

the

Transformation Lab

Experiment Using *E. coli* and pFluoroGreen

Maryland Loaner Lab Teacher Packet



*Based on a kit produced by
EDVOTEK®. Adapted by
Towson University.*

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Transformation Lab

Loaner Lab Overview

The Transformation Lab **has Three Parts:**

- A Pre-laboratory classroom activity that allows students to explore how bacteria grow and how to inoculate bacteria using sterile technique. The Pre-laboratory should also include a discussion of the concepts listed under the *Introduction* section found on pages 3-5 to adequately prepare students to understand this lab.
- A laboratory activity that allows students to investigate some basic principles of molecular biology and an introduction to genetic engineering. Plasmids containing the pFluoroGreen gene and the ampicillin resistance gene will be used to transform *E. coli* bacteria cells.
- The Post-laboratory activity includes a 24 or 48 hour period (again, depending on the temperature) for growth of the transformed bacteria cells and for analyzing results. This includes a transformation efficiency calculation and assessment of sterile conditions maintained during the experiment. Recommended extension activities are found on page 21.

This laboratory activity meets Exercise 6A: Bacterial Transformation - Ampicillin Resistance for the Advanced Placement Biology Laboratory 6 under the Molecular Biology requirements. [Note* The second part of this AP Lab, Exercise 6B: Restriction Enzyme Cleavage of DNA and Electrophoresis, objectives are met with the Loaner Lab titled "The Case of the Crown Jewels" or with "The Chestnut Tree Lab" (uses a DNA marker for sizing DNA bands).] Students are required to know the objectives under the section titled "**Before doing this laboratory you should understand:**" before doing the Transformation Lab.

Materials

Material	Number	Comments	Return?
Teacher packet and laminated student sheet packet	1	Contains all of the information necessary to conduct the lab.	Yes
Foam microtube racks	10	1 per group	Yes
Lab markers	10	Used to label tubes and plates	Yes
1000ul micropipettes	10	1 per group	Yes
10ul fixed volume minipipettes	10	1 per group	Yes
Blue micropipette tip boxes	5	1 per 2 groups	Return box and unused tips
Yellow micropipette tip boxes	1	1 per class	Return box and unused tips
White sterile toothpicks	10 sets	2 toothpicks per group	Only if unused
Sterile yellow loops	20	2 per group. Kept in bag with white sterile toothpicks.	Only if unused
Clear microcentrifuge tubes	20	2 per group	No

Sterile yellow loops	5	Used by teacher to prep 5 <i>E. coli</i> source plates	No
Large agar plates *	5	1 per 2 groups (Teacher will prep)	No
<i>E.coli</i> source slant pack**	1	Instructions in pack	No
Small agar plates (non-striped)	20	2 per group	No
Small agar plates (striped)	20	2 per group	No
“p” tubes	10	1 per group. Keep Refrigerated until used.	No
“LB” tubes	10	1 per group. Keep Refrigerated until used.	No
“CaCl ₂ ” tubes	10	1 per group. Keep Refrigerated until used.	No
White plastic tube floater	1	Used with water bath.	Yes
Insulated bucket (red)	1		Yes
Thermometer in plastic holder	1		Yes
UV lamps	5	1 per 2 groups.	Yes
Bioscience video and lesson plan	1		Yes
Container of disinfectant wipes	1	Use to disinfect equipment	Yes

Supplied by the Teacher:

Description	Quantity	Comments
Student worksheets	1 set per student	
Gloves	1 pair per student	
Goggles	1 pair per student	
Crushed ice	1 cup per 2 groups + 1 large container	Crushed ice is preferred over ice cubes.
Styrofoam cups	5	To hold crushed ice.
Stopwatch or timer	1	Use for incubation times.

Water bath*	1	Needed for 2 separate incubation steps.
Incubator**	1	37 ⁰ C for growth of <i>E. coli</i> source plates and post-lab grow out of transformed bacterial cultures.
Waste containers	1 per group	
Bleach bucket	1	Containing 10% bleach solution to kill bacteria on plates, waste containers and workstations.

*If an actual water bath is not available then a makeshift water bath can be made using the red insulated bucket provided in the kit. A separate container that can be heated in a microwave or on a hot plate will be needed for adding hot water to the insulated bucket for maintaining the makeshift water bath's temperature. A crock-pot can also be used as a water bath.

**If an incubator is not available then the lab can still be performed with delayed results. The optimal temperature for growing out *E.coli* source plates is 37°C for 24 hours before use. If a 37°C incubator is not available then *E.coli* source plates can be grown out at room temperature (warmest spot in room) for 48 hours before use. The post-lab activity that involves growing out the transformed bacteria will need to be done at room temperature (warmest spot in room) if an incubator is not accessible; the results will take an extra 24 hours.

IMPORTANT: The teacher must also supply all the materials for the Pre-Lab Activity found on page 11 if he/she wishes to perform this activity with the students.

Safety: The classroom teacher must instruct students with basic laboratory safety rules and provide gloves and goggles for student use with the laboratory activity.

Allergy Warning: This lab involves the use of 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.

AP Laboratory Objectives

Objectives

According to the AP Biology Laboratory manual the following objectives will be met:

Before doing this laboratory you should understand:

- The principles of bacterial transformation
- The conditions under which cells can be transformed
- The process of competent cell preparation
- How a plasmid can be engineered to include a piece of foreign DNA
- How plasmid vectors are used to transfer genes
- How antibiotic resistance is transferred between cells

After doing this laboratory you should be able to:

- Use plasmids as vectors to transform bacteria with a gene for antibiotic resistance in a controlled experiment
- Describe the biological process of transformation in bacteria
- Calculate transformation efficiency
- Be able to use multiple experimental controls
- Design a procedure to select positively for antibiotic-resistant transformed cells

Teachers and students who will be performing The Transformation Lab activity using the Maryland Loaner Lab must first complete the Pre-Laboratory classroom activity that involves a discussion of concepts. The conceptual aspects of the curriculum will be reinforced with the laboratory activity.

Transformation Lab

Introduction

The purpose of the Pre-Laboratory is to familiarize the students with the basic biology of bacterial cells and plasmid DNA, the concept of transformation, and the understanding of selection for the acquired genes. The larger topic of use and application of transgenic organisms (bacterial, plant, and mammal) will also be addressed. The final subject and activity material will involve an overall discussion of sterility, plating bacterial cells (quadrant streaking method), and the growth of bacterial cells to form colonies, which will be used in the laboratory for efficiency purposes.

E.coli and Plasmids

The bacterium **Escherichia coli** (*E.coli*) is used extensively in the laboratory for recombinant DNA research. It is a common inhabitant of the human colon and can be found easily in many areas of our environment. It has a single circular **chromosome** that contains about five million DNA base pairs, only 1/600th the haploid amount of DNA in a human cell. *E.coli* may also contain small circular DNA molecules called **plasmids** (1,000-200,000 DNA base pairs). These plasmids carry genetic information and are **extrachromosomal**, meaning they are not part of the bacterial genome. Plasmids are found in many species of bacteria and yeast and do not carry genetic information considered essential to the life of the organism, rather they tend to carry genetic information that gives the cell a survival advantage such as genes for resistance to antibiotics, resistance to normally poisonous heavy metals, for the degradation of unusual chemicals, or for killing of other bacteria. Some plasmids replicate only when the bacterial cell replicates and some will replicate **autonomously**, or independent of cell division. By using plasmids the scientist can change or transform the genetic information available to the bacterial cell. When plasmids contain multiple genes, these genes will always be transferred together. In the laboratory we will use a plasmid called **pFluoroGreen** (plasmids are always denoted by a lowercase "p" before the name), which has a gene for Ampicillin resistance (AMP^R) and a gene for GFP (green fluorescent protein). The gained ampicillin resistance will allow the *E.coli* to grow on media that contains ampicillin and the GFP will allow it to **bioluminesce** (to glow, light produced by a living organism).

Bacterial Characteristics

Some strains of *E.coli* are helpful and necessary for a healthy life and are, therefore, **non-pathogenic**. Others can cause disease and can even be deadly, these are called **pathogens** or **pathogenic**.

Non-pathogenic *E.coli* are used in molecular biology because of a number of useful characteristics. Growing conditions for these bacteria are simple and can be set up easily. These include an optimal temperature of 37°C, equal to 98.6 F or normal body temperature, no special oxygen or nitrogen levels, and no special light conditions. These bacteria are also able to express a variety of proteins from genes of different lengths without any inhibition of growth.

Another useful characteristic of all bacteria, including *E.coli*, is its reproduction by direct cell division, or **asexual reproduction**. Because there is no exchange of DNA during reproduction, there is no risk of recombination or loss of genetic material. One bacterial cell will divide and replicate and produce two completely identical cells. These will then each divide, producing four cells, again, identical to the original cell. These four cells divide and produce eight identical cells, and so on and so on. This is called **exponential growth** and involves the log-phase of the bacterial life cycle. As long as there are enough nutrients and space, this process will continue.

These characteristics are advantageous when selecting for bacteria which contain the gene of interest. Bacteria are grown in **media**, which contains its nutrients and supports growth. Bacteria is easily grown in a suspension culture in liquid media called Luria-Bertani (**LB**) **Broth** or grown in a petri dish with a mixture of LB Broth and agar on a solid surface. When bacteria are added onto solid media, one individual bacterial cell will start to divide and form a

colony which is a visible spot. The colony contains many individual bacterial cells, possibly millions, started from one individual cell. All of the cells in the colony have the exact same DNA and gene expression. By ensuring the cells are plated at a low enough density, the colonies will grow with enough space in between so they can be picked off the media individually. These individual colonies will then be grown in large volumes so the protein of the inserted gene can be harvested.

Transformation

Inserting a foreign plasmid into a bacteria cell which results in a new genetic trait is called **transformation**. Bacterial transformation involves the transfer of genetic information on a plasmid by the direct uptake of this **exogenous**, or foreign, DNA into the bacteria cell of interest that results in the acquisition of a new genetic trait that is stable and heritable. Because bacterial cells contain enzymes called endonucleases, better known as restriction enzymes, which degrade linear pieces of foreign DNA starting at the ends and working inwards, the DNA is contained on a plasmid. Plasmids are circular and immune to endonuclease attack. Plasmids are also used because they will be replicated and transmitted to daughter cells. Plasmids can be naturally occurring DNA molecules or can be **recombinant DNA molecules**. Plasmids can be engineered through a process that starts with cutting DNA with restriction enzymes to include a piece of foreign DNA that contains a specific gene of interest. Plasmids are very convenient **vectors** for introducing new genetic material, or transferring genes, into bacterial cells. By putting a gene of interest into a plasmid the transformed bacteria will express that gene and scientists can use that expression for the production of a needed protein or for selection purposes. Bacterial transformation is essential to the field of molecular biology in that it allows for the propagation, genetic expression, and isolation of recombinant DNA molecules.

Competency

There are a few species of bacteria that go through the transformation process naturally. Most bacteria need to be manipulated so they become **competent**, or able to take up the plasmid. Competency is a physiologic state, which changes the structure and permeability of the cell membrane so the plasmid DNA can enter the cell. Competence can be induced in *E.coli* by treating with chloride salts of the metal cations calcium, magnesium, and rubidium and with the assistance of sudden cycles of heat and cold. This process is called **chemical competency**. The cations in the solution disrupt the bacterial cell wall and membrane to create small holes through which DNA can enter. The cold conditions also serve to create gaps in the lipid structures of the cell membrane that allow DNA to enter. The heat shock produces a thermal gradient that sweeps into the cell and brings along the DNA; after this step the DNA has entered the competent cell. Competency can also be achieved through the use of electrical pulses called **electroporation**, which transiently changes the cell membrane structure and allows foreign DNA into the cells without killing most of the cells. Electroporation can also be used to introduce foreign DNA into other types of cells such as plants and animal cells. It tends to be more efficient than chemical competency, but it requires a special instrument called an electroporator. Rapidly growing (log-phase) cells take up foreign DNA most efficiently after either of these treatments that make the cells competent and able to be transformed.

Selection

Bacteria that have taken up the plasmid and transformed must be **selected**, or isolated. The plasmid is too small to be seen, so it is useful for it to contain a gene that expresses a characteristic that can be seen or interpreted. One such characteristic is antibiotic resistance. By growing the bacteria on solid media which contains an antibiotic, only the cells that were transformed with a plasmid containing the gene for antibiotic resistance will grow and the others will die. Plasmids can have multiple genes inserted into them. The genes on a plasmid cannot be separated during transformation. If a plasmid had a gene for antibiotic resistance and a gene for the expression of some protein, then the cells that survive on an antibiotic plate would be known to also contain the other gene of interest. In the case of this particular lab, the cells that survive on the media plates that contain ampicillin will have obtained the gene for ampicillin resistance from the plasmid used in the transformation and will also have the gene for GFP and will glow when exposed to long wave UV light.

Ampicillin

Many plasmids used in bacterial transformations contain genes that provide resistance to various antibiotics. Ampicillin resistance (AMP^R) is a commonly used gene when transforming bacteria. Ampicillin is a derivative of penicillin and inhibits bacterial growth by interfering with the synthesis of cell walls. The gene for ampicillin resistance produces an enzyme called beta-lactamase that is secreted by the cell and breaks down the ampicillin in the surrounding media. Because of this extracellular secretion from transformed cells, some untransformed cells can grow in the zones around colonies of transformed cells and are called "satellite colonies". They are much smaller and found around the larger transformed colonies.

Green Fluorescent Protein (GFP)

The green fluorescent protein (GFP) gene is used because of the bioluminescence (ability of an organism to produce color and light) that is produced when the gene is expressed and energy is transferred to it within its host. This energy transfer results in a change in conformation (shape) of the protein, producing the light and color. The GFP gene naturally occurs in specialized photogenic cells in the umbrella of the jellyfish *Aequorea Victoria*. This fluorescent protein can be expressed in *E.coli* bacteria and will produce a green light and can especially be seen when exposed to a long wave U.V. light source.

Transformation Efficiencies

Transformation efficiencies are a way to determine how many cells were transformed per microgram (ug) of plasmid DNA used. The colonies (on plate #4 for this lab) we count after the overnight incubation originally grew from one transformed cell, called a transformant. The calculation for transformation efficiency is:

$$\frac{\text{Number of transformants (colonies)}}{\text{ug of plasmid DNA}} \times \frac{\text{final volume at recovery (ml)}}{\text{volume plated (ml)}} = \text{number of transformants/ug of plasmid DNA}$$

(In the lab we will use 0.05ug of pFluoroGreen DNA, our final volume will be 0.50ml, and we will plate a volume of 0.20ml)

(An example of 40 transformants using the lab conditions and volumes would produce 2000 transformants/ug of DNA)

In research laboratories the transformation efficiencies will usually be between 1×10^4 to 1×10^7 transformants per ug of DNA. Transformations are never 100% efficient. The efficiencies can be lowered due to shortened incubation times or temperature variations while making the cells competent, shorten incubation times when uptake of the plasmid DNA is taking place, or possibly by a shorten "recovery" phase before plating transformed bacteria for overnight growth. There are many possible factors that can decrease the transformation efficiencies, which have to be optimized and controlled in the laboratory.

Applications of Transformation and Similar Techniques

The process of bacterial transformation, and ones very similar for eukaryotic cells, is used every day in biotechnology. By incorporating a gene of interest, the scientist can express proteins that can be beneficial to the organism and/or useful in other areas of science. Because the gene of interest is always inserted with a selectable marker, it's very easy to determine which of the organisms have been successfully transformed.

Bacteria are used for expression of simple proteins, which require little or no processing by the cell and are naturally secreted. Pharmaceutical companies use large quantities of bacteria grown in bioreactors to produce therapeutic proteins such as insulin or human growth hormone. Since these proteins were produced from genes naturally found in humans there is a lower risk when using them as human treatments. Purified proteins can also be used to make vaccines for the prevention of disease.

Plants can also be genetically engineered by using a similar process to transformation. Because plants are eukaryotic and contain a nucleus, a slightly different method is used to insert the gene of interest. Plants can be engineered to be resistant to pesticides, to increase crop yield, to tolerate normally adverse weather conditions, or to even produce pharmaceuticals. Plants that are genetically engineered can also produce fruits and vegetables that have longer and more stable shelf-lives in the grocery store, making it more profitable for farmers and store owners. It is also possible to take an adverse market condition, like lowering sales of tobacco, and turn tobacco plants into a profitable crop by transforming them to produce a pharmaceutical.

Genetically engineered animals can be used for both research and for production of proteins. By using the modified transformation technique, scientists can produce animals that simulate human diseases so that different treatments can be attempted. Research on animals also provides the scientist a way to better test toxicity and efficacy of pharmaceuticals developed in chemical labs. By genetically engineering the animal, the reaction of exposure to the test pharmaceutical can better mimic how the drug will react in a human. Farm animals can also be genetically engineered to allow them to grow larger and leaner, increasing the profit for the farmer and making it more nutritious for the consumer. Transgenic animals can also be used as "bioreactors" to produce large quantities of specific proteins in their milk and/or eggs due to the introduction of a specific gene to their natural genomes. Transgenic goats that have a human gene called AT III can be used to produce a protein that prevents blood from clotting. This transgenic method is faster and cheaper than the more complex ways to produce this needed protein.

Science is constantly developing and modifying the basic technique of transformation. One such modification is used in a promising treatment for otherwise untreatable diseases through a process called gene therapy. Theoretically a molecule can be developed which contains a gene that will help cure a disease or treat an otherwise untreatable symptom. Through a process similar to transformation, it is thought this molecule can be safely delivered to a human and improve the quality of life. To date the promise of gene therapy has not yet reached its full potential.

Transformation lab

Pre-Laboratory Activity

NOTE: Groups using the loaner lab must first complete the Pre-Laboratory classroom activity. The conceptual aspects of the curriculum will be reinforced with the laboratory activity.

The purpose of the Pre-Laboratory is to familiarize the students with sterility issues and with the procedure for streaking out (inoculating) microbial cultures on solid media agar plates. A discussion of bacterial cell growth and replication along with the definition of what colonies are will be major topics of the Pre-Laboratory.

The Objectives of the Pre-Lab are:

- Describe media (solid and liquid) and its purpose for bacterial growth
- Introduction to basic sterile technique and to understand its purpose
- Become familiar with Quadrant streaking method for bacterial cell plating
- Execute plate streaking procedure independently after seeing demonstration
- Verbally communicate procedure, purpose, and results

Pre-Laboratory Materials:

- One plastic lid per student (small yogurt container lids are recommended)
- One glass Pyrex measuring container
- One 3 oz. Box of Jell-O (will fill approximately 10 small yogurt lids) Add 5 oz. water to the glass measuring container and bring to a boil in microwave (approximately 3 minutes on high power). Add the entire contents of Jell-O box and stir to dissolve. Microwave an additional 30 seconds and again stir to completely dissolve. Carefully pour into the plastic lids and fill almost to the top. Let sit for a half and hour and carefully transfer to a refrigerator and let sit for 2-3 hours. **It is recommended that the plates be poured half a day prior to the Pre-Laboratory exercise or the day before use.**
NOTE: If LB agar (to make your own plates) or pre-poured agar plates are accessible they would be ideal to use for this activity instead of using Jell-O to make mock agar plates (optional). Also, if small Petri dishes are available they can be used instead of the yogurt container lids (optional).
- Squirt bottle of thick colored liquid (bright yellow mustard is recommended) to represent a bacterial culture.
- Box of toothpicks (flat, not round ended)

Pre-Laboratory Engagement (5 Minutes)

Give each student a pre-poured "Jello-O" plate and four flat toothpicks. Organize students in small groups. Each group will share a squirt bottle of thick colored liquid to be spread which will represent a bacterial culture (mustard is recommended because it is brightly colored and thick). Propose to the students that they will engage in an activity that will spread a very tiny amount of bacteria out on their agar plates, so that they will be able to see individual colonies grow. Challenge the students with the question of "How would you spread out a drop of bacterial culture (mustard) so that it became the thinnest in the small space on your plate?" Allow students to make suggestions within their groups. Have each group verbally present their potential solutions to the class.

Pre-Laboratory Explanation (10 Minutes)

What is Quadrant streaking?

The quadrant streaking method is used to obtain isolated, independent bacterial colonies. A colony is a group of cells that all originated from the same source (one original cell), therefore all the cells in a single colony are identical and contain the same genetic information. A colony may contain millions of individual bacterial cells. The

ability to produce single isolated colonies is important for several reasons including helping to identify organisms, purifying bacterial strains, and isolating pure genetic clones. With respect to transformation, the production of isolated colonies allows researchers to identify and manipulate bacterial cells with very specific characteristics. For example, after performing a transformation, researchers must be able to select cells from a colony that have been successfully transformed. For the purposes of our laboratory, students will be using an *E.coli* source plate that was streaked out using the "Quadrant Streaking" method to isolate the single colonies required for this lab activity. This method allows sequential dilution of the original microbial culture over the entire surface of solid media agar in a petri dish. As the original sample is diluted by streaking it over successive quadrants, the numbers of cells decrease. Usually by the third or fourth quadrant only a few cells are transferred on the toothpick and these produce only a few isolated colonies.

Media and Bacterial Growth

Media provides nutrition for bacteria and allows for growth. Media comes in both liquid and solid forms, both of which will be used in the laboratory activity. Solid media has the additive of agar, a gelatin-like substance derived from seaweed, which changes the consistency to be solid. It is still very soft much like "Jell-O" and students will need to be very gentle when working with it or it will tear or be gouged. The solid media agar allows bacteria to grow on its surface to produce discrete colonies that can be visualized as opposed to liquid media, which appears cloudy as bacteria grows but individual colonies cannot be seen. Additional selective media (such as antibiotics) can be added to further isolate and identify organisms with particular characteristics.

Aseptic Technique

One of the most important components of the Quadrant Streaking Method is avoiding contamination through the use of proper sterile technique. Bacteria are commonplace in our environment and in order to selectively grow a particular type of bacteria, researchers must take many precautions to avoid contamination. Several of the common precautions taken when working with bacteria to avoid contamination include sterilizing metal instruments with flames or using pre-sterilized (wrapped) instruments, keeping agar plates closed as much as possible and not talking over open plates (our mouths are full of various bacteria), not touching sterilized objects with non-sterile objects (such as one's hands), and to work as quickly as possible with sterile objects and not subject them to potential contamination in the environment.

Pre-Laboratory Exploration (10 Minutes)

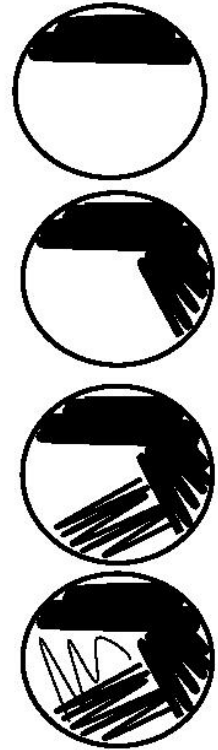
Students will perform a mock version of Quadrant Streaking in order to become familiar with the basic technique. Please keep in mind that in this Pre-Lab activity Jell-O plates are used in place of sterile agar plates and toothpicks are used in place of sterile loops (which would be sterilized between each quadrant streak). Although students will not be sterilizing their instruments in this activity they can practice good aseptic technique by changing toothpicks between quadrants and avoiding talking over their plates.

Step 1. Place a dime sized drop of mustard at the top of the plate and using one of the flat toothpicks, spread it back and forth across the small quarter-section of the plate. Use the flat side of the toothpick and avoid gouging the Jell-O.

Step 2. Turn the plate 45° and using a NEW toothpick, streak a few times into the last area and then into the new unused quarter-section of the plate.

Step 3. Turn the plate again 45° and using the third NEW toothpick streak a few times into the second area that was last struck out, and again into a new unused quarter-section of the plate.

Step 4. For the final and last time, use a fourth NEW toothpick and streak into the third area a few times and then streak out into the last unused area of the plate and make the last streaks independent "zig zag" streaks. All four areas that were struck out should not be touching except where you struck into the previous area.



Assessment (10 Minutes)

Ask the students to write a summary of what they learned and to describe the technique of streaking out microbial cultures on solid media agar plates. Have the students also describe the application of using isolated bacterial colonies for the purpose of bacterial transformation and to discuss the importance of using sterile technique.

Transformation lab

Laboratory Explanation

The purpose of the laboratory activity is to apply the concepts outlined in the Pre-Laboratory activity to an actual application in which students will perform a bacterial transformation.

The Objectives of the Laboratory Activity are:

- Make a strain of *E.coli* competent for bacterial transformation.
- Transform competent *E.coli* cells with a plasmid that contains genes that the bacterium does not originally possess (GFP-green fluorescent protein and ampicillin resistance genes).
- Observe bacterial cells for successful transformation: acquired fluorescent trait and ability to grow on media plates that contain ampicillin (observing phenotypic proof of a genotypic change in *E.coli*).
- Calculate transformation efficiency and evaluate results for maintaining sterile conditions during the experiment.

Developing the Concept for the Laboratory Activity

The Pre-Lab activity covered basic inoculation of bacterial cultures (using the quadrant streaking method), a discussion of bacterial cell growth, and the importance of using sterile technique. Students will share *E.coli* source plates that you prepared either 24 hours (if grown in 37°C) or 48 hours (if grown at room temperature) in advance to the laboratory activity. The reason for using freshly inoculated bacterial cultures is because bacterial cells that are in their rapidly growing or log-phase can more efficiently be transformed. Older cultures will yield poorer transformation efficiencies.

Additional Pre-Laboratory Activities

Before students perform the laboratory activity, discuss how to use micropipettes properly (see Micropipette Challenge Explanation). Students will practice using the variable volume micropipette by performing The Micropipette Challenge prior to the lab. Also explain to students how to use the smaller fixed volume 10ul minipipettes: they do not have two stops on their plunger as the variable volume micropipettes. They only have one stop. Make sure a tip is on the end of the minipipette and press down on the plunger, insert tip into liquid, release plunger, and transfer 10ul of liquid to desired tube.

The Laboratory Activity

Students should be instructed about proper safety rules when working with bacteria in the lab (even though bacteria used in this lab in non-pathogenic). Safety goggles are to be provided by the teacher and students should be encouraged to wash their hands after working with the lab materials. After use, any lab materials that came into contact with bacteria should be disinfected by the teacher before disposal in the garbage. To disinfect objects, soak overnight in a bucket with a 10% bleach solution (soak tubes and plates opened). Lab benches should also be wiped down with a 10% bleach solution after the lab activity.

Recommendations will be provided below for both the preparation of the lab and for the individual steps of the lab activity; many of these will help provide higher transformation efficiencies.

When the kit is received, the "LB", "CaCl₂" and "p" tubes are to be refrigerated until they are used. In preparation for the lab, the "p" tube contents need to be hand flicked into the bottom of the tubes because they contain a very small volume of plasmid DNA. When setting up the student workstations, the "LB" tubes can be placed at room temperature in the foam tube racks. The "CaCl₂" and "p" tubes **must** be placed on ice in cups at student workstations. Cups with ice are best set up using crushed ice for better surface area coverage on the tubes, which helps to maintain the temperature. If crushed ice is not available, use ice cubes with a small amount of water to

create a very cold water bath with the cubes at least partially touching the sides of the tubes. A larger container to be filled with ice and shared for the entire class is used in step #9.

To start the lab, there are 10 workstations and students need to be assigned a group number #1-10. They will label their tubes and plates with this number. The flat lids of the tubes, not the sides of the tubes, should be labeled.

In **step #1**, labeled tubes should be placed into a cup with ice to chill. Allow a few minutes for this to occur and use this time to explain good sterile technique to be used throughout this experiment. To maintain higher transformation efficiencies and to provide uncontaminated results, the following is suggested. Most importantly, do not touch anything sterile (tips, loops, toothpicks, etc.) to anything non-sterile. Be careful not to touch these sterile objects to the counters, bump them into the arm of another group member, touch the ends you will use with your hands, etc. It is also important not to speak over sterile tips, open tubes, or open plates. We naturally have bacteria in our mouths and when we speak we spray a fine mist of saliva, which contains bacteria, into the immediate area. To prevent contamination maintain the most sterile conditions possible.

In **step #3**, students will need to know how to properly open the wrapped sterile toothpicks to maintain their sterility. The straight end of the toothpick (instead of the bent end) will be pushed out of the paper backing of the wrapper so scissors are not needed. Make sure students understand that they can't touch the bent end of the toothpick or it will no longer be sterile. When scraping the media agar plates instruct students to be very gentle to prevent digging or gouging of the agar. They will want to use the bent end of the toothpick to scrape an area a few centimeters long to get enough cells from the *E.coli* source plates. They should be able to see the cells on the toothpick (the yellowish bacteria culture). Separate toothpicks are used for the two different tubes. When they are twisting and shaking the toothpicks into the CaCl_2 in the tubes, instruct them to be very careful not to drop the toothpick—because they will not be able to use it again or they may contaminate their results. When shaking the toothpicks they should try to get as many cells off as possible, not all will come off.

In **step #4**, students are asked to "flick the tubes" to mix the contents thoroughly. They are attempting to break up clumps of cells in solution. This involves holding the capped tube with one hand and using the other to snap or flick the tube with a fingernail. Flicking will mix the contents better than simple inversion of the tube.

In **step #5**, students will use the 10ul fixed volume minipipettes to add the plasmid DNA to the "+" tube. Students should always make sure they use a tip with these minipipettes. They are not like the micropipettes with two different stops on the plunger. The fixed volume minipipettes only have one stop on the plunger, otherwise they work the same way. To release the tips they have to be manually pulled off. There isn't an ejector button as on the micropipettes.

In **step #7**, students will be labeling their small media plates. The agar plates without a stripe on the side do not contain the antibiotic ampicillin. The plates with a stripe have ampicillin. Students should flip the covered plates and label the agar side (now facing upwards) with the lab markers. The final cultures will be grown with the plates inverted this way to avoid condensation that forms in the lids from dripping into the culture.

In **step #8**, all tubes will be placed into one plastic tube holder provided with the kit and put into the 42°C water bath for a strict 90 seconds. Then in **step #9**, the entire tube holder will be placed on ice in the larger container for a strict 2 minutes before handing the tubes back. While students are doing **step #10**, adjust the water bath to 37°C and monitor it with the thermometer provided (if using the insulated bucket, small amounts of heated water will need to be added to maintain 37°C).

In **step #11**, tubes will be placed into a 37°C water bath. This period is called the "recovery phase" during which cells are recovering from the stress of being made competent and undergoing transformation. They are adjusting to

be able to grow and divide again. It is convenient during these 30 minutes to use the bottom of the flow chart and have students write for each of the four plates the **expected results** (see the flow chart with short answers). **Plate #1** is considered the positive control because it contains cells that were not transformed (no plasmid DNA added) and were grown on plates without ampicillin. There are so many colonies that the culture appears as a "lawn" or total plate coverage of the yellowish *E.coli* bacteria. This shows that original cells being used in this experiment are able to grow when ampicillin is not present. **Plate #2** is considered the negative control because it is the same situation as in plate #1 where these are also cells that were not transformed (no plasmid DNA added), but in this case were grown on plates with ampicillin which killed them—so no growth is visible on this plate. This shows that the bacterial cells being used are sensitive to ampicillin and only the transformed cells given the ampicillin resistance gene will grow on plates with ampicillin. **Plate #3** is a mixed culture plate with both untransformed and transformed cells—mostly untransformed cells so only one or two transformed cells may be visible under long wave UV light. Both will grow on a plate without ampicillin. Again, total bacterial coverage will be seen as a "lawn" on the plate. Bacterial growth on **plate #4** will ONLY be transformed cells as they are able to grow on plates with ampicillin because they have gained the ampicillin resistance gene. The growth on the plate will be seen as individual colonies and are especially visible with the long wave UV lights. It is this number of colonies that will be used for the transformation efficiency calculation.

Steps #13 and #14 have to be done in a very specific way for proper results to occur. Make sure students understand that the "-" is going to be plated on plates #1 and #2, and that the "+" is going to be plated on plates #3 and #4. Instruct the students how to open the sterile wrapped inoculation loops: pull apart the ends of the wrapper which has the straight needle-like end of the loop—not the circle end which will be used. They cannot touch the circle end to anything or it will no longer be sterile.

When they are about to streak out their plates, have them gently rotate the liquid on the plates to cover the entire agar surface first. They will not be streaking out using the quadrant method from the Pre-lab activity. Instead they will streak the entire plate in one direction and then repeat after turning the plate in a 90° angle. The same loop will be used for plate #2 **ONLY** after plate #1 has been inoculated. The same is for plate #4 being inoculated **after** plate #3 with the same loop. If the plates are mixed up there is a potential to transfer ampicillin to a plate intended to not contain ampicillin and results will be affected.

In **step #15**, plates should be flipped over for overnight growth **ONLY** after all the liquid has been absorbed in to the agar. Place into a 37°C incubator if available for 24 hours or leave at room temperature for 48 hours before **students observe results**: checking all four plates for expected results, counting colonies from plate #4 (will vary, but anywhere from 10-40 colonies is good) for the transformation efficiency calculation, and observing if any plates have unusual (large, different colored, or fuzzy) colonies which would be contamination that occurred during the lab (reflecting on how well they maintained sterile conditions). The long wave UV lamps will help to detect especially small bioluminescent colonies (transformants); use in a darkened room. The transformant colonies are usually visible to the naked eye but the bioluminescent quality is enhanced by using the UV lamps. Decontaminate all materials that came into contact with bacteria before disposal, including student plates after results are read.

Note: If student plates are being left at room temperature for 48 hours to grow, the resulting colonies may be very small and only seen with the UV lamps. These plates may need to be grown an additional 24 hours at room temperature to really be able to see the colonies as they will have grown larger.

Transformation Lab

Micropipette Challenge Explanation (for Variable Volume Micropipettes)

NOTE: The Micropipette Challenge is an additional Pre-Lab activity that will allow students practice in using micropipettes. This activity may be performed the day of the laboratory activity or any time in advance. If your students are unfamiliar with the use of micropipettes provide additional time to do this activity before students perform the lab. All students must be reminded about the proper usage of micropipettes to prevent damage to the equipment and also to provide students with accurate results during the lab activity. **Be sure that everyone understands how to operate the micropipettes. It is worthwhile to check each student for correct technique before beginning the lab activity.**

Micropipettes

Micropipettes are precision instruments designed to measure and transfer small volumes. They are expensive and must be used with care. Their accuracy is dependent upon their proper use. Different brands of micropipettes vary in the volume range they will measure, the type of tips they fit, and the type of device used to set the volume.

Solution Preparation for The Micropipette Challenge

Make up the colored water solutions (yellow and blue) by adding food coloring to water (for 10 ml of water, add 1-2 drops of food coloring). Allow 1 ml of each colored solution for each student group and place in small test tubes.

Student Stations for The Micropipette Challenge

1 empty small test tube test tube rack 1000 ul micropipette micropipette tips
2 tubes of 1 ml each (pre-made yellow and blue solutions)

Basic Directions for Micropipette Use

Golden Rules of Pipetting

- 1. Don't rotate the volume adjuster beyond the upper or lower range of the pipette, this can damage it.*
- 2. Don't use a pipette without a tip on it. If this happens, liquid gets into the opening of the pipette and can damage the mechanism inside.*
- 3. Don't lay down a pipette that has a tip filled with liquid. If this happens, liquid can get inside the pipette and can damage it.*
- 4. Use new pipette tips between different samples to prevent contamination.*

Setting the Volume

All micropipettes have a volume control dial. Determine whether the volume window on your pipette shows tenths of microliters (0.1 ul) or whole microliters in the smallest place so that you can read the scale correctly (it varies with different brand micropipettes). Each set of micropipettes comes with a laminated card with specific instructions for setting their volumes.

Drawing Up and Expelling Liquid

Variable volume micropipettes have 2 stops as you depress the plunger to expel liquid. The first stop corresponds to the volume set in the window. The second stop gives a little puff of air to blow out any remaining liquid upon delivery. To draw liquid into the pipette tip depress the plunger control only to the first stop. If you go to the second stop you will draw too much liquid into the tip. The most common pipetting error is to go past the first stop to the second stop for drawing liquid into the tip (which gives an inaccurate volume). When you are letting the liquid out of the tip then you go to the second stop.

Using the Micropipette:

1. Select the pipette that includes the volume range you will need.
2. Adjust the pipette to the desired volume by turning the dial. Do not turn beyond the volume range for the pipette.
3. Press a new tip onto the pipette firmly (gently tap the pipette into a tip while in the box). Get a tip without touching it with your hands; this is to prevent contamination of the samples.
4. To draw liquid into the micropipette tip:
 - a) Depress the plunger to the first stop to measure the desired volume and hold in that position.
 - b) Holding the pipette vertically, immerse the tip 1-3 mm into the liquid to be transferred.
 - c) Draw the fluid into the tip by slowly releasing the plunger. Wait 1-2 seconds to be sure that the full volume of sample is drawn into the tip. If you see air bubbles there is a problem with your volume and you will need to repeat this step to get the correct volume (either your tip wasn't immersed far enough down into the liquid or you perhaps raised your arm while releasing the plunger).
5. To dispense the liquid:
 - a) Place the tip into the container where the liquid is to be released, near the bottom.
 - b) Slowly depress the plunger to the second stop to blow out all of the liquid in the tip. Be careful to not to suck liquid back into the tip by releasing the plunger while the tip is in the liquid you just dispensed.
 - c) Eject the tip when done into a waste container by pressing the separate ejector plunger found on the top or side of the micropipette (depends on the brand of micropipette).

Transformation Lab

Teacher Laboratory Preparation

Maryland Loaner Lab will supply reagents, equipment, and instructions outlined for 10 student workstations.

Teachers must supply the students with the following handouts (laminated worksheets are provided in the kit for photocopying): Micropipette Challenge, Laboratory Protocol, Flow Chart, and Student Worksheet

Prepare Student Stations (10):

- One pair of safety goggles per student (**provided by teacher**)
- One lab marker
- One foam microtube rack
- One disposable cup (waste container for tips) (**provided by teacher**)
- Two empty microcentrifuge tubes (will be labeled by students)
- One tube: "LB" kept at room temperature in foam rack
- One tube each: "CaCl₂" and "p" **both kept in ice bucket** ("p" tubes will need to be hand flicked to get contents in bottom of tube)
- One 1000ul micropipette
- One 10ul fixed volume minipipette
- One blue box of 1000ul micropipette tips (1 box/2 student groups)
- One yellow box of 10ul minipipette tips (1 box/10 student groups)
- 2 petri dishes with solid media (no stripe on side)
- 2 petri dishes with solid media (with stripe on side)
- 2 yellow sterile loops (wrapped)
- 2 white sterile toothpicks (wrapped)
- One *E.coli* bacteria source plate (1plate/2 student groups)

Activity:	Time needed:
Preparing student stations	30 minutes
Pre-Lab activity	35 minutes
Micropipette Challenge	15 minutes
Laboratory activity	90 minutes
Post-Lab activity	20 minutes



Shared Equipment for Multiple Student Stations:

- Cups with ice for student stations and a larger container with ice for the entire class (for step #9) (**provided by teacher**).
- Water bath and thermometer with plastic tube floater to hold all student tubes (for steps #8 & #9). (A water bath can be made using the red insulated bucket provided and water heated in a microwave or hot plate)

Storage of Student Media Plates:

There will be a sleeve of petri dishes with solid media that have no stripe on the plates (do not contain ampicillin) and a sleeve of petri dishes with solid media that have a stripe on the plates (contain ampicillin). Both will need to be stored in the refrigerator with plates inverted (agar side facing up) until used.

Preparation of *E. coli* Source Plates:

It is crucial that the *E. coli* source plates be prepared as described here. If you have a 37°C incubator, it is optimal for the source plates to be inoculated and grown out at 37°C for 24 hours before use. If an incubator is not available, then grow them at room temperature (keeping at the warmest available spot such as the back or top of a refrigerator) for 48 hours before use. A vial of reconstitution medium and a vial of Fluorocells™ (which contains the *E. coli*) are included in each kit for the teacher to inoculate the 5 large petri dishes labeled "E.coli" either 24 or 48 hours before they are to be used. Using a sterile pipet, add 2 ml of cell reconstitution medium to the vial of Fluorocells™. Replace the rubber stopper and cap. Mix by gently inverting the vial until the freeze-dried plug is dissolved. Incubate the vial of cells for 30-60 minutes in a 37°C incubation oven. (If an incubation oven is not available, a 37°C water bath may be used.) Growth should be evident (broth should be slightly turbid or cloudy). If growth is not evident, incubate for a longer period of time. To inoculate the 5 petri dishes (or source plates), transfer 50-75 ul of cells to each petri dish using a sterile pipet. Place the cap back on the vial of Fluorocells™. Unwrap the sterile yellow loop (by opening the end of the package that has the straight end of the loop, and by not touching the end of the loop with a circle on it which will be used to inoculate the plates). Without touching over the open large petri dish labeled "E.coli", use the quadrant streaking method outlined in the Pre-Lab activity (see picture above). Use a new sterile loop to inoculate each of the other 4 large petri dishes labeled "E.coli". When all 5 "E.coli" source plates have been inoculated, invert the plates so that the agar side faces up (to prevent condensation that often appears on the lids from dripping into cultures). The plates should either be placed in a 37°C incubator for 24 hours or kept at room temperature for 48 hours. The vial of Fluorocells™, pipets, and loops should be soaked overnight in a 10% bleach solution to decontaminate before disposal.

Transformation Lab

Student Worksheet Answer Key

1. What is *E.coli*? What does "non-pathogenic" mean?

E.coli is a type of bacteria found in many places in our environment, from the soil to our intestines. It is frequently used in the laboratory for recombinant DNA research (research that involves DNA molecules formed by joining DNA segments from different sources). Non-pathogenic means non-disease causing.

2. What is a plasmid?

Plasmids are small, circular, extrachromosomal DNA molecules found in bacteria cells. They can replicate independently of the bacteria genome. Plasmids frequently carry genes for antibiotic resistance.

3. When a plasmid is inside a cell it is "extrachromosomal", what does this mean?

"Extra" means in addition to. Extrachromosomal means that the plasmid exists separately from the chromosome of the bacteria cell. The plasmid carries its own genetic information (for example genes for antibiotic resistance).

4. What is the name of the plasmid used in this protocol? What two genes does it contain?

The plasmid used in this experiment is called pFluroGreen. It contains two genes: AMP_R (provides resistance to the antibiotic ampicillin) and GFP (the gene for a green fluorescent protein).

5. How might a plasmid contribute to a selective advantage for a bacterial cell?

A plasmid carrying genetic information that a bacteria cell does not normally have, could provide a selective advantage for that cell. The plasmid would be providing beneficial genetic information to the bacteria cell. An example would be if a plasmid carried a gene for antibiotic resistance and allowed the bacteria cell to live in the presence of that antibiotic (when it would normally die). The cell would have gained antibiotic resistance.

6. What is transformation? What does exogenous DNA mean?

Transformation is the insertion of a foreign plasmid into a bacteria cell which results in newly acquired genetic trait(s) for that cell. Exogenous means foreign or found outside.

7. What is competency for a cell? How is it achieved?

Competency for a cell is a process by which bacteria is manipulated to undergo transformation. It is a change in physiologic state of the cell. Changes occur in the structure and permeability of the cell membrane (small holes are created) that makes the cells able to take up the plasmid DNA. This process is achieved via controlled growth conditions, sudden temperature change, and the use of certain chemicals.

8. What is media when used with cells? What are the two forms it comes in?

Media is nutrients for bacterial growth. It comes in two forms: liquid (broth) and solid (agar).

9. What is selection? What are we selecting for in this experiment?

Selection is the isolation of bacteria cells that contain the plasmid with genes of interest. In this experiment we are selecting for the bacteria cells that have become resistant to ampicillin by growing the cells on agar plates which contain ampicillin. They gained resistance by being transformed with the plasmid that contained the AMP_R gene.

10. What does bioluminescence mean?

Bioluminescence means to glow as a biological function or process. It is when a living organism produces light and color (glows).

11. What is transformation efficiency? What would cause it to be lowered? Transformation efficiency is a number calculated which represents how well the bacteria cells were transformed. This number would be lowered if the steps of transformation were not done properly, such as the incubation times were shortened, if the temperatures used for various steps were slightly off, or if fresh bacterial cultures were not initially used.

Transformation Lab

Extension Activities

The following extension activities may be used to expand upon the concepts introduced during the Pre-Lab and Laboratory activities.

- I. Ask the students to investigate and write a brief report on one application or use of **genetic engineering** in any of the biotechnology areas (including medical, environmental, forensic, microbial, agricultural, animal, or marine biotechnology).

[Suggested resources: www.accessexcellence.org/AB, www.actionbioscience.org, academicinfo.net/biotech.html, www.biotechinstitute.org, www.Agbio.com and www.nal.usda.gov/bic]

- II. Introduce to students the topic of **regulatory biotechnology** and open this into a discussion including the comments and concerns that students may voice about genetically modified organisms (GMO). When discussing regulatory biotechnology include things such as the role of the Environmental Protection Agency (EPA) in regulating products of biotechnology, the Federal Drug Administration's (FDA) role in regulating food and food additives produced by biotechnology and in regulating pharmaceutical products, the federal government agencies involved with preventing the release of bioengineered plants into the environment, and also the function of legal patents for biotechnology products.

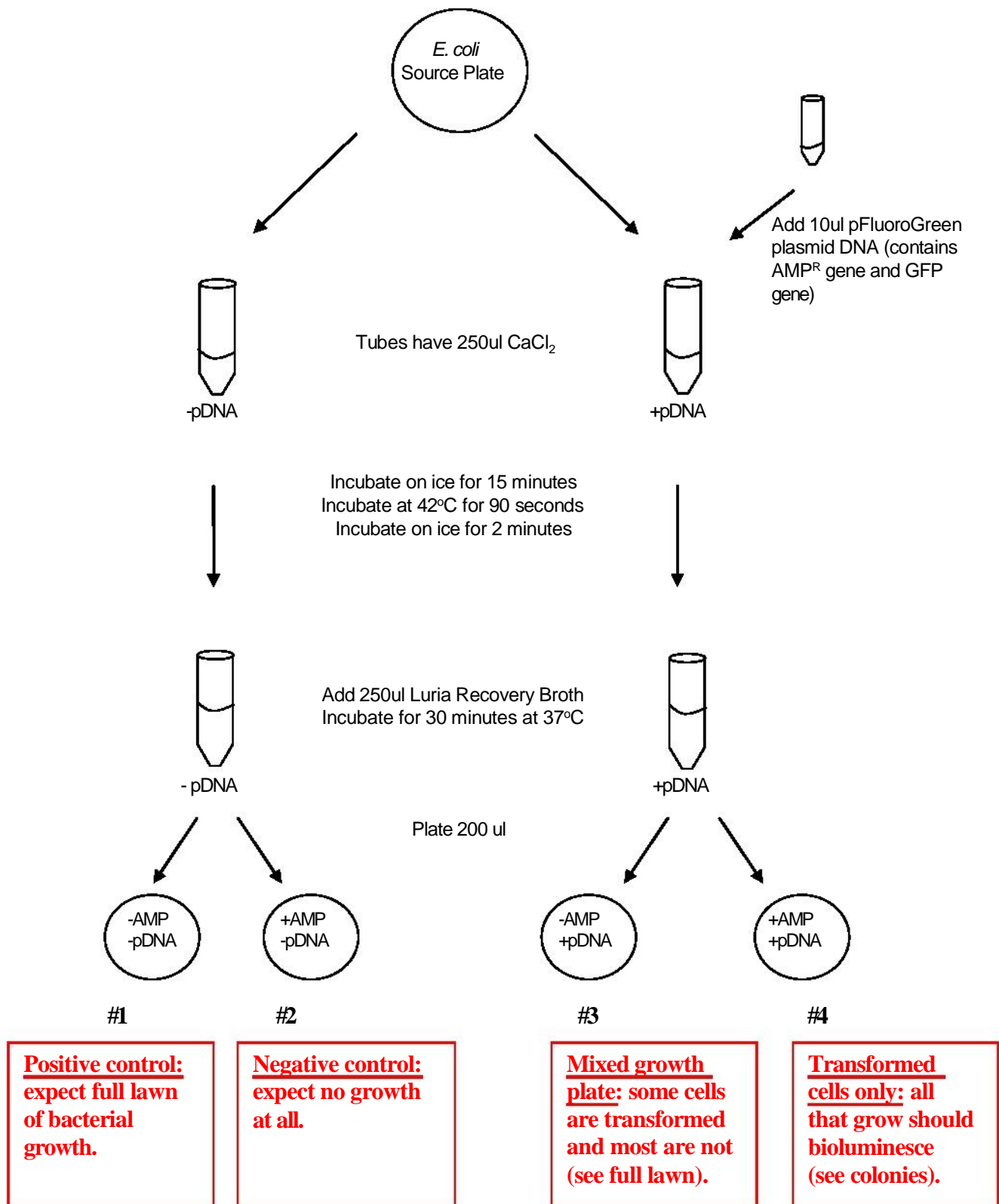
[Suggested resources: www.fda.gov, www.epa.gov, www.aphis.usda.gov (The Animal and Plant Health Inspection Service), binas.unido.org/binas (Biosafety Information Network and Advisory Service), and www.er.doe.gov (Department of Energy)]

- III. Work with students to define **bioethics** and how it relates to biotechnology. Allow students to develop arguments on both sides of issues that reflect potential ethical problems with biotechnology research. Involve students in the discussion of ethical issues with genetic testing, the use of embryonic stem cells, and cloning. Address the relationship between science and public policy.

[Suggested resources: <http://bioethics.georgetown.edu/> (Bioethics Research Library Georgetown University), <http://bioethics.od.nih.gov/> (Bioethics Resources on the Web National Institutes of Health), <http://www.bioethics.gov/> (Presidential Commission for the Study of Study of Bioethical Issues)]

Transformation Lab

Flow Chart (With Expected Results for Each Plate: Teacher's Notes)



MICROPIPETTE CHALLENGE

Laboratory science often involves working with very small volumes of liquid; frequently millionths of liters are used. One millionth of a liter is equal to one microliter, abbreviated 1 μL . (**1 liter = 1,000 ml = 1,000,000 μL**)

You can imagine that it would be very difficult to measure such small volumes without a very accurate and precise instrument. The instrument most often used to measure microliters is called a micropipette. Micropipettes differ in the volume of liquid they can accurately measure.

To help you become accustomed to using micropipettes you will be given two tubes and asked to measure liquid from each. One tube has blue food coloring and the other has yellow food coloring. Practice using the micropipettes by adding the amounts listed below to an empty tube. Notice how very small the volumes really are. Remember to change pipette tips between different color solutions, so not to contaminate them. (Option: you could pipette all the yellow first, and then change the tip and pipette the blue last) Watch each other pipette and check each other's technique. Make sure you do not see bubbles or your volumes could be incorrect. Ask your teacher for help if you have questions about using the micropipettes. Discuss with your class what is considered good micropipette technique and how to use micropipettes properly.

Example:

Amount to add to tube	Color	Record the setting as it appears in the window
150 μL	blue	150

Add the following amounts to an empty tube - all of the amounts will be added to the same tube:

Amount to add to tube	Color	Record the setting as it appears in the window
250 μL	blue	
125 μL	yellow	
370 μL	blue	
495 μL	yellow	

How many microliters (μL) should you have in the test tube when you are done?

How many milliliters (mL) should you have in the test tube when you are done?

Transformation Lab

Laboratory Protocol

NAME:

1. Label the lid of one tube "+" (will get plasmid DNA) and the lid of another tube "-" (will not get plasmid DNA) using the lab markers. Also, label tubes with assigned group number. Place labeled tubes in the cups of ice to chill.
2. Using a micropipette, add 250ul of cold CaCl_2 to **each** chilled tube and place back in ice. (**Change tips between tubes**).
3. Using a sterile toothpick--**following the specific directions from your teacher on how to open the wrapped toothpick**-- transfer a small swatch of colonies from the *E.coli* source plate to each tube (**use a separate toothpick for each tube**). Twist, shake, and scrape the toothpicks in the CaCl_2 to get most of the cells off the toothpicks and into the solution in the tubes (for about 20 seconds).
4. With both tubes capped, flick the tubes for a full minute to mix thoroughly. Place both tubes back in ice.
5. To the **bottom** of the tube labeled "+" **ONLY**, add 10ul pFlurooroGreen plasmid DNA (tube marked "p") with the small 10ul minipipette (**Be sure to use a tip**). Flick the capped tube to mix for about 20 seconds.
6. Incubate both tubes in ice for 15 minutes.
7. While tubes are incubating, label four agar plates with your initials and date as such: (Label plates on the bottom agar side)
 1. -pDNA, -AMP (plate without stripe)
 2. -pDNA, +AMP (plate **with stripe**)
 3. +pDNA, -AMP (plate without stripe)
 4. +pDNA, +AMP (plate **with stripe**)
8. Place both tubes in the **42°C** water bath (all tubes will be placed in plastic floater) for **90 seconds** to heat shock.
9. **Immediately** place the entire plastic floater with all tubes in the larger **ice bucket for 2 minutes**. Return each student group's tubes to their foam racks.
10. Using a micropipette, add 250ul of Luria-Bertani Recovery Broth (tube labeled "LB") to each tube and gently hand mix by inverting the tubes (**change micropipette tips between tubes**).
11. Incubate both tubes in the 37°C water bath for 30 minutes for a recovery period.
12. After recovery period is over, take the tubes from the water bath back to the student's foam racks.

13. **Plating of the tube labeled "-":**

Take 200ul and **drop onto the top of the agar** (without touching the agar) of labeled **plate #1** and another 200ul for **plate #2** (these samples have no plasmid DNA). (**Tips can be reused if they didn't touch the agar plates**)

Using a sterile inoculating loop, spread cells in one direction the full length of the plate then turn the plate 90° and spread again the full length. **You can use the inoculating loop for both plates but do plate #1 first, then #2.** Cover both plates and let sit to allow the liquid to be absorbed before turning plates over.

14. **Plating of the tube labeled "+":**

With a new pipette tip, take 200ul and **drop onto the top of the agar** of labeled **plate #3** and another 200ul to **plate #4** (these have plasmid DNA added). Spread the cells the same way as in step #13 and **spread plate #3 before you reuse the loop on plate #4.** Again, cover plates and let sit to absorb the liquid before turning plates over. Stack plates inverted when dry.

15. Incubate overnight at 37°C (15-20 hours). Next day read results and calculate transformation efficiency.

Transformation Lab

Student Worksheet

NAME:

1. What is *E.coli*? What does "non-pathogenic" mean?
2. What is a plasmid?
3. When a plasmid is inside a cell it is "extrachromosomal", what does this mean?
4. What is the name of the plasmid used in this protocol? What two genes does it contain?
5. How might a plasmid contribute to a selective advantage for a bacterial cell?
6. What is transformation? What does exogenous DNA mean?
7. What is competency for a cell? How is it achieved?
8. What is media when used with cells? What are the two forms it comes in?
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