circle and triangle) can create different colored proteins.

CONTROL OF GENE EXPRESSION

Scientists can regulate the expression of recombinant proteins using a genetic "on/off" switch called an inducible promoter (Figure 6). These sequences allow precise control because expression of the gene will only "turn on" in the presence of a small molecule like arabinose, tetracycline, lactose, or IPTG (isopropyl-ß-D-thiogalactopyranoside).

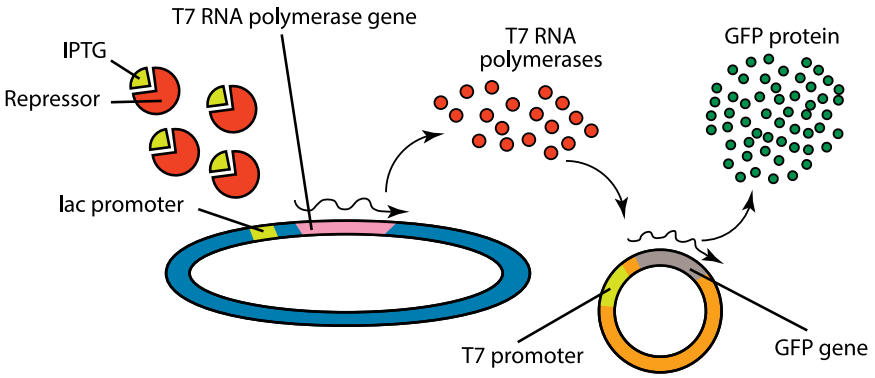


Figure 6: Model of the Activation of an Inducible Promoter.

The lac operon represents an iconic example of bacterial gene regulation using inducible promoters. The lac operon is a control system in bacterial cells that regulates the use of lactose, a sugar found in milk. It acts like a switch that turns on or off depending on the presence of lactose. When lactose is absent, the lac repressor protein binds to the operator region of the lac operon, preventing the transcription of the genes responsible for breaking down lactose. But when lactose is available, the sugar binds to the repressor protein, changing its conformation. The lac repressor releases from the operator and the lac operon switches "on," enabling the bacteria to break down lactose for energy. Scientists have studied the lac operon extensively, and it has become a classic example in biology to understand how genes are controlled and regulated in living organisms.

The inducible promoter system used in this experiment was modified from the lac operon. The host bacteria used in this experiment have been genetically engineered to contain the gene for a special RNA polymerase (T7), which is controlled by the lac promoter. T7 RNA polymerase specifically binds to the T7 promoter that is found on the GFP plasmid that is transformed into the bacteria. Under normal circumstances, lac repressor binds to the lac promoter and blocks expression of the T7 polymerase. Without T7 polymerase, the recombinant protein cannot be expressed, and cells will not fluoresce.

To turn on transcription, we will add IPTG, a molecule that mimics the structure of lactose and binds to lac repressor. This inactivates lac repressor, allowing T7 polymerase to be expressed. T7 polymerase specifically recognizes the T7 promoter found in the GFP-containing plasmid. Gene expression is then activated, resulting in the transcription of large quantities of mRNA. Finally, the mRNA is translated to produce GFP protein in the cytoplasm, causing the cells to fluoresce.

TRANSFORMATION EFFICIENCY

After cells are transformed with the GFP plasmid, they are spread on nutrient agar plates that contain the correct selection antibiotic. The presence of the antibiotic restricts the growth of any untransformed bacteria, allowing for the identification of transformed bacteria. To demonstrate the effectiveness of selection, transformed bacteria are plated on both selective and nonselective agar medium. We will observe very different results on each plate. 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.

In practice, transformation of the plasmid into the bacteria 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.

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

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 7:
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 7). 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 mL 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.

EXPERIMENT OVERVIEW

In this experiment, students learn how to transform *E. coli* bacteria with pFluoroGreen™, a plasmid containing genes for ampicillin resistance and GFP. Cells are treated with calcium chloride to make them chemically competent before they are transformed with the plasmid using the heat shock protocol. Plasmid-containing bacteria are selected for by spreading the cell suspension on selective media plates containing ampicillin, upon which only bacteria expressing ß-lactamase can grow. From their results, students calculate the transformation efficiency. To explore the principles of gene expression and inducible promoters, some cells will be exposed to the small molecule IPTG, whereas others will not. Because GFP protein will only be expressed in the presence of IPTG, this experiment will demonstrate differential gene expression.

Through bacterial transformation students will gain hands-on experience with fundamental concepts in genetics and molecular biology, including DNA as the molecule of heredity, gene transfer, differential gene expression, and acquired traits producing phenotypic changes. At the end of the activity, students will have experience observing and analyzing acquired traits (ampicillin resistance and fluorescence) as exhibited by transformed bacterial cells. Students should also possess an enhanced understanding of the abstract concepts of transformation and gene expression.

Experiment Overview

LABORATORY SAFETY

Wear gloves and safety goggles

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

- *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% beach 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.

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

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.

