INVESTIGATION 8

BIOTECHNOLOGY: BACTERIAL TRANSFORMATION*

How can we use genetic engineering techniques to manipulate heritable information?

BACKGROUND

Are genetically modified foods safe? There is ongoing debate about whether it is safe to eat fruit and vegetables that are genetically modified to contain toxins that ward off pests. For instance, biotechnologists have succeeded in inserting a gene (Bt) from the bacterium *Bacillus thuringiensis* into the corn genome. When expressed, the Bt gene produces a toxin that kills caterpillars and controls earworms that damage corn — but is the corn safe for human consumption?

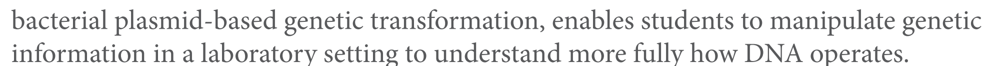
Genetic information passed from parent to offspring via DNA provides for continuity of life. In order for information in DNA to direct cellular activities, it must be transcribed into RNA. Some of the RNAs are used immediately for ribosomes or to control other cellular processes. Other RNAs are translated into proteins that have important roles in determining metabolism and development, i.e., cellular activities and phenotypes (traits). When the DNA of a cell changes, the RNAs and proteins they produce often change, which in turn changes how that cell functions.

DNA inside a cell can change several ways. It can be mutated, either spontaneously or after the DNA replication machinery makes an error. Biotechnologists may cause an intentional mutation in a cell's own DNA as a way to change how that cell behaves. The most powerful tool biotechnologists have, though, is the ability to transfer DNA from one organism into another and make it function there. With this tool, they can make cells produce novel protein products that the cells did not make previously.

Stimulate student interest in the investigation by describing applications of genetic engineering. For example, insulin that people take to control their blood sugar levels is often made from engineered bacteria. Some vaccines, as well as enzymes used for manufacturing denim jeans, are also made using engineered cells. In the near future, engineered bacteria and other cells being developed could help clean up spilled oil or chemicals, produce fuel for cars and trucks, and even store excess carbon dioxide to help slow global climate change. Ask students to think of other possible applications of biotechnology. Remind students, however, that human manipulation of DNA raises several ethical, social, and medical issues, such as the safety of genetically modified foods.

The techniques required for gene transfer in higher plants and animals are complex, costly, and difficult even in the research laboratory. However, the techniques of gene transfer in *Escherichia coli* (*E. coli*) bacteria are simple and appropriate for the teaching and learning laboratory (Rapoza and Kreuzer 2004). One common technology,

* Transitioned from the *AP Biology Lab Manual* (2001)



bacterial plasmid-based genetic transformation, enables students to manipulate genetic information in a laboratory setting to understand more fully how DNA operates.

In this investigation, students will first acquire the tools to transform *E. coli* bacteria to express new genetic information using a plasmid system and apply mathematical routines to determine transformation efficiency. (Competent bacterial cells are able to take up exogenous genetic material and are capable of being transformed, and the procedure provided is designed to promote competence. An excellent preparation of competent cells will yield approximately 10^8 transformed colonies per microgram of plasmid; a poor preparation will yield approximately 10^4 or less transformed colonies per microgram of plasmid.) Students then have the opportunity to design and conduct individual experiments to explore transformation in more depth. For example, students can select a factor *of their choice* and explore its ability to induce mutations with observable phenotypes, or they can investigate if bacteria take up more plasmid in some environmental conditions and less in others. They also can explore answers to questions about plasmids and transformation that might have been raised during the initial investigation.

This investigation also provides students with the opportunity to review, connect, and apply concepts that they have studied previously, including cell structure of bacteria; structure and function of cell membranes, enzymes, and DNA and RNA; transcription and translation; the operon model of the regulation of gene expression; evolution and natural selection; and interactions between organisms and their environment.

Interspersed within each investigation are supplemental activities designed to keep students on track and to provide opportunities for them to take a deeper dive into the concepts. You may assign these activities for homework or ask students to do them as they work through the investigation.

PREPARATION

Materials and Equipment

Supplies for plasmid transformation systems may be purchased in kits from commercial vendors or purchased individually, depending on your current inventory. A partial list of suppliers is provided in the Supplemental Resources section. At minimum, plasmids should contain the gene for ampicillin resistance (pAMP), as experimental procedures typically use ampicillin to select transformed cells. In addition, plasmids with colored marker genes like beta-GAL and fluorescence markers like green fluorescent protein (GFP) and its cousins make it possible to measure gene expression directly, to follow cell populations as they grow or move, and to find cells that have taken up a second plasmid that we cannot see easily. Thus, you have freedom in choosing a plasmid transformation system.

The following materials are included in a typical eight-station ampicillin-resistant plasmid system. The list will vary depending on the system used. Materials and supplies needed for each student workstation are provided in the student version of this investigation. Students are encouraged to set up their own workstations. Note that you might need additional materials such as agar plates and nutrient agar for the student inquiry investigations.

Materials Included in Eight-Station Kit

- *E. coli* (1 vial or slant)
- Plasmid (pAMP), hydrated (20 μg)
- Ampicillin, lyophilized (30 μg)
- Transformation solution (50mM $CaCl_2$, pH 6), sterile (15 mL)
- LB nutrient agar powder, sterile (to make 500 mL) (20 g) or prepared agar
- LB nutrient broth, sterile (10 mL)
- Pipettes, sterile (50)
- Inoculation loops, sterile (10 µL, packs of 10 loops)
- Petri dishes, sterile, 60 mm (packs of 20)
- Multicolor 2.0 mL microcentrifuge tubes (60)

Accessories Required but Not Included in Kit

- Microcentrifuge tube holders
- Clock or watch to time 50 seconds
- Microwave oven/water bath
- Thermometer that reads 42°C
- 1 L flask
- 500 mL graduated cylinder
- Distilled water
- Crushed ice and containers
- 10% solution household bleach
- Permanent marker pens
- Masking tape
- Biohazardous waste disposal bags or plastic trash bags

Optional Accessories

- Micropipettes, adjustable volume, 2–20 µL (and pipette tips)
- Parafilm laboratory sealing film
- 37°C incubator oven*

*If an incubator is not available, try using an electric blanket or construct a homemade incubator with a cardboard box and a low voltage lightbulb inside. Otherwise, incubate agar plates with bacteria 48–72 hours at ambient room temperature. Another option is to use a seedling heating mat with thermostat available from garden supply catalogs; the advantage is that they are sealed against water damage and can be repurposed for other lab activities, including their original purpose, germinating seeds.

Student Workstation

- *E. coli* starter plate
- Poured agar plates 2 LB and 2 LB/amp
- Transformation solution ($CaCl_2$, pH 6.1) kept *ice cold*
- LB nutrient broth
- Sterile inoculation loops
- 100–1000 µL sterile bulb pipettes
- 1–10 µL micropipettes with sterile tips
- Microcentrifuge tubes
- Microcentrifuge tube holder/float
- Container full of crushed ice
- Marking pen

Common Workstation

- Plasmid (pAMP), hydrated (20 μg)
- 42°C water bath and thermometer
- 37°C incubator or equivalent
- 20 μL adjustable volume micropipettes and tips (optional)
- 10% household bleach
- Biohazardous waste disposal bags
- Masking or lab tape

PREPARATION

Advance Preparation Quick Guide for Teachers

Step	Objective	Time Required	When
Step 1	Prepare agar plates.	1 hr.	3–7 days prior
Step 2	Rehydrate *E. coli.* Streak starter plates. Rehydrate plasmid DNA, if necessary.	2 min. 15 min. 2 min.	24–36 hours prior
Step 3	Aliquot solutions. Set up workstations.	10 min.	Immediately prior

Advance Preparation for Step 1: 3–7 Days Before the Transformation

1. Prepare nutrient agar (autoclave-free).
The agar plates should be prepared at least three days before the investigation(s) are performed. Plates should be left out at room temperature for two days and then refrigerated until use. (Two days at room temperature allows the agar to cure, or dry, sufficiently to readily take up the liquid transformation solution.) **Hint**: If time is short, incubate the plates at 37°C overnight. This will dry them out as well, but it shortens their shelf life.

Refrigerated plates are good for up to 30 days.

To prepare the agar, add 500 mL of distilled water to a one liter or larger Erlenmeyer flask. Add the entire content of the LB nutrient agar packet. Swirl the flask to dissolve the agar and heat to boiling in a microwave *or* water bath *or* by using a hot plate with stir bar. Heat and swirl until all the agar is dissolved. **CAUTION: Be careful to allow the flask to cool a little before swirling so that the hot medium does not boil over onto your hand.**

When all the agar is dissolved, allow the LB nutrient agar to cool so that the outside of the flask is just comfortable to hold (approximately 50°C.). While the agar is cooling, you can label the plates and prepare the ampicillin as outlined below in

Step 3. **CAUTION: Do not let the agar cool so much that is begins to solidify. Keeping the flask with liquid agar in a water bath at 45–50°C can help prevent the agar from cooling too quickly.**

Preprepared nutrient agar also can be purchased. However, it will have to be melted before it can be poured into plates. To do this, the plastic bottles containing solid agar can be microwaved at a low temperature (such as using the "poultry defrost" option) for several minutes. Be sure to loosen the cap slightly to expel any air. At high microwave temperatures, the agar can boil over. Another option is to place the bottles in a hot water bath; however, this will take up to 45 minutes or so to melt the agar. **CAUTION: Be careful when handling the bottle(s). They will get hot!**

2. Prepare ampicillin.
Ampicillin is either shipped dry in a small vial or already hydrated. If shipped dry, you need to hydrate the ampicillin. Do this by adding 3 mL of transformation solution to the vial to rehydrate the antibiotic. Use a sterile pipette.

   Note: Excessive heat (≥60°C) will destroy ampicillin. With this in mind, here's the tricky part: the nutrient agar solidifies at 27°C, so you must be careful to monitor the cooling of the agar and then pour the plates from start to finish without interruption. Keeping the flask with liquid agar in a water bath set to 45–50°C can help prevent the agar from cooling too quickly. Before adding ampicillin to the flask of agar, make sure you can hold the flask in your bare hand (approximately 50°C). If your hand tolerates the temperature of the flask, so will the antibiotic!

3. Label plates.
While the agar is cooling, reduce preparation time by labeling the plates. Label with a permanent marker on the *bottom* of each plate close to the edge. For each class using an eight-station kit, label 16 plates *LB* and 16 plates *LB/amp*.

4. Pour nutrient agar plates.
<u>First, pour LB nutrient agar into the 16 plates that are labeled LB.</u> If you do not do this and add ampicillin to the flask with agar, you will not be able to make control plates containing just nutrient agar.

   Fill each plate to about one-third to one-half (approximately 12 mL) with agar and replace the lid. You may want to stack the plates and let them cool in the stacked configuration.

   Second, add the hydrated ampicillin to the remaining LB nutrient agar. Swirl briefly to mix. Pour into the 16 plates labeled *LB/amp* using the same technique.

   Plates should set within 30 minutes.

5. Store the plates.
After the plates have cured for two days at room temperature, they may be either used or stored by stacking them in a plastic sleeve bag slipped back down over them. The stack is then inverted, the bag taped closed, and the plates stored *upside down* at 4°C until used. (The plates are inverted to prevent condensation on the lid, which may drip onto the agar.)

Advance Preparation for Step 2:
24–36 Hours Before the Transformation

1. Rehydrate bacteria.
Some *E. coli* cultures come prepared (or can be purchased) in a slant and will not have to be rehydrated. For bacteria that must be rehydrated, use a sterile pipette to add 250 µL of transformation solution directly to the vial. Recap the vial and allow the cell suspension to stand at room temperature for 5 minutes. Then shake the mix before streaking on the LB starter plates. Store the rehydrated bacteria in the refrigerator until used (within 24 hours for best results and no longer than three days).

2. Streak starter plates.
Starter plates are needed to produce bacterial colonies of *E. coli* on agar plates. Each lab team will need its own starter plate as a source of cells for transformation. LB plates should be streaked for single colonies and incubated at 37°C for 24–26 hours before the transformation investigation begins.

Using *E. coli* and LB agar plates, streak one starter plate for each of your student lab groups in order to generate single colonies from a concentrated suspension of bacteria. A small amount of the bacterial suspension goes a long way. Under favorable conