the Transformation Lab
Experiment Using *E. coli* and pFluoroGreen

Maryland Loaner Lab Teacher Packet

Email MDLL@towson.edu to request a Word version of this document.

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

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# Bacterial Transformation

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### Bacterial Transformation

### Standards

#### Next Generation Science Standards

**Performance Expectations:** Students’ ability to complete the following performance expectation(s) will be supported by participation in this activity.

**HS-LS1-1:** Construct an explanation based on evidence for how the structure of DNA determines the structure of protein, which carry out the essential functions of life through systems of specialized cells.

**HS-LS3-1:** Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions characteristic traits passed from parents to offspring.

<table>
<thead>
<tr>
<th>Dimension</th>
<th>NGSS Code or citation</th>
<th>Corresponding student task in activity</th>
</tr>
</thead>
</table>
| Disciplinary Core Idea | **LS1.A Structure and Function**  
  - Systems of specialized cells within organisms help them perform the essential functions of life. (HS-LS1-1)  
  - All cells contain genetic information in the form of DNA molecules. Genes are regions in the DNA that contain the instructions that code for the formation of proteins, which carry out most of the work of cells (HS-LS1-1) | Students transform bacterial cells with a novel gene that confers antibiotic resistance and the ability to glow. As part of the activity, students explore how to make competent cells and how plasmids can be used to deliver novel genes into a bacterial cell. |
| Practice           | **LS3.A Inheritance of Traits**  
  - Each chromosome consist of a single very long DNA molecule, and each gene on a chromosome is a particular segment of that DNA. The instructions for forming species’ characteristics are carried in DNA. All cells in an organism have the same genetic content, but the genes used (expressed) by the cell may be regulated in different ways. Not all DNA codes for a protein; some segments of DNA are involved in regulatory or structural functions, and some have no known function. (HS-LS3-1) | Students explore how inserting novel genes that code for specific proteins into new organisms can enable an organism to produce protein they normally would not produce. |

**Practice Asking Questions and Defining Problems**  
Students explore the idea of genetic engineering and the creation of transgenic organisms. They are asked to reflect...
## Bacterial Transformation

### Standards

<table>
<thead>
<tr>
<th>Activity</th>
<th>Description</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Define a design problem</td>
<td>Define a design problem that involves the development of a process or system of interacting components and criteria and constraints that may include social, technical, and/or environmental.</td>
<td>on the use of genetic engineering to create transgenic pets, as well as other transgenic organisms.</td>
</tr>
</tbody>
</table>

#### Planning and Carrying out Investigations

- Plan an investigation individually and collaboratively and in the design identify independent and dependent variables and controls, what tools are need to do the gathering, how measurements will be recorded, and how many data are need to support a claim.
- Conduct an investigation and/or evaluate and/or revise the experimental design to produce data to serve as the basis for evidence that meet the goals of the investigation.

Students will perform a bacterial transformation and insert a vector containing the pFluoroGreen plasmid into E. coli bacterial cells. Prior to beginning, students are asked to identify what controls are being used in the procedure and why they are necessary.

#### Analyzing and Interpreting Data

- Analyze data using tools, technologies, and/or models (e.g. computational, mathematical) in order to make valid and reliable scientific claims or determine an optimal design solution.
- Analyze data to identify design features or characteristics of the components of a proposed process or system to optimize it relative to criteria for success.

Students will analyze the results of the transformation process for each of the four plates. They will explain the results of each plate (i.e. growth versus no growth, lawn versus colonies).

Students will calculate the transformation efficiency and discuss ways in which the efficiency can be improved.

#### Using Mathematics and Computational Thinking

- Use mathematical, computational, and/or algorithmic representations of phenomena or design solutions to describe and/or support claims and/or explanations.

Students will calculate the transformation efficiency and discuss ways in which the efficiency can be improved.

#### Crosscutting Concept

**Patterns**

- Patterns of performance of designed systems can be analyzed and interpreted to reengineer and improve the system.

Students explore transformation efficiency and consider how changes to the system could increase or decrease the efficiency.
## Bacterial Transformation

### Standards

<table>
<thead>
<tr>
<th>Cause and Effect</th>
<th>Students explore why each plate had different results (lawn versus colonies, growth versus no growth). They reflect on why transformation efficiencies may differ among groups.</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Students suggest cause and effect relationships to explain and predict behaviors in complex natural and designed systems.</td>
<td></td>
</tr>
</tbody>
</table>

### Nature of Science

**Science Addresses Questions about the Natural and Material World**

- Science and technology may raise ethical issues for which science, but itself, does not provide answers and solutions.
- Scientific knowledge indicates what can happen in natural systems—not what should happen. The latter involves ethics, values, and human decisions about the use of knowledge.
- Many decisions are not made using science alone, but rely on social and cultural contexts to resolve issues.

### Connections to Common Core State Standards

**English Language Arts/Literacy**

- RST.9-10.2
- RST.9-10.3
- RST.9-10.4
- RST.9-10.7
- RST.11-12.2
- RST.11-12.3
- RST.11-12.4
- RST.11-12.7
- RST.11-12.9

**Mathematics**

- PRACTICE.MP1
- PRACTICE.MP3
- PRACTICE.MP4
- PRACTICE.MP6
Bacterial Transformation

Standards

Advanced Placement Biology Standards

<table>
<thead>
<tr>
<th>Standard</th>
<th>Enduring Understanding 1A: Change in the genetic makeup of a population over time is evolution.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Essential Knowledge 1.A.2: Natural selection acts on phenotypic variations in populations.</td>
</tr>
<tr>
<td>Enduring Understanding 3A: Heritable information provides for continuity of life.</td>
<td>Essential Knowledge: 3.A.1: DNA, and in some cases, RNA, is the primary source of heritable information.</td>
</tr>
<tr>
<td></td>
<td>Essential Knowledge: 3B.1: Gene regulation results in differential gene expression.</td>
</tr>
<tr>
<td>Enduring Understanding 3C: The processing of genetic information is imperfect and is a source of genetic variation.</td>
<td>Essential Knowledge 3.C.1: Changes in genotype can result in changes in phenotype.</td>
</tr>
<tr>
<td></td>
<td>Essential knowledge 3.C.2: Biological systems have multiple processes that increase genetic variation.</td>
</tr>
</tbody>
</table>

Science Practice 3.1: The student can pose scientific questions.
Science Practice 5.3: The student can evaluate the evidence provided by datasets in relation to a particular scientific question.
Science Practice 6.4: The student can make claims and predictions about natural phenomena based on scientific theories and models.
Science Practice 7.1: The student can connect phenomena and models across spatial and temporal scales.
Bacterial transformation serves as an essential basis to a number of DNA technologies. In transformation, bacteria take up exogenous (foreign) DNA and produce the genetic products (proteins) encoded in the foreign DNA. Transformation enables inexpensive and reliable production of important medical products such as insulin, human growth hormone, and other replacement hormone and gene therapies.

In bacteria, the haploid genome is a single circular chromosome. This differs from eukaryotic genomes like those of plants and animals, where the genetic material is diploid and arranged into linear chromosomes. Bacteria can also possess additional nonessential pieces of circular DNA called plasmids. Plasmids contain fewer genes than the genome but can offer the cell unique characteristics. For example, a plasmid which encodes for resistance to an antibiotic allows the cell with the plasmid to survive when the antibiotic is present in the environment.

In genetic research, plasmids must be large enough to manipulate, small enough for the cell to take up, and include a marker gene after the targeted gene. The marker gene allows the researcher to readily identify which cells successfully acquired the new genetic material. For example, if a phosphorescence gene follows the gene for insulin production, then glowing cells successfully took up the plasmid and can produce insulin.

Acquiring exogenous plasmids can occur in one of three ways. Cells which possess a plasmid are generally labeled as F+ cells whereas those that lack that same plasmid are F-. Some cells undergo conjugation, in which an F+ cell replicates and transmits the plasmid to an F- cell. In transduction, which rarely occurs in nature, a bacteriophage (bacterial virus) accidentally packages bacterial DNA and injects it into a new cell. In transformation, competent cells take up “naked” DNA in the medium. Not all cells can become competent, but some bacteria such as Escherichia coli can become competent by undergoing either heat or electric shock after treatment with calcium chloride or other chlorine salts.

This lab activity provides the protocols and materials for students to transform E. coli with a plasmid which contains the gene for ampicillin resistance (AMP R) and a jellyfish gene which codes for a phosphorescing protein (GFP). In this case, bacteria can be grown on a selective medium, which encourages growth of bacteria with certain characteristics (resistant to ampicillin) but discourages those without that characteristic. If successful, students will have some colonies which phosphoresce and can grow on media treated with ampicillin. Additionally, this lab supports student exploration of differential gene expression. The GFP gene requires the presence of isopropyl-beta-D-thiogalactopyranoside (IPTG), which induces the transcription of the mRNA to make the phosphorescing protein. Without IPTG in the medium, transformed cells will not transcribe nor translate the protein, and so will not glow. This demonstrates differential gene expression.

Inquiry based Instruction

Students learn best when they explore and discover the information for themselves. Additionally, the Next Generation Science Standards and the newest AP Biology materials support inquiry in learning. Therefore, teachers are strongly encouraged to allow students to explore the concepts before explaining, and to permit students to use the protocols and materials to design the answer to a
Bacterial Transformation

Introduction for Teachers

question. To assist, Towson University’s Center for STEM Excellence has provided a scenario and pre-lab materials to encourage inquiry based learning.

Loaner Lab Learner Goals
The lesson aims to have students explore the use of DNA technologies and transformation in a potential real-world situation. These goals include objectives of the AP Laboratory Objectives, which are marked with an asterisk (*). To achieve this overarching goal students will:

1. *Describe and apply the principles of bacterial transformation. (Exploration Chart, Teach Assistant)
2. *Understand the conditions under which cells can be transformed. (Exploration Chart, Teach Assistant)
3. *Describe the process of competent cell preparation. (Exploration Chart)
4. *Explain how a plasmid can be engineered to include a piece of foreign DNA. (Exploration Chart)
5. *Explain how plasmid vectors are used to transfer genes. (Exploration Chart, Post Lab)
6. *Describe how antibiotic resistance is transferred between cells. (Exploration Chart, Teach Assistant, Post Lab)
7. Understand and describe the concepts and applications of plasmids, vectors, aseptic techniques, and transgenic organisms. (Exploration chart, Wet Lab)
8. Demonstrate competence with aseptic technique to successfully conduct a bacterial transformation. (Wet Lab)
9. Explain the use of marker genes in transformation. (Exploration Chart, Post-Lab)
10. Explain the use of selective media. (Exploration Chart, Post-Lab)
11. *Design a procedure to select positively for antibiotic-resistant transformed cells. (Exploration Activity)
12. Evaluate if a novel situation is scientifically realistic, based on information learned in this activity and lab. (Extension)
13. *Use plasmids as vectors to transform bacteria with a gene for antibiotic resistance in a controlled experiment. (Wet Lab)
14. *Calculate transformation efficiency. (Post Lab)
15. Be able to use multiple experimental controls. (Wet Lab)

Students are required to know the objectives under the section titled "Before doing this laboratory you should understand:" before doing the Transformation Lab. 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.

The Transformation Lab has Four Parts:

- A pre-laboratory classroom activity that allows students to explore the concepts of and
elements involved in transformation, transgenic animals, how bacteria grow, and how to inoculate bacteria using aseptic technique. The pre-laboratory exploration should also include a discussion of the concepts listed under the Introduction and Exploration sections found on pages S1-S6 to adequately prepare students to understand this lab.

- Pre-laboratory technique exercises that allow students to practice aseptic techniques, learn how to streak plate cultures, and practice using the micropipettes.

- 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 72 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 in the Facilitation Guide.
Bacterial Transformation

Materials and Supplies

Supplied by the Teacher:

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Student worksheets</td>
<td>1 set per student</td>
<td></td>
</tr>
<tr>
<td>Gloves</td>
<td>1 pair per student</td>
<td></td>
</tr>
<tr>
<td>Goggles</td>
<td>1 pair per student</td>
<td></td>
</tr>
<tr>
<td>Crushed ice</td>
<td>1 cup per 2 groups + 1 large container</td>
<td>Crushed ice is preferred over ice cubes.</td>
</tr>
<tr>
<td>Styrofoam cups</td>
<td>5</td>
<td>To hold crushed ice.</td>
</tr>
<tr>
<td>Stopwatch or timer</td>
<td>1</td>
<td>Use for incubation times.</td>
</tr>
<tr>
<td>Water bath*</td>
<td>1</td>
<td>Needed for 2 separate incubation steps.</td>
</tr>
<tr>
<td>Incubator**</td>
<td>1</td>
<td>37°C for growth of <em>E. coli</em> source plates and post-lab grow out of transformed bacterial cultures.</td>
</tr>
<tr>
<td>Waste containers</td>
<td>1 per group</td>
<td></td>
</tr>
<tr>
<td>Bleach bucket</td>
<td>1</td>
<td>Containing 10% bleach solution to kill bacteria on plates, waste containers, and workstations.</td>
</tr>
</tbody>
</table>

*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.
# Bacterial Transformation

## Materials and Supplies

### Materials in Kit

<table>
<thead>
<tr>
<th>Material</th>
<th>Number</th>
<th>Comments</th>
<th>Return Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Teacher packet and laminated student sheet packet</td>
<td>1</td>
<td>Contains all of the information necessary to conduct the lab.</td>
<td>Return</td>
</tr>
<tr>
<td>Foam microtube racks</td>
<td>10</td>
<td>1 per group</td>
<td>Return</td>
</tr>
<tr>
<td>Lab markers</td>
<td>10</td>
<td>Used to label tubes and plates</td>
<td>Return</td>
</tr>
<tr>
<td>1000µl micropipettes</td>
<td>10</td>
<td>1 per group</td>
<td>Return</td>
</tr>
<tr>
<td>20µl micropipette</td>
<td>1</td>
<td>For teacher to aliquot pFlouroGreen</td>
<td>Return</td>
</tr>
<tr>
<td>10µl fixed volume minipipettes</td>
<td>10</td>
<td>1 per group</td>
<td>Return</td>
</tr>
<tr>
<td>Blue micropipette tip boxes</td>
<td>5</td>
<td>1 per 2 groups</td>
<td>Return box and unused tips</td>
</tr>
<tr>
<td>Yellow micropipette tip boxes</td>
<td>1</td>
<td>1 per class</td>
<td>Return box and unused tips</td>
</tr>
<tr>
<td>White sterile toothpicks</td>
<td>10 sets</td>
<td>2 toothpicks per group</td>
<td>Return if unused</td>
</tr>
<tr>
<td>Sterile yellow loops</td>
<td>20</td>
<td>2 per group. Kept in bag with white sterile toothpicks.</td>
<td>Return if unused</td>
</tr>
<tr>
<td>Clear microcentrifuge tubes</td>
<td>20</td>
<td>2 per group</td>
<td>Return if unused</td>
</tr>
<tr>
<td>Sterile yellow loops, bagged individually</td>
<td>5</td>
<td>Used by teacher to prep 5 E. coli source plates</td>
<td>Return if unused</td>
</tr>
<tr>
<td>Large agar plates **</td>
<td>5</td>
<td>1 per 2 groups (Teacher will prep)</td>
<td>Do not return. Properly Dispose **</td>
</tr>
<tr>
<td>BactoBead™ EDVO-Kit</td>
<td>1</td>
<td>Instructions in pack</td>
<td>Return if unused</td>
</tr>
<tr>
<td>Small agar plates (non-striped)</td>
<td>10</td>
<td>1 per group</td>
<td>Do not return. Properly Dispose **</td>
</tr>
<tr>
<td>Small agar plates (striped)</td>
<td>20</td>
<td>2 per group</td>
<td>Do not return. Properly Dispose **</td>
</tr>
<tr>
<td>Small agar plates (double striped)</td>
<td>10</td>
<td>1 per group</td>
<td>Do not return. Properly dispose.</td>
</tr>
</tbody>
</table>
## Bacterial Transformation
### Materials and Supplies

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>“p” tubes</td>
<td>10</td>
<td>1 per group. <em>Keep frozen after aliquoted and until used.</em></td>
</tr>
<tr>
<td>“Rec” tubes</td>
<td>10</td>
<td>1 per group. <em>Keep frozen after aliquoted and until used.</em></td>
</tr>
<tr>
<td>“CaCl₂” tubes</td>
<td>10</td>
<td>1 per group. <em>Keep frozen after aliquoted and until used.</em></td>
</tr>
<tr>
<td>CaCl₂ Container</td>
<td>1</td>
<td>For teacher to aliquot for student stations <em>Keep frozen until used.</em></td>
</tr>
<tr>
<td>Recovery Broth Container</td>
<td>1</td>
<td>For teacher to aliquot for student stations. <em>Keep frozen until used.</em></td>
</tr>
<tr>
<td>pFlouroGreen Tube</td>
<td>1</td>
<td>For teach to aliquot for student stations. <em>Keep frozen until used.</em></td>
</tr>
<tr>
<td>White plastic tube floater</td>
<td>1</td>
<td>Used with water bath.</td>
</tr>
<tr>
<td>Insulated bucket (red)</td>
<td>1</td>
<td>Return</td>
</tr>
<tr>
<td>Thermometer in plastic holder</td>
<td>1</td>
<td>Return in container</td>
</tr>
<tr>
<td>UV lamps</td>
<td>5</td>
<td>1 per 2 groups.</td>
</tr>
<tr>
<td>Bioscience video and lesson plan</td>
<td>1</td>
<td>Return</td>
</tr>
<tr>
<td>Container of disinfectant wipes</td>
<td>1</td>
<td><em>Use to disinfect equipment</em></td>
</tr>
</tbody>
</table>

**See instructions in teacher’s manual for proper disposal**

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

### Proper Disposal: This lab involves the use of live bacterial cultures. Therefore, all bacterial cultures and materials contaminated with bacteria or DNA must be either autoclaved according to the autoclave manufacturer’s directions OR soaked overnight in a 10%
bleach solution. It is the teacher’s responsibility to ensure proper disposal of all cultures and contaminated materials!

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.
Bacterial Transformation

Background Information for Teachers

The purpose of the pre-laboratory exploration 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.

The purpose of the pre-laboratory techniques activity is to familiarize students with aseptic techniques, micropipette skills, and streak plating. Practicing these skills and techniques prior to performing the wet lab improves student confidence and reduces error in the lab.

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<sup>R</sup>) 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 are 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 more efficiently after either of these treatments.

**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\textsuperscript{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 *Aquorea victoria*. This fluorescent protein can be expressed in *E.coli* bacteria and will produce a green light that can especially be seen when exposed to a long wave UV light source.

**Transformation Efficiencies**

Transformation efficiencies are a way to determine how many cells were transformed per microgram (µg) 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:
Bacterial Transformation

Background Information for Teachers

\[
\text{Number of Transformants} \times \frac{\text{Final volume at recovery (ml)}}{\text{vol of plates (ml)}} = \text{Number of transformants per } \mu \text{g}
\]

(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 0.25ml)

(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 1x10^4 to 1x10^7 transformants per µg 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, shortened incubation times when uptake of the plasmid DNA is taking place, or possibly a shortened "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. In our test of this lab, three of five IPTG plates incubated at 37°C showed 1-10 transformed colonies and two of five IPTG plates incubated at 20°C for three days showed 1-18 transformed colonies.

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.
Bacterial Transformation

Facilitation Guide

Engage: (Use with Student Pages S1-3)

Note: There is a Powerpoint presentation available on our website (www.towson.edu/cse) with these images and videos embedded.

1. Show students the picture of Alba the Bunny and ask them to speculate how a bunny could be this color. If they could purchase dogs, cats, fish, etc. that glowed in the dark like this, would they? Why or why not?

2. Show them the picture of the spider goat. Tell the class that this goat secretes the spider silk protein in her milk. Scientists later remove the spider silk protein for many applications.

3. What do these animals have in common? How did they become transgenic (having foreign DNA in their cells)?

4. Show students the short video from the National Science Foundation about the “Spider Goats”. It provides many uses for their silk proteins and gives a brief introduction to the topic. https://www.youtube.com/watch?v=ktgACq4zcAU

5. Tell students that the same basic concepts used in the spider goat and in Alba are regularly used with certain bacteria that are able to take up foreign DNA and the make products we need. Products include the genes themselves as well as proteins, medicines, and hormones.

Explore (Use with Student Pages S4-7.)

6. Students need to understand how transformation works and why we use it. Students should complete the Exploration Chart as they learn about the topics. There are many ways you can allow students to explore these concepts. You can share the Web Resources with the class and have them explore the concepts in pairs or individually. Alternatively, you can assign different groups to research and present information on each of the assigned topics.

7. Introduce the scenario to the students. Then have them use their resources and their new learning to instruct the new lab assistant, Brad.

Scenario: The biotech company called BTSilk wants to break into the silk manufacturing field by using bacteria to produce spider silk. Spider goats have been very effective, but raising goats is expensive and time consuming. Additionally, the spider silk is only produced when the goats are milking. In contrast, bacteria could potentially produce more silk, faster, and cheaper! BTSilk researchers there have been working on a way to insert the gene into the bacterium Escherichia coli and verify that it was successfully done. The current research team is developing a plasmid containing the spider silk gene, the A. victoria phosphorescing gene (GFP), and an ampicillin resistant (ampR) gene. GFP is an inducible gene, which means that it is unavailable for transcription unless conditions turn the gene “on”. The GFP gene
becomes available for transcription in the presence of the molecule IPTG. Many plasmids, such as the one being developed by the research team, are designed for transformation to include the amp_\text{R} gene. Bacterial cells with the amp_\text{R} gene can survive in the presence of the antibiotic, ampicillin, while untransformed cells cannot. Cultures are therefore plated on medium containing ampicillin in order to isolate the transformed cells.

The plasmid development team believes they have the first phase of the plasmid completed. This prototype plasmid contains the GFP gene and the ampicillin resistance gene. While that team continues with plasmid development by inserting the spider silk gene, our lab in BTSilk has been tasked with the successfully transforming E. coli with the two-gene prototype plasmid and confirming that transformation was successful.

You have access to the plasmid which contains the ampR gene, GFP gene, and BTSilk gene on it and to a variety of growth medium to culture the bacterial cells. The growth media you can use are growth medium with ampicillin, growth medium without ampicillin, and growth medium with ampicillin and IPTG. You’ll be following the actual transformation lab procedures detailed by colleagues involved in other transformation projects. Your job is to develop a lab protocol to test if the bacteria successfully took up the plasmid.

You actually have two jobs, though. First, it seems that the research assistant they just hired and assigned to you is brand new to working with bacteria and biotechnology. Brad doesn’t really understand how bacteria take up the DNA into their cells. So, you need to teach him about bacteria transformation, plasmid vectors, and marker genes. Because you’re working with bacteria it would be helpful to also be sure Brad understands how to use aseptic techniques in the lab. Finally, it would be a good idea to review the idea of bacterial resistance since your plasmid contains the gene which makes E. coli resistant to the antibiotic ampicillin. Your second job is to develop a lab protocol to test if the bacteria successfully took up the plasmid.

8. After students understand the concepts above, ask them to develop the plan for this lab. If they need guidance, help them to realize that the question they need to answer is “How do I know if the bacteria successfully took up the plasmid?” They will need to identify positive and negative controls, will use four different plates (one without ampicillin or IPTG, two with ampicillin but no IPTG, one with ampicillin and IPTG).

**Explain (Use Student Pages S8-10 for steps 9 and 10. Use Student Pages 11-14 for Step 11.)**

9. Review the concepts with the class, making sure that students understand the concepts. This video provides an overview of several concepts introduced here and of the lab: [http://www.pbslearningmedia.org/resource/biot11.sci.life.gen.transbact/transforming-bacteria/](http://www.pbslearningmedia.org/resource/biot11.sci.life.gen.transbact/transforming-bacteria/)

10. Explain that there are some techniques which require practicing in order to do them well. Work through the Pre-Lab Techniques Activity, checking that students are successful in streaking their
plates and using the micropipettes. Please note that students will be adding 250 µl of bacterial suspension to their plates. Students need to spread the suspension across the plate, but the volume plated may not require actual streak plating.

11. Run the Wet Lab, following the protocol provided. We found that cultures incubated at 37°C had better success and had large enough colonies to readily see the phosphorescence after 24 hours. Cultures grown at room temperature (20°C) required three days before the colonies were large enough to readily see phosphorescence.

Extend

12. Your lab assistant, the same one who needed some explanation of the concepts before you actually tried the lab, is also a big Spider Man fan. He wants to know if the basic premise behind Spider Man’s transformation into a spider-human hybrid is accurate or science fiction or fantasy. Answer his question, supporting it with details and information from this lab. You may use a CER chart.

13. This link (http://www.topsecretwriters.com/2013/01/will-there-be-glow-in-the-dark-pets-in-the-future/) connects to a story asking if there will be glow-in-the-dark-pets in the future. Ask students to read the article, apply the concepts from this lab, and respond to this article.

14. You may offer students the opportunity to research the ethical controversy that surrounded Alba and hold a class debate about transgenic art, transgenic animals, and bacteria used to make products.

15. Ask students to explore pharm animals. These traditional farm animals are genetically engineered to produce medicines.


17. 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

18. 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.

Evaluate (Use with Student Page S15.)

1. Students can complete the post lab activity or submit a formal lab report.
The purpose of this pre-lab activity is to introduce and develop an understanding of the concepts of transformation, bacterial competence, plasmids construction and use in gene transfer, antibiotic resistance, transgenic organisms, and aseptic techniques. The initial activity aims to engage student interest and connect to real-world transgenic animals as art, pets, and resources. The exploration activity encourages students to explore the information before direct explanation is offered by the instructor.

**Materials Needed:**

- PowerPoint Slide Presentation
- Student Worksheets
- Student access to the Internet and/or other resources
Bacterial Transformation

Pre-Lab: Techniques (Streak Plate)

NOTE: Groups using the Loaner Lab should complete the pre-laboratory Explore and Explain classroom activity outlined in steps 1-10 of the Facilitation Guide (pages 14-17) prior to beginning the lab.

The purpose of the pre-laboratory techniques activity is to familiarize the students with aseptic techniques, the procedure for streaking out (inoculating) microbial cultures on solid media agar plates, and micropipette skills. 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: Techniques 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
- Demonstrate accurate skills with the micropipette
- Verbally communicate procedure, purpose, and results

Pre-Laboratory Techniques 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 an 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 vinaigrette is recommended) to represent a bacterial culture.
- Box of toothpicks (flat, not round ended)
- Second materials set for Micropipette Challenge (see directions below)

Pre-Laboratory Techniques Engagement (5 Minutes)

Give each student a pre-poured "Jello-0" 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 (vinaigrette 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 (vinaigrette) 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 Techniques 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 struck 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.

We recommend teaching students the quadrant streaking method prior to running this lab. Please note that students will be adding 250 µl of bacterial suspension to their plates. Students need to spread the suspension across the plate, but the volume plated may not require actual streak plating.

**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.
NOTE: The Micropipette Challenge is a 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 µl micropipette

Micropipette tips  
1 ml pre-made yellow solution  
1 ml pre-made blue solution

Basic Directions for Micropipette Use

Setting the Volume
All micropipettes have a volume control dial. Determine whether the volume window on your pipette shows tenths of microliters (0.1 µl) 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).

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 the 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 samples to prevent contamination.
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.

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 "Rec", "CaCl$_2$" and "p" bottles are to be frozen until they are used. The teacher will aliquot the “Rec”, “CaCl$_2$" and “p” into the labeled tubes and should be frozen until used. In preparation for the lab, "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 "Rec" tubes can be placed at room temperature in the foam tube racks. The "CaCl$_2$" 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 tops of the tubes, not the sides of the tubes, should be labeled. The bottoms of the tubes should also be labeled with group number and other contents (i.e., + or – DNA, + amp, + IPTG).
The following instructions are for use when students are in the lab, working through the laboratory procedures.

In **Student 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 aseptic 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. To minimize heat buildup in the cell suspension, suggest that students refrain from holding the tubes at the bottom as this will generate heat and warm the cells. Hold the tubes away from the cell suspension.

In **Student Step #3**, students will need to know how to properly open the wrapped sterile toothpicks to maintain their sterility. Push one end of the toothpick through the packaging, making sure students understand that they can't touch the collecting 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 collecting end of the toothpick to scrape an area a few centimeters long OR 5 colonies 1-1.5 mm in diameter from the *E.coli* source plates. They should be able to see the cells on the toothpick (the yellowish bacteria culture). When they are twisting and shaking the toothpick into the CaCl₂ in the tube, 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 toothpick they should try to get as many cells off as possible.

In **Student Step #4**, students are asked to pipet up and down to mix the contents thoroughly. They are attempting to break up clumps of cells in solution.

In **Student Step #6**, students will use the 10µl 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 **Student Steps #7, 8, 9 & 12**, make sure students keep their tubes on ice for the full 10 minutes. Timing is very important throughout this lab. If the *E. coli* is heated too long, it will die, but the *E. coli* transforms best when it is shocked, so the timing is very important on icing, heating, and recovery time.

- During **Student Step #7**, students should label their agar plates on the bottom (agar side). Ask students to put their group number clearly on each plate bottom, then to label as follows:
• In **Student 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 **Student 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 **Student 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 10 minutes to use the bottom of the flow chart and have students consider and discuss the **expected results** for each plate. (See the flow chart with short answers).

<table>
<thead>
<tr>
<th>Plate #</th>
<th>Marks on Plate</th>
<th>Label as...</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No stripe</td>
<td>-pDNA, -AMP</td>
</tr>
<tr>
<td>2</td>
<td>One of the single stripe plates</td>
<td>-pDNA, +AMP</td>
</tr>
<tr>
<td>3</td>
<td>Second of the single stripe plates</td>
<td>+pDNA, +AMP</td>
</tr>
<tr>
<td>4</td>
<td>Two stripes</td>
<td>+pDNA, +AMP, +IPTG</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source Plate</th>
<th>Expected results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plate #1</td>
<td>Positive control. Shows cells that were not transformed. Plate does not contain ampicillin. <em>E. coli</em> bacteria growth is expected.</td>
</tr>
<tr>
<td>Plate #2</td>
<td>Negative control. Shows cells that were not transformed. Plate does contain ampicillin which kills cells. No growth is visible.</td>
</tr>
<tr>
<td>Plate #3</td>
<td>Only transformed cells grow here because the plate has ampicillin which kills non-transformed cells, but the cells do not glow because the medium lacks the inducer IPTG. <em>E. coli</em> bacteria growth is expected.</td>
</tr>
<tr>
<td>Plate #4</td>
<td>Only transformed cells grow here because the plate has ampicillin which kills non-transformed cells, and the cells will glow because the medium has the inducer IPTG. <em>E. coli</em> bacteria growth is expected.</td>
</tr>
</tbody>
</table>

**Student Steps #14, 15 & 16** 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 **Student Step #17**, 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 48-72 hours before **students observe results**: checking all four plates for expected results, counting colonies from plate #4 (will vary, but anywhere from 3-10 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.
Bacterial Transformation

Laboratory Preparation for Teachers

Maryland Loaner Lab will supply reagents, equipment, and instructions outlined for 10 student workstations. **Teachers must supply the students with the handouts (laminated worksheets are provided in the kit for photocopying):** Student Introduction Reading and Worksheets, Streak Plate Challenge, Micropipette Challenge, Laboratory Protocol, Flow Chart, Post-Lab.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Time Needed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preparing Student Stations</td>
<td>45 minutes</td>
</tr>
<tr>
<td>Pre-Lab: Explore &amp; Explain</td>
<td>40 minutes</td>
</tr>
<tr>
<td>Pre-Lab: Streak Plate</td>
<td>35 minutes</td>
</tr>
<tr>
<td>Micropipette Challenge</td>
<td>15 minutes</td>
</tr>
<tr>
<td>Laboratory Activity</td>
<td>90 minutes</td>
</tr>
<tr>
<td>Post-Lab Activity</td>
<td>15 minutes + Extension time (varies)</td>
</tr>
</tbody>
</table>

**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 microcentrifuge tube: “Rec” kept at room temperature in foam rack
- One microcentrifuge tube each: "CaCl₂" and "p" both kept **in ice cup at student station** ("p" tubes will need to be hand flicked to get contents in bottom of tube)
- One 1000µl micropipette
- One 10µl fixed volume minipipette
- One blue box of 1000µl micropipette tips (1 box/2 student groups)
- One yellow box of 10µl minipipette tips (1 box/10 student groups)
- 1 petri dishes with solid media (no stripe)
- 2 petri dishes with solid media (with one stripe)
- 1 petri dish with solid media (with two stripes)
- 2 yellow sterile loops (wrapped)
- 2 white sterile toothpicks (wrapped)
- One *E.coli* bacteria source plate (1 plate/2 student groups)

**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 & #11). (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), a sleeve of petri dishes with solid media that have one stripe on the plates (contain ampicillin), and a sleeve of petri dishes with solid media that have two stripes on the plates (contain ampicillin and IPTG). All three sleeves will need to be stored in the refrigerator with plates inverted (agar side facing up) until used.

Preparation of five *E. coli* Source Plates:
You will prepare the isolated *E. coli* host transformation colonies 16-24 hours (maximum) prior to experiment.

To prepare *E. coli* cells:

1. Use aseptic technique to transfer one BactoBead™ to the edge of source plate and replace lid on source plate. The kit should contain 6 BactoBead™. Use 1 per source plate. Dispose of unused BactoBead™ with *E. coli* source plates.

2. Add 10 µl of sterile water or sterile nutrient (recovery) broth to BactoBead™ and allow dissolve on surface. This should take several minutes.

3. Once the BactoBead™ has dissolved, use a sterile inoculating loop to make a primary streak on the agar, as seen below in Figure 1.

4. Repeat steps 1-3 for the remaining four source plates.

5. Label the plates “*E. coli*”. Invert and incubate overnight (16-18 hours) at 37°C in an incubation room or 24 hours at room temperature (find a warm spot, such as the top of refrigerator).

*Fig 1. Note:* The BactoBead should be dissolved in Plates 2-5 but are shown for reference. The “red arrows” show where you begin streaking with your inoculation loop.
Aliquot the Student Station Microcentrifuge Samples

Either the night before you plan to run the lab or the day of the lab, you will need to aliquot the liquid samples for each student station.

1. Put the 10 “CaCl$_2$” microcentrifuge tubes in a foam rack. Using the 1000 µl micropipette and a large tip, dispense 1 mL (1000 µl) CaCl$_2$ into each of the 10 CaCl$_2$ microcentrifuge tubes. The same tip can be used for this entire process. Carefully close each tube and either store in the freezer until class or put on ice for the class to use the same day.

2. Put the 10 “p” microcentrifuge tubes in a foam rack. Using the 20 µl and a small tip, transfer 12 µl of the pFlouroGreen into each microcentrifuge tube. Cap tightly and store in the freezer until ready to use.

3. Put the 10 “Rec” microcentrifuge tubes in a foam rack. Use the 1000 µl micropipette and a large tip, dispense 1.5 ml of Recovery Broth into each microcentrifuge tube. Carefully cap and store in the freezer until class or put on ice if using the same day.
Bacterial Transformation

Laboratory Flow Chart Teachers

Add 500 µl of CaCl₂ then add 5 well isolated 1-1.5mm diameter colonies from Source Plate. Then transfer 250 µl to –pDNA and 250 µl to +pDNA.

Tubes contain 250µl CaCl₂ and E. coli

-pDNA

+DNA

Incubate on ice for 10 min.
Incubate at 42°C for 90 s.
Incubate on ice for 2 min.

Add 250µl Recovery Broth.
Incubate for 10 min at 37°C.

Plate 1: No plasmid. Will grow lawn of cells. Demonstrates E. coli will grow on medium.
Plate 2: Ampicillin, no plasmid. No cell growth. Demonstrates E. coli susceptible to amp.
Plate 3: Ampicillin, with plasmid. Will grow scattered, non-glowing colonies. Demonstrates transformed E. coli survived amp.
Plate 4: Plasmid with Ampicillin, IPTG. Grows glowing colonies. Demonstrates transformed E. coli glow with IPTG to induce gene expression.

Add 10µl pFlouroGreen plasmid DNA (contains AMP<sup>R</sup> gene and GFP gene).
Now that Brad and you have reviewed the concepts and techniques available to you, you’re ready to plot out the general steps of the procedure. You will follow the accepted protocols of transformation, but you need to understand the question you’re answering and how you’ll answer it.

4. **What research question are you trying to answer? Why?**
   
   How will we know if the transformation is successful?

5. **You have four plates available to you in this investigation (one with no ampicillin and no IPTG, two with ampicillin but not IPTG, and one with ampicillin and IPTG). You also have access to *E. coli* (-) and can also expose *E. coli* to the plasmid in a transformation process (+). What will you plate on each plate, + or -? Why**
   
   - You will plate – on the plate without ampicillin (Plate 1) to confirm *E. coli* is growing.
   - You will plate – on the first plate with ampicillin (Plate 2) to confirm *E. coli* is susceptible to ampicillin.
   - You will plate + on the second plate with ampicillin (Plate 3) to confirm that transformed *E. coli* survive ampicillin.
   - You will plate + on plate with IPTG and ampicillin (Plate 4) to see if the transformed bacteria glow.

6. **What plates are your controls for this experiment? What are they controlling for?**
   
   Plates 1 & 2 are controls. Plate 1 confirms growth and Plate 2 confirms that ampicillin kills *E. coli*.

7. **What are your predicted results?**
   
   Answers will vary
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. **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<sub>R</sub> (provides resistance to the antibiotic ampicillin) and GFP (the gene for a green fluorescent protein).*

4. **4. 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.*

5. **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.*

6. **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.*

7. **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<sub>R</sub> gene.*

8. **Why did the cells only glow when IPTG was present?**

   *The GFP gene is an inducible gene. This means that it is usually “off” unless a chemical prompts it to switch “on”. IPTG serves as the chemical to switch the gene “on”, so the protein is only transcribed and translated when IPTG is present.*
9. What were your experimental results? Sketch your results below, and describe what happened on each plate.

Plate 1: Lawn of bacterial growth because nothing inhibited *E. coli*. No glowing because no plasmid or inducer.

Plate 2: No growth because no plasmid, so amp killed *E. coli* plated.

Plate 3: Limited growth because only transformed cells survive amp. No glowing because no inducer present.

Plate 4: Limited growth because only transformed cells survive amp, but these should glow because IPTG inducer is present.

10. Were your transformations successful? How did you know? Provide evidence and reasoning to support your claim.

   If bacteria grow on plates 3 and 4 then these bacteria were transformed. Only cells which took up the plasmid can survive when ampicillin is present, so growth on 3 and 4 means success.

   a. If not, what might have happened to interfere with success?
      Bacteria might not have taken up the plasmid, temperatures or times could be inaccurate, if no glowing cells on 4 then contamination might have occurred. Should be noted that transformation is not a very efficient process, so not all groups will have success and those that do will likely have only a few cells (colonies).

   b. If not, how might you change your protocol to improve success?
      Answers will vary, but if students know they contaminated their plates, they could discuss aseptic technique. If the temperatures or times were inaccurate, they could talk about adjusting those. They might have other ideas as well.

11. Calculate bacterial efficiency using the formula provided.
    Answers will vary.

12. 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 used.*
In 2000 the French artist, Eduardo Kac, partnered with a scientific team from France’s National Institute of Agronomic Research in Avignon resulted in the first glow-in-the-dark pet. Alba the GFP (green fluorescent protein) Bunny looked albino during the day, but under black lights, she glowed fluorescent green thanks to a gene from the Aequorea victoria jellyfish.

Transgenic organisms have foreign DNA in their cells. Alba became the world’s first transgenic bunny, developed as transgenic art. Kac intended to bring Alba home to live with him and his family as a pet in the family’s home in Chicago, but animal rights activists created such a protest that the scientists were hesitant to release Alba to go to her new home. Another concern voiced at Alba’s birth was the use of these technologies and research for “frivolous” activities like transgenic art.

Not long after Alba made news, researchers introduced the “spider goat”. These goats secrete spider silk in their milk. The golden orb-weaver spider provided the gene for the spider silk, and technological advancements permitted researchers to insert it in the goats where it is only active in the udder during milk production. After milking, scientists extract the silk proteins from the milk and use it to make highly specialized products including suture materials, wound dressings, and ropes. Here’s a link to a NSF video introducing the spider goats and the products developed from their spider silk: https://www.youtube.com/watch?v=ktgACq4zcAU

How does a rabbit get a jellyfish gene or a goat a spider gene? Essentially, a researcher extracts the gene from the organism which has the desired gene. In Alba’s case, researchers extracted DNA from the A. victoria jellyfish. They then used a process called RT-PCR with the appropriate primer to target the GFP phosphorescing gene. PCR uses a series of heating and cooling cycles to many copies of the selected gene, but eukaryotic genes consist of exons (“good” genetic material) interrupted by introns (“interrupting” genetic material). To ensure the foreign DNA is translated correctly by the host organism, scientists actually use the RNA coding for the protein as a template to make DNA. The enzyme which does this, reverse transcriptase, came from retroviruses. So, RT-PCR is a two-part process where first reverse transcriptase produces an intron-free form of the gene, then PCR makes many copies of the gene. The resulting DNA product is called cDNA. Next, the cDNA was inserted into a vector to deliver it to the host cell. The host cell, in this case, the fertilized rabbit egg which would grow into Alba the Bunny, now has new genetic material and can express a new trait, that of phosphorescing.
Bacterial Transformation

Introduction

But why do this? Researchers are looking for ways to insert new genes into existing organisms to replace genes that don’t work, to have a reliable source of necessary proteins and hormones, and to manufacture products which might cure cancers, AIDS, or other illnesses. In 2013, researchers created a vector with a possible cure for feline AIDS with the phosphorescing gene. If the cell successfully took up the vector, then both genes would be present and the cell would glow under black lights or UV lights. Bacteria are common hosts for taking up a target gene and producing the product. They are inexpensive to grow, some relatively readily take up foreign DNA vectors, and they read cDNA to produce the same product as the original eukaryotic donor.

There are some notable differences between developing transgenic multicellular animals and unicellular prokaryotic organisms. For the development of Alba and the spider goats, researchers carefully inserted the new genetic sequences into a fertilized egg, then implanted the egg into the mother. The resulting bunny or goat had the new genetic material in all of its cells. Bacteria naturally undergo horizontal gene transfer, which is when cells acquire new genetic material after their normal genome is intact and the cell is fully formed. The goat equivalent would be to give an adult goat new genetic material in all of its cells. Bacterial horizontal gene transfer occurs in one of three ways: conjugation, where an plasmid-containing bacterium forms a bridge and passes a copy of the replicated plasmid to a new cell which previously lacked a plasmid; transduction, where a bacteriophage (virus) accidentally packed host cell DNA in the viral capsule and subsequently the bacteriophage injected the cell’s DNA into the new host; and, transformation, where a competent cell takes up “naked” DNA found in the medium or substrate. Bacterial transformation is readily accomplished in a lab setting, and is relatively reliable.
Bacterial Transformation

Introduction

Student Worksheets: Transformation

Introduction:

1. The glowing green rabbit, Alba the GFP Bunny, was the first transgenic, glowing pet. Would you be interested in having a glowing pet cat or dog? What about a pet fish that doesn’t usually glow green, but we could genetically engineer to glow green? Why or why not?

2. After viewing the video found here (https://www.youtube.com/watch?v=ktgACq4zcAU), what do you think about goats that can secrete spider silk in their milk?

3. What do the spider goats have in common with Alba? How do you think this relates to bacterial transformation?
Bacterial Transformation

Exploration Chart:

Before you can delve into this lab, you must understand some technologies and concepts. These are listed below. As you study or explore, jot your notes down about each of these concepts.

<table>
<thead>
<tr>
<th>Explore question</th>
<th>Notes</th>
<th>Your questions?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. What is growth medium and how is it different from selective medium?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. What are aseptic techniques? Why are they important?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. What are plasmids? How are they used as vectors?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. What are marker genes and when are they used?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. What is bacterial resistance to antibiotics? Will a resistant bacteria grow when exposed to the antibiotic?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. What is bacterial transformation? What is bacterial competence? What is PCR?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. What are restriction enzymes? How do you think they would be used in developing plasmid vectors?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Congratulations on your new assignment!

The biotech company called BTSilk wants to break into the silk manufacturing field by using bacteria to produce spider silk. Spider goats have been very effective, but raising goats is expensive and time consuming. Additionally, the spider silk is only produced when the goats are milking. In contrast, bacteria could potentially produce more silk, faster, and cheaper! BTSilk researchers have been working on a way to insert the gene into the bacterium Escherichia coli and verify that it was successfully done. The current research team is developing a plasmid containing the spider silk gene, the A. victoria phosphorescing gene (GFP), and an ampicillin resistant (amp\(_R\)) gene. GFP is an inducible gene, which means that it is unavailable for transcription unless conditions turn the gene “on”. The GFP gene becomes available for transcription in the presence of the molecule IPTG. Many plasmids, such as the one being developed by the research team, are designed for transformation to include the amp\(_R\) gene. Bacterial cells with the amp\(_R\) gene can survive in the presence of the antibiotic, ampicillin, while untransformed cells cannot. Cultures are therefore plated on medium containing ampicillin in order to isolate the transformed cells.

The plasmid development team believes they have the first phase of the plasmid completed. This prototype plasmid contains the GFP gene and the ampicillin resistance gene. While that team continues with plasmid development by inserting the spider silk gene, our lab in BTSilk has been tasked with the successfully transforming E. coli with the two-gene prototype plasmid and confirming that transformation was successful.

You have access to the plasmid which contains the amp\(_R\) gene, GFP gene, and BTSilk gene on it and to a variety of growth medium to culture the bacterial cells. The growth media you can use are growth medium with ampicillin, growth medium without ampicillin, and growth medium with ampicillin and IPTG. You’ll be following the actual transformation lab procedures detailed by colleagues involved in other transformation projects. Your job is to develop a lab protocol to test if the bacteria successfully took up the plasmid.

You actually have two jobs, though. First, it seems that the research assistant they just hired and assigned to you is brand new to working with bacteria and biotechnology. Brad doesn’t really understand how bacteria take up the DNA into their cells. So, you need to teach him about bacteria transformation, plasmid vectors, and marker genes. Because you’re working with bacteria it would be helpful to also be sure Brad understands how to use aseptic techniques in the lab. Finally, it would be a good idea to review the idea of bacterial resistance since your plasmid contains the gene which makes E. coli resistant to the antibiotic ampicillin. Your second job is to develop a lab protocol to test if the bacteria successfully took up the plasmid.
You, the Teacher... teaching your assistant
Your first task is to explain the concepts to your lab assistant, Brad. He tends to be a visual learner, so sketch each concept below for him, using brief captions to highlight important aspects of each.

a. Bacterial transformation

b. Plasmid vectors

c. Marker genes

d. Aseptic techniques
Bacterial Transformation

Planning Your Investigation

Now that Brad and you have reviewed the concepts and techniques available to you, you’re ready to plot out the general steps of the procedure. You will follow the accepted protocols of transformation, but you need to understand the question you’re answering and how you’ll answer it.

1. What research question are you trying to answer? Why?

2. You have four plates available to you in this investigation (one with no ampicillin and no IPTG, two with ampicillin but not IPTG, and one with ampicillin and IPTG). You also have access to *E. coli* (-) and can also expose *E. coli* to the plasmid in a transformation process (+). What will you plate on each plate, + or -? Why

3. What plates are your controls for this experiment? What are they controlling for?

4. What are you predicted results?
Bacterial Transformation

Streak Plate Challenge

The task: Quadrant Streak Plating
You will use Jell-O plates, toothpicks, and a substrate to practice aseptic techniques and completing a Quadrant Streak Plating. In the real lab, you’ll use agar and sterile transfer loops instead of Jell-O and toothpicks.

Remember to
• Select a new toothpick for each quadrant of your plate.
• Not to talk over your plates or to excessively expose them to contaminants.
• Not pierce the surface of your plate with your toothpick.

Step 1. Place a dime sized drop of vinaigrette 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.

Questions:
1. Why did you have to change toothpicks between quadrants?
2. What would have happened to your “bacteria” if you broke the surface of the agar?
3. How will this technique be used in the lab?
4. Why is it important to maintain sterile conditions as much as possible?
Bacterial Transformation

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\(\mu\)L. \(1\) liter = 1,000 ml = 1,000,000 \(\mu\)l

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:

<table>
<thead>
<tr>
<th>Amount to add to tube</th>
<th>Color</th>
<th>Record the setting as it appears in the window</th>
</tr>
</thead>
<tbody>
<tr>
<td>150(\mu)l</td>
<td>blue</td>
<td>150</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Amount to add to tube</th>
<th>Color</th>
<th>Record the setting as it appears in the window</th>
</tr>
</thead>
<tbody>
<tr>
<td>250(\mu)l</td>
<td>blue</td>
<td></td>
</tr>
<tr>
<td>125(\mu)l</td>
<td>yellow</td>
<td></td>
</tr>
<tr>
<td>370(\mu)l</td>
<td>blue</td>
<td></td>
</tr>
<tr>
<td>495(\mu)l</td>
<td>yellow</td>
<td></td>
</tr>
</tbody>
</table>
Bacterial Transformation

Micropipette Challenge

Questions:

1. How many microliters (µL) should you have in the test tube when you are done?
2. How many milliliters (mL) should you have in the test tube when you are done?
3. Should you change tips between colors? Why or why not?
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 500µl of cold CaCl₂ to the – tube only.

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 the –DNA tube. Twist, shake, and scrape the toothpicks in the CaCl₂ to get most of the cells off the toothpicks and into the solution in the tubes (for about 20 seconds).

4. In each tube, pipet up and down for 60 seconds to mix thoroughly. Place both tubes back on ice.

5. With a new tip, transfer 250 µl of the cell suspension in the -DNA to the +DNA tube.

6. To the bottom of the tube labeled "+" ONLY, add 10µl pFluoroGreen plasmid DNA (tube marked "p") with the small 10µl minipipette (Be sure to use a tip). Flick the capped tube to mix for about 20 seconds.

7. Incubate both tubes in ice for 10 minutes. While tubes are incubating, label four agar plates with your initials and date as such: (Label plates on the bottom agar side)

<table>
<thead>
<tr>
<th>Plate #</th>
<th>Marks on Plate</th>
<th>Label as...</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No stripe</td>
<td>-pDNA, -AMP</td>
</tr>
<tr>
<td>2</td>
<td>One of the single stripe plates</td>
<td>-pDNA, +AMP</td>
</tr>
<tr>
<td>3</td>
<td>Second of the single stripe plates</td>
<td>+pDNA, +AMP</td>
</tr>
<tr>
<td>4</td>
<td>Two stripes</td>
<td>+pDNA, +AMP, +IPTG</td>
</tr>
</tbody>
</table>

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 250µl of Luria-Bertani Recovery Broth (tube labeled "Rec") 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 10 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 250µl and **drop onto the top of the agar** (without touching the agar) of labeled plate #1 and another 250µl 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 250µl and **drop onto the top of the agar** of labeled plate #3 and another 250µl 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 (24-72 hours). Next day read results and calculate transformation efficiency.
Bacterial Transformation

Lab Flow Chart for Students

**E. coli source plate**

Add 500 µl of CaCl\textsubscript{2} then add 5 well isolated 1-1.5mm diameter colonies from Source Plate. Then transfer 250 µl to −pDNA and 250 µl to +pDNA.

**−pDNA**

Tubes contain 250µl CaCl\textsubscript{2} and *E. coli*

Incubate on ice for 10 min.

Incubate at 42°C for 90 s.

Incubate on ice for 2 min.

Add 250µl Recovery Broth.

Incubate for 10 m in at 37°C.

**−pDNA**

Plate 250 µl.

Plate 1: No plasmid. What will happen here?

Plate 2: Ampicillin, no plasmid. What will happen here?

**+pDNA**

Add 10µl pFlouroGreen plasmid DNA (contains Amp\textsuperscript{R} gene and GFP gene).

Plate 3: Ampicillin, with plasmid. What will happen here?

Plate 4: Plasmid with Ampicillin, IPTG. What will happen here?
Bacterial Transformation

Post Lab

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

2. What is a plasmid?

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

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

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

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

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

8. Why did the cells only glow when IPTG was present?
9. What were your experimental results? Sketch your results below, and describe what happened on each plate.

Plate 1:  
Plate 2:  
Plate 3:  
Plate 4:  

10. Were your transformations successful? How did you know? Provide evidence and reasoning to support your claim.

   a. If not, what might have happened to interfere with success?

   b. If not, how might you change your protocol to improve success?

11. Scientists are often interested in comparing efficiency. Put simply, efficiency is the amount successful divided by the total amount attempted. The formula is below for you to calculate the transformation efficiency.

\[
\frac{\text{Number of Transformants}}{\mu g \text{ of DNA}} \times \frac{\text{Final volume at recovery (ml)}}{\text{vol of plates (ml)}} = \text{Number of transformants per } \mu g
\]

12. What is transformation efficiency? What would cause it to be lowered?
Bacterial Transformation

Web Resources

**Microbiology Lab Techniques** (Note, these videos use Bunsen burners and teach flaming the loop; the kit includes disposable loops)

*Aseptic Technique: (BioRadLifeScience, 6 min): [https://www.youtube.com/watch?v=bRadiLXkqoU](https://www.youtube.com/watch?v=bRadiLXkqoU)*

Aseptic Technique (BRCCVirginia, 2.22 min): [https://www.youtube.com/watch?v=tBmNitxvgyc](https://www.youtube.com/watch?v=tBmNitxvgyc)

Streak Plate (BioRadLifeScience, 3:40 min): [https://www.youtube.com/watch?v=0heifCiMbfY](https://www.youtube.com/watch?v=0heifCiMbfY)


**Transformation**

Overview of Transformation (New England Biolabs, 1:41 min): [https://www.youtube.com/watch?v=7Ul9RVYG5CM](https://www.youtube.com/watch?v=7Ul9RVYG5CM)

DNA Transformation History (DNA Learning Center, click-through animation): [https://www.dnalc.org/resources/animations/transformation1.html](https://www.dnalc.org/resources/animations/transformation1.html)

*These resources provide excellent coverage of the information! They could potentially be assigned for students to view and use to complete the chart. The other, shorter resources are also very good, but their coverage of the material is a less thorough.*