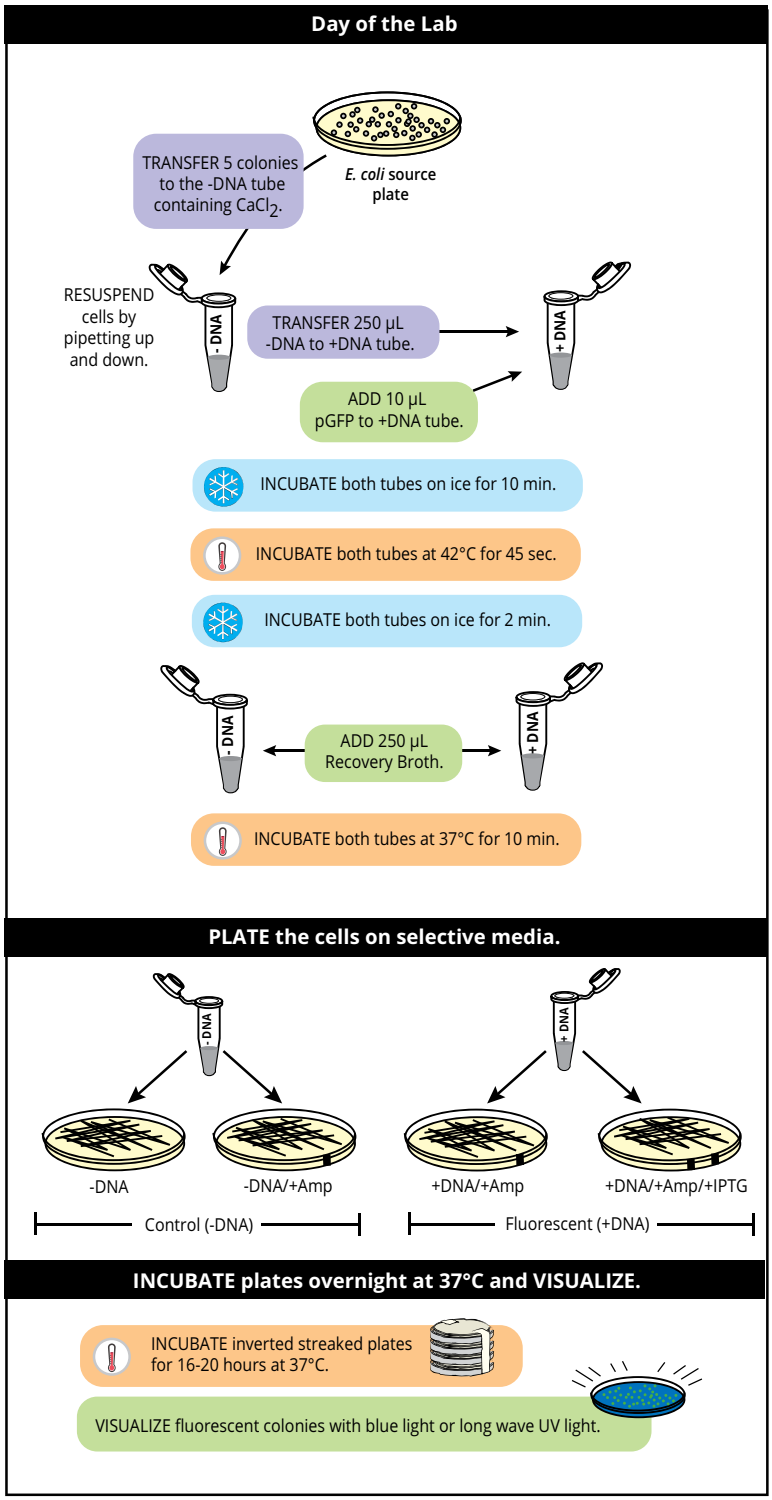
Before starting the Experiment:
- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

During the Experiment:
- Record your observations in your lab notebook or in the Student Handout in Appendix E.
- Record any challenges faced while performing the experiment.

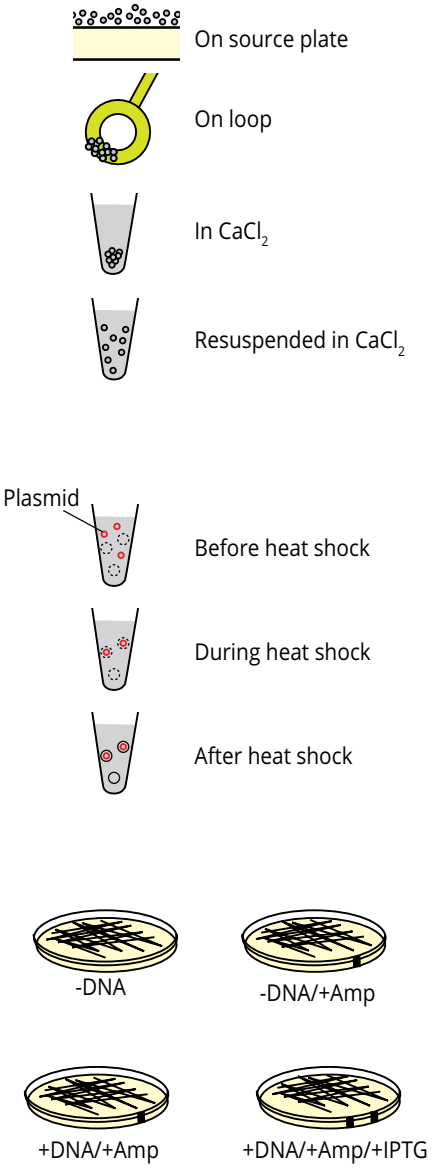
After the Experiment:
- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

Experiment Overview, continued

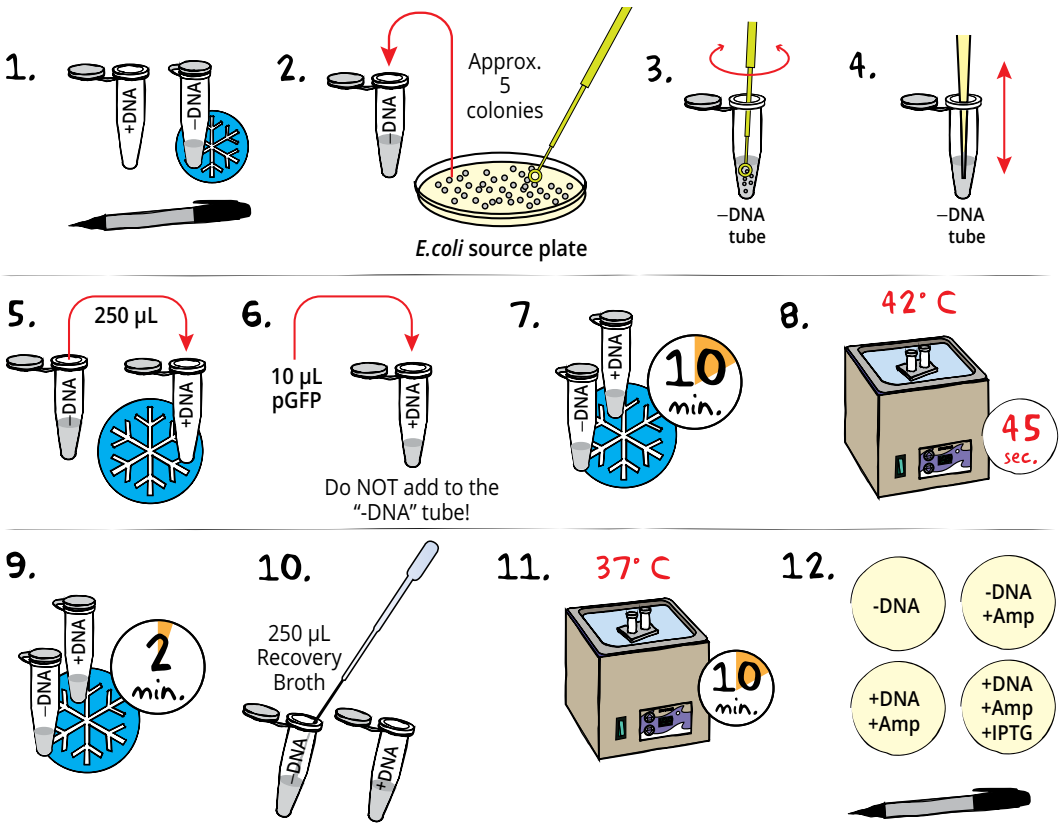
In this experiment, host *E. coli* bacteria is transformed with pGFP. The bacteria will be grown for 18-22 hours on LB-agar "source plates", collected using a sterile loop, and made competent in $CaCl_2$. Next, the plasmid will be added to half of the cells before they are briefly heat shocked. Finally,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



Explain what the *E. coli* are doing:



Transformation of *E. coli* with Green Fluorescent Protein



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

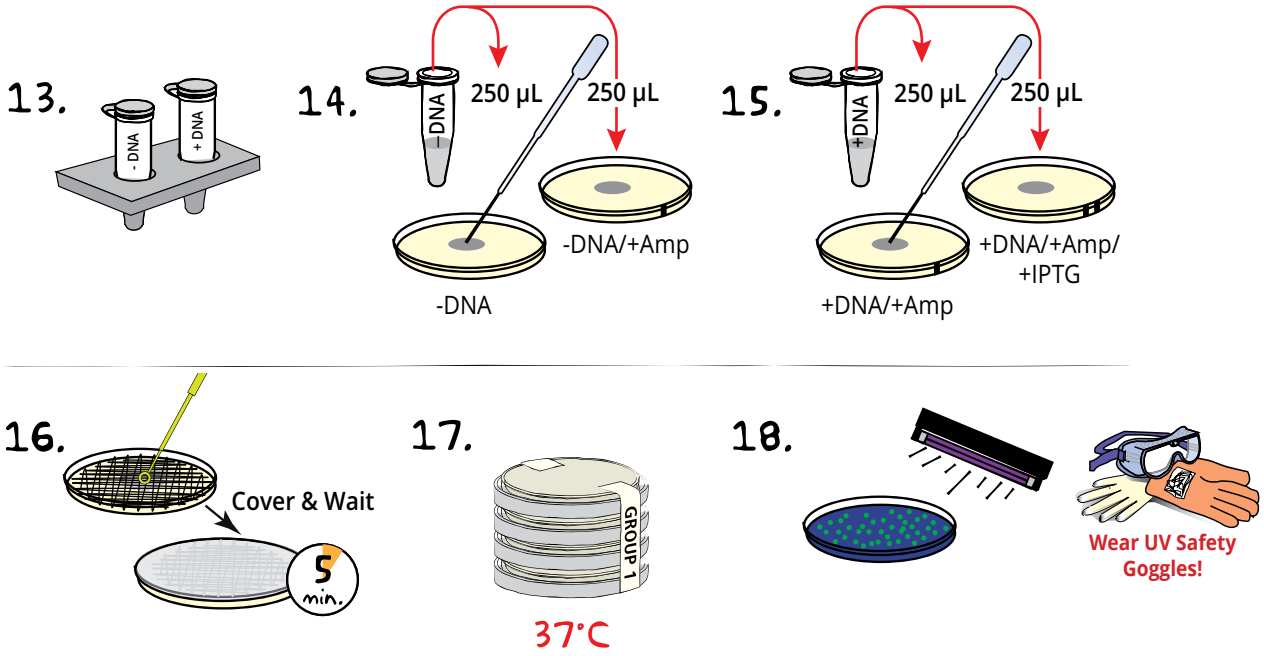
IMPORTANT:
For best results, ensure that the $CaCl_2$ is ice cold and that the cells are rapidly moved from ice to 42°C and then immediately back to ice. Follow all times exactly.

Make sure to keep the actual labels small!

1. **LABEL** the microcentrifuge tube containing ice cold $CaCl_2$ as "-DNA" and the empty microcentrifuge tube as "+DNA".
2. Using a sterile inoculation loop, **TRANSFER** approx. 5 well-isolated colonies (each colony should be approx. 1-1.5 mm in size) from the *E. coli* source plate to the "-DNA" tube.
3. **TWIST** the loop between your fingers to free the cells. **ENSURE** that all cells have been removed from the loop.
4. **RESUSPEND** the bacterial cells in the $CaCl_2$ solution by pipetting up and down 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** both tubes on ice.
6. **ADD** 10 µL of pGFP to the tube labeled "+ DNA" and gently flick to mix. DO NOT add plasmid to the "-DNA" tube.
7. **INCUBATE** the tubes on ice for 10 minutes.
8. **PLACE** the transformation tubes in a 42°C water bath for exactly 45 seconds.
9. Immediately **RETURN** the tubes to the ice bucket and **INCUBATE** for 2 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 10 minutes in a 37°C water bath.
12. While the cells are recovering, **LABEL** the bottom of four agar plates as indicated below.
    - **-DNA** *(plate with no stripe)*
    - **-DNA/+Amp** *(plate with one stripe)*
    - **+DNA/+Amp** *(plate with one stripe)*
    - **+DNA/+Amp/+IPTG** *(plate with two stripes)*

continued

Transformation of *E. coli* with Green Fluorescent Protein , 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 and -DNA/+Amp plates.
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 and +DNA/+Amp/+IPTG plates.
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. **PLACE** the plates in the inverted position (agar side on top) in a 37°C bacterial incubation oven for overnight incubation (16-18 hours). If you do not have an incubator, colonies will form at room temperature in approximately 48-72 hours.
18. **VISUALIZE** the transformation and control plates using a long wave UV light or a blue light. For each of the plates, **RECORD** the following:
    - The number of colonies on the plate.
    - The color of the bacteria under UV or blue light.

NOTE: We recommend recording the results and taking a photo for your lab notebook.

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. We recommend documenting these results with a photograph.

   Control Plates: (-) DNA
   - -DNA
   - -DNA/+Amp

   Transformation Plates: (+) DNA
   - +DNA/+Amp
   - +DNA/+Amp/+IPTG

2. **DESCRIBE** what you observe. You may wish to **DRAW** the plates if you do not have a photograph. 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/+IPTG. 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 or by marking a photograph.

2. **DETERMINE** the transformation efficiency using the following formula:

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

Related Instructional Video

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 223/AP08:

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?

Extension Activities

After performing the initial transformation, students can design their own explorations to further their understanding in areas like gene transfer by transformation, gene expression, organism fitness, or the effects of mutation on DNA. Below are some open-ended questions that students can research for further exploration. These topics should be researched, and new hypotheses should be formed and tested.

Gene transfer by transformation can be affected by a variety of factors. How would you change conditions to improve transformation efficiency?

- Amount of DNA
- Time and/or temperature of heat shock
- Different salt ($RbCl_2$ and $CaCl_2$ are common)
- Preparation of cells – traditional transformation vs. enhanced protocol.

The production of the green fluorescent protein is controlled by the lac operon.

- What effects do mutations have on gene expression? Add mutagens to the cells or to the plasmid before transformation and look for the effects.
- Cotransformation of plasmids that express different genes (fluorescent or chromogenic, different antibiotic resistance).
- Is gene expression affected in the absence of selective pressure (antibiotic)?

Bacteria respond to stress and environmental changes.

- Does the plasmid give a selective advantage over untransformed bacteria? Grow up cultures of both transformed and untransformed bacteria and plate on plain LB plates.
- How do transformed vs non-transformed cells grow on different media?

Study Questions

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

1. On which plate(s) would you expect to find bacteria most like the *E. coli* on the source plate? Explain.

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

3. What is the purpose of the control plates? Explain the difference between the controls and why each one is necessary.

4. Why would one compare the -DNA/+Amp and +DNA/+Amp plates?"

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 doesn't the recovery broth used in this experiment contain ampicillin?

3. What is the difference in the amino acid structure of the green and blue fluorescent proteins?

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

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

6. What is the source of the fluorescence? Why are cells on the +DNA/+AMP/+IPTG plate fluorescent while cells on the +DNA/+AMP plate not fluorescent?

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 five additional resources:

1. An alternative enhanced transformation protocol (Appendix A) that improves student results. This protocol replaces steps 1-5 in the student's experiment and requires two additional pre-transformation steps so **determine beforehand which procedure your class will follow and plan accordingly.**

2. A transformation tips and tricks section (Appendix B) that complements the student protocol on pages 11 and 12. 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.

3. A transformation troubleshooting guide (Appendix C) 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.

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

5. A student transformation worksheet (Appendix E) for students to record their experiment and their results.

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	17
Prepare *E. coli* Source plates	20 minutes to streak plates; 16-18 hours to incubate plates	The day before performing the experiment	19
Dispense plasmid DNA, $CaCl_2$, and recovery broth	30 minutes	One day to 30 min. before performing the experiment	20

ADDITIONAL PREPARATION FOR *ALTERNATIVE* ENHANCED TRANSFORMATION:

What to do:	Time Required:	When?	Page
Prepare *E. coli* Starter Culture	70-90 minutes	Up to 3 days before the experiment	25
Prepare Competent Cells	30 minutes	Up to 2 days before the experiment	25

DAY OF THE EXPERIMENT:

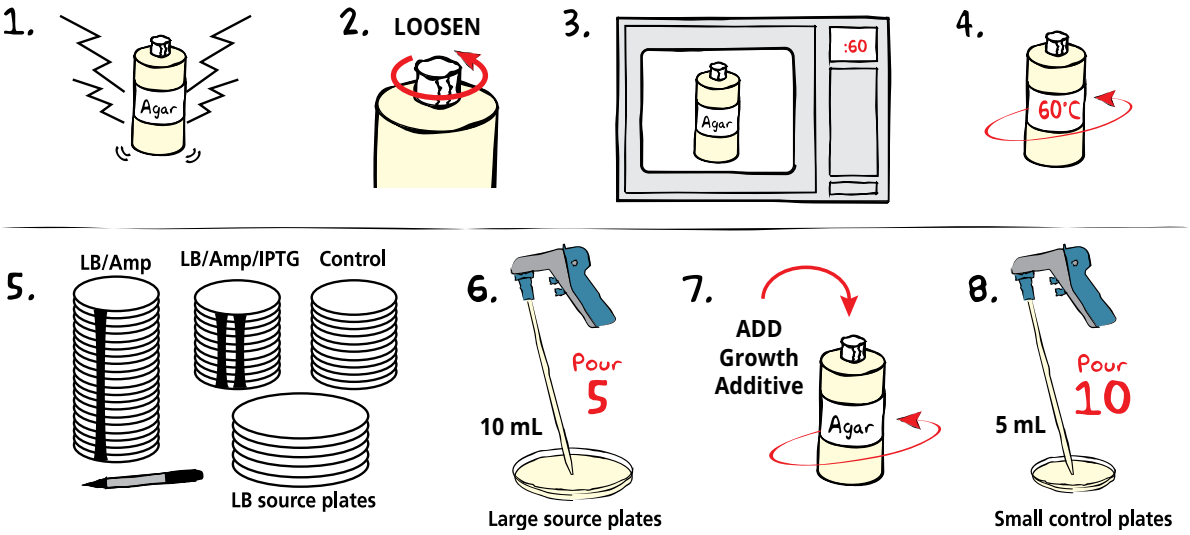
What to do:	Time Required:	When?	Page
Equilibrate waterbaths at 37°C and 42°C; incubator at 37°C	10 minutes	One to two hours before performing the experiment	20
Perform laboratory experiment	50 minutes	The class period	11
Incubate cells at 37°C	16-18 hours	Overnight after the class period	12

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	13
Discard any contaminated materials	45 minutes - overnight	After the students have analyzed their results	13

Pouring LB-Agar Plates

One bottle of ReadyPour™ Luria Broth Agar will make 5 large LB source plates, 10 LB plates, 20 LB/Amp plates and 10 LB/Amp/IPTG plates.



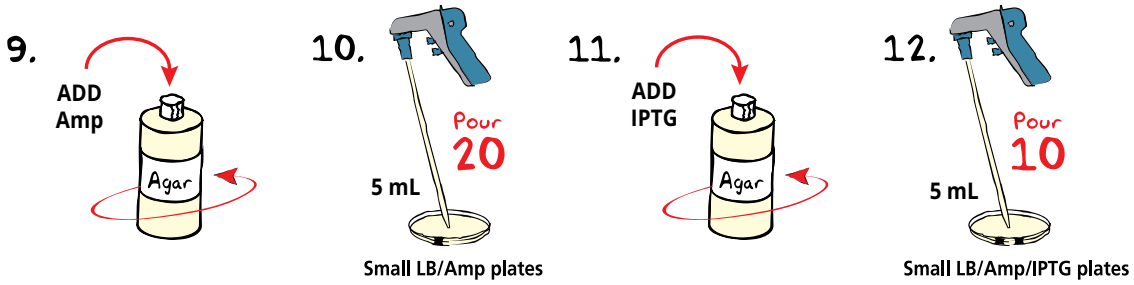
1. **BREAK** solid ReadyPour™ LB Agar into small chunks by vigorously squeezing and shaking the plastic bottle.
2. **LOOSEN**, but DO NOT REMOVE, the cap on the ReadyPour™ Agar bottle. This allows the steam to vent during heating. ***CAUTION: Failure to loosen the cap prior to heating may cause the bottle 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** the small (60 x 15 mm) petri dishes with a permanent marker.
   - **OPEN** the first sleeve and neatly **STACK** all 20 plates.
   - Next, "**STRIPE**" the 20 plates by placing the marker at the bottom of the stack and dragging it vertically to the top plate. These plates will be used for LB/Amp plates.
   - **OPEN** the second sleeve and neatly **STACK** ten plates.
   - STRIPE the 10 plates with two lines. These will be the LB/Amp/IPTG plates. DO NOT label the remaining 10 plates. These will be the control LB plates. (You should also have 5 large petri dishes for the LB source plates).
6. **POUR** 10 mL of the cooled ReadyPour™ Agar into each of the five large petri dishes (source plates) using a 10 mL pipet and pipet pump.
7. **ADD** the entire amount of the Growth Additive to the cooled ReadyPour™ Agar. **RECAP** the bottle and SWIRL to mix the reagents. ONLY ADD REAGENTS TO COOLED AGAR. Reagents like ampicillin and IPTG degrade at high temperature.
8. Using a fresh 10 mL pipet, **POUR** 5 mL of the agar into the 10 unlabeled petri plates.

Wear Hot Gloves and Goggles during all steps involving heating.

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.

continued

Pouring LB-Agar Plates, continued



9. **ADD** the entire amount of the Ampicillin to the ReadyPour™ Agar bottle. Make sure all of the powder is transferred from the tube. **RECAP** the bottle and **SWIRL** to mix the reagents.
10. Using a fresh 10 mL pipet, **POUR** 5 mL of the LB/Amp medium into the 20 small petri plates with one stripe.
11. Use a micropipette and clean tip to **TRANSFER** the *entire volume* (~65 µL) of IPTG liquid to the ReadyPour™ Agar bottle. **RECAP** the bottle and **SWIRL** to mix the reagents.
12. Using a fresh 10 mL pipet, **POUR** 5 mL of the LB/Amp/IPTG medium into the 10 small petri plates with two stripes.
13. **COVER** and **WAIT** for the LB-agar plates to solidify. For optimal results, leave plates at room temperature overnight.
14. **STORE** plates in the refrigerator (4°C) until needed. Plates should be inverted and placed in a sealable plastic bag to ensure that they do not dry out.

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

NOTE: If plates are prepared more than one day before use, they should be left on the bench overnight to dry. The following day, store plates 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.

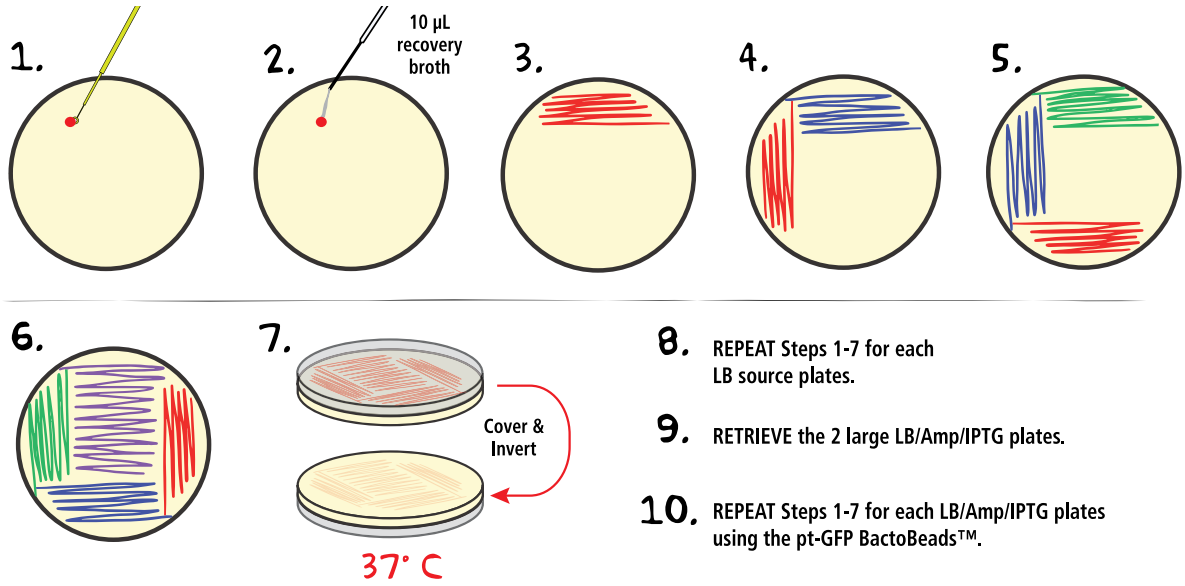
QUICK REFERENCE: POURING LB AGAR PLATES

- 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.

Related Instructional Video

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.

Related Instructional Video

Pre-Lab Preparations

Each Group Requires:

- Sharing - one of five *E. coli* source plates
- 1 tube $CaCl_2$
- 1 tube pGFP plasmid DNA
- 1 tube Recovery broth
- 2 one-striped plates
- 1 two-striped plate
- 1 unstriped plate
- 4 pipets (1 mL)
- 3 sterile inoculating loops

Classroom Equipment:

- Waterbath(s)
- Incubation Oven

DAY OF THE LAB:

1. **EQUILIBRATE** waterbaths at 37°C and 42°C; **SET** the incubator at 37°C. **CONFIRM** the temperature with a thermometer.

2. **PREPARE** ice or ice-water baths for each group. Small ice cubes will help to rapidly cool the bacteria after the heat shock.

3. If performing the original transformation procedure, **DISPENSE** 500 µL of $CaCl_2$ into microcentrifuge tubes for each of the 10 groups and **PLACE** on ice. **OR** if performing the enhanced transformation procedure, **DISPENSE** 800 µL of $CaCl_2$ into microcentrifuge tubes for each of the 10 groups and **PLACE** on ice.

4. **DISPENSE** 600 µL 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 pipetting station for students to share. ***NOTE: Maintain sterile technique while aliquoting Recovery Broth.***

Related Instructional Video

Preparation of pGFP Plasmid DNA

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

5. **PLACE** the tube of pGFP Plasmid DNA on ice to thaw.

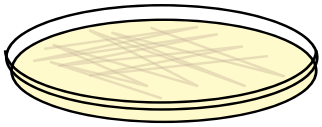
6. **LABEL** 10 microcentrifuge tubes "pGFP".

7. Before dispensing, **TAP** the tube of pGFP until all the sample is at the tapered bottom of the tube.

8. Using an adjustable volume micropipette, **DISPENSE** 12 µL of the plasmid DNA to each of the microcentrifuge tubes labeled "pGFP". **CAP** the tubes and **PLACE** them on ice.

NOTE: If prepared ahead of time, the pGFP and $CaCl_2$ aliquots can be stored at 4°C for up to 24 hours. Always provide plasmid DNA and $CaCl_2$ on ice to assist the heat shock procedure.

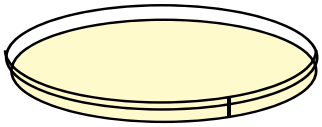
Experiment Results and Analysis



-DNA
plated with non-transformed cells (no DNA)

Result: No fluorescent cells visible. White colonies. Will likely look like a smeared layer of cells (lawn).

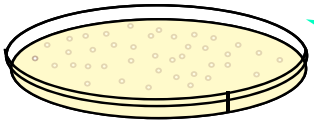
Demonstrates: Host bacterial cells are viable in the absence of ampicillin.



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

Result: No growth

Demonstrates: Cells are sensitive to ampicillin. Without pGFP, they are not ampicillin-resistant.

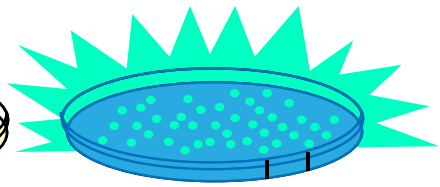


+DNA/+AMP
plated with transformed cells (pGFP)

Result: white colonies. May look like a smeared layer of cells.

Demonstrates: Cells become resistant to Ampicillin when transformed with the pGFP.

GFP protein is not produced in the absence of IPTG.



+DNA/+AMP/+IPTG
plated with transformed cells (pGFP)

Result: Individual colonies that will fluoresce when exposed to long wave UV light.

Demonstrates: Cells become resistant to Ampicillin when transformed with the pGFP. Production of GFP protein is turned on in the presence of IPTG.

Results Photo of the +DNA/+Amp/+IPTG plate

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.

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

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 Edvotek® Enhanced Transformation Protocol
B Transformation Tips and Tricks
C Troubleshooting Guide
D Pre-Transformation Practice - Bacteria Colony Collection
E Plate Culture Transformation - Student Worksheet

Safety Data Sheets:

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

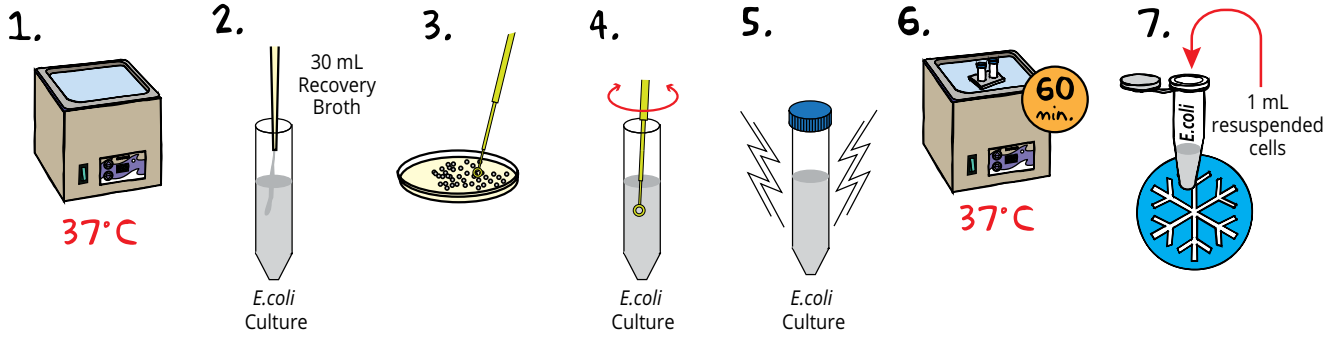
Appendix A

Edvotek® Enhanced Transformation Protocol

This procedure creates a liquid culture of competent cells that can produce superior transformation results. It also offers additional flexibility for the teacher and students. It requires additional recovery broth and a specially formulated competent cell solution (both provided in the kit components). It also requires the use of a centrifuge. In this protocol, you will prepare the starter cultures while your students will prepare the competent cells and perform the transformation. However, if class time is limited, you may decided to prepare the competent cells as part of the teacher's prelab.

PREPARATION OF *E. coli* STARTER CULTURES

Performed by the teacher. Prepare at least 70 to 90 min. before needed (20-30 min. active, 60 min. incubation).



1. **PREPARE** a 37°C waterbath.
2. **ADD** 30 mL of Recovery Broth to a 50 mL conical. **LABEL** the tube "*E. coli* Culture".
3. **SWIPE** a loop through a dense section of the bacterial culture. You want to collect a clump of bacteria approximately the size of a match head.
4. **RESUSPEND** the bacteria in the *E. coli* Culture tube prepared in step 2 by twisting the loop back and forth until all bacteria have been removed from the loop.
5. **SHAKE** or vortex the tube briefly to ensure that the bacteria are completely resuspended.
6. **INCUBATE** the *E. coli* culture for 60 min. in a 37°C waterbath.
7. **LABEL** 20 snap-top microcentrifuge tubes as "*E. coli*" and aliquot 1 mL of resuspended cells into each tube. **PLACE** the tubes on ice until they are needed for the experiment.

OPTIONAL STOPPING POINT: The *E. coli* can be stored for up to 24 hours after aliquoting. Centrifuge the cells at maximum speed for 5 minutes, then carefully pour off the supernatant. Finally, store the bacteria at 4°C until needed by the students. This will complete steps 1-3 of the Preparation of Competent Cells (below). If you opt for this stopping point begin this next section at step 4.

ADDITIONAL PRELAB PREPARATIONS

1. **DISPENSE** 300 µL Competent Cell Solution into ten microcentrifuge tubes, **LABEL**, and **PLACE** on ice.
2. **COMPLETE** steps 1-8 on page 21 before students begin "Preparation of Competent Cells" on the next page.

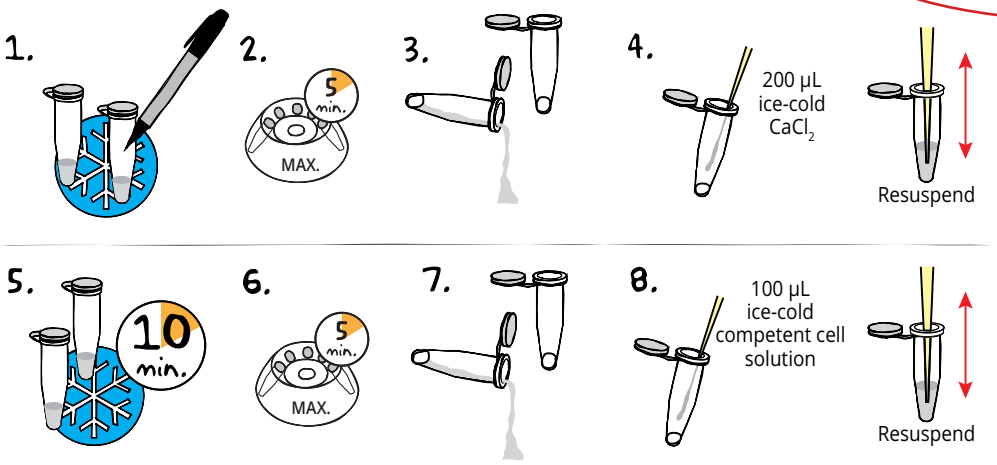
NOTE: Several of these reagents will be used during competent cell preparation and during transformation so encourage students to label all items with their group ID.

Related Instructional Video

Appendix A, continued

Performed by the students.
30 min. Requires a centrifuge.

PREPARATION OF COMPETENT CELLS



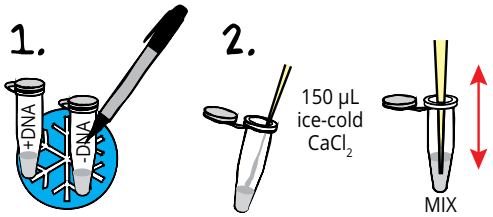
NOTE: Keep tubes on ice as much as possible during this module.

1. **OBTAIN** two 1.5 mL tubes of *E. coli* starter culture. **LABEL** tubes with their initials or group number.
2. **CENTRIFUGE** the tubes at maximum speed for 5 minutes to pellet the cells.
3. Carefully **POUR** off the supernatant. DO NOT DISTURB THE CELL PELLET!
4. **ADD** 200 µL of ice-cold $CaCl_2$ solution to each tube. Gently **RESUSPEND** the cells by slowly pipetting up and down several times. Save the remaining $CaCl_2$ on ice for later.
   NOTE: It is important that the cells are fully resuspended. Continue to gently pipette until no clumps are seen in the $CaCl_2$ solution.
5. **INCUBATE** the tubes on ice for 10 minutes.
6. **CENTRIFUGE** the tubes at maximum speed for 5 minutes to pellet the cells.
7. Carefully **POUR** off the supernatant. DO NOT DISTURB THE CELL PELLET! ***NOTE: At this point the cells are fragile. Keep the cells on ice and pipette slowly and gently.***
8. Slowly **ADD** 100 µL of ice-cold Competent Cell Solution (CCS) to each tube. Gently **RESUSPEND** the cells in the ice-cold competent cell solution by slowly pipetting up and down several times. Immediately **PLACE** the tubes on ice and proceed to Transformation.

OPTIONAL STOPPING POINT: The competent cells can be stored for up to 48 hours in the freezer after they have been resuspended in competent cell solution.

TRANSFORMATION

Performed by the students.
45 min.



1. **RETRIEVE** two tubes of competent cell and place immediately on ice. **LABEL** one tube "+DNA" and the other tube "-DNA".
2. **ADD** 150 µL ice-cold $CaCl_2$ solution to both tubes. **MIX** by gently pipetting up and down several times.
3. **CONTINUE** with the experimental protocol on page 11 starting with step 6.

Appendix B
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 C
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 D
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.

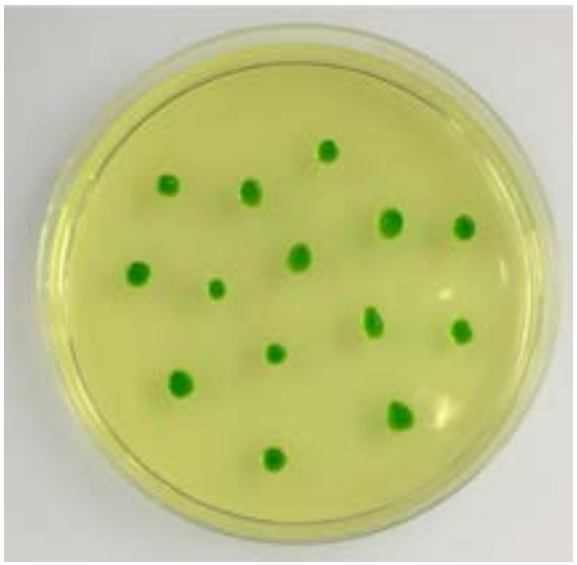
1. Make 10 Jell-O® practice petri plates.
   - In a large breaker mix Jell-O® powder and water according the package directions.
   - Quickly pour the Jell-O® into petri plates, filling about half way.
   - Allow plates to solidify in the fridge for 30-60 minutes.
   - Add 10 dots using whatever liquid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

Related Instructional Video



Appendix E
PLATE CULTURE TRANSFORMATION - Student Worksheet

APPENDIX A - EXPERIMENT NOTES:

- Ice-cold $CaCl_2$ added to "-DNA" (Step 2) Added ❑
- Colonies added to "-DNA" (Steps 3 & 4) Added ❑
  - Number of colonies collected _____
  - Was agar plate scratched or gauged? Yes / No
    (agar can inhibit transformation)
  - Did loop appear free of cells at the end of Step 4? Yes / No
- Half of cell suspension added to "+DNA" (Step 5) Added ❑
- Plasmid added to "+DNA" (Step 6) Added ❑
  - Plasmids contain genes for ampicillin resistance and the following colors _____.
- Incubation on ice - 10 minutes (Step 7) Start_____ End_____
- Heat shock at 42 °C - 45 seconds (Step 8)* Start_____ End_____
- Incubation on ice - 2 minutes (Step 9) Start_____ End_____
- Recovery at 37 °C - 10 minutes (Step 11) Start_____ End_____
- Plate Incubation at 37 °C – 24-48 hours (Step 17) Start_____ End_____

***NOTE:**
For best results, bacteria need to go from ice to 42 °C and back to ice as rapidly as possible. Bring your ice bucket with you to the water bath!

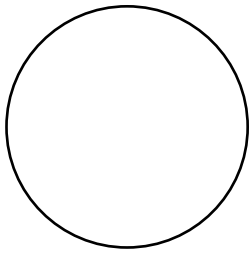
ADDITIONAL NOTES:

Appendix E

PLATE CULTURE TRANSFORMATION - Student Worksheet

EXPERIMENT OBSERVATION:

-DNA Plate

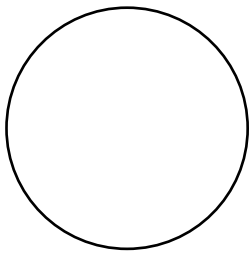


Draw or add photo here↑

How much bacterial growth do you observe? If colonies are present, determine a count.

What color(s) are the bacteria by eye? What color(s) are the bacteria with UV or blue light?

-DNA/+Amp Plate

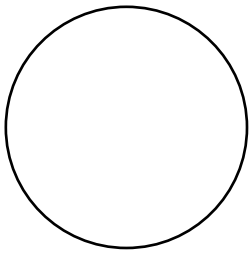


Draw or add photo here↑

How much bacterial growth do you observe? If colonies are present, determine a count.

What color(s) are the bacteria by eye? What color(s) are the bacteria with UV or blue light?

+DNA/+Amp Plate

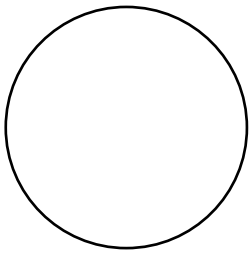


Draw or add photo here↑

How much bacterial growth do you observe? If colonies are present, determine a count.

What color(s) are the bacteria by eye? What color(s) are the bacteria with UV or blue light?

+DNA/+Amp/+IPTG Plate



Draw or add photo here↑

How much bacterial growth do you observe? If colonies are present, determine a count.

What color(s) are the bacteria by eye? What color(s) are the bacteria with UV or blue light?

Appendix E
PLATE CULTURE TRANSFORMATION - Student Worksheet

EXPERIMENT ANALYSIS:

1. For your +DNA/+AMP/+IPTG plate record the following*:

- Number of transformants: ____
- Nanograms of plasmid DNA added:_____
- Final recovery volume (mL):____
- Volume plated (mL):_____
- Calculated transformation efficiency:_____

* *You may need to refer to the quick reference box on page 13 for some numbers and for the transformation efficiency equation.*

2. Why do different members of your class have different transformation efficiencies?

3. If you did not get any results, what factors could be attributed to this fact? *(HINT: Refer to the Experiment Notes on page 31 and the Troubleshooting Guide (Appendix C).*

4. What would you change if you performed this experiment again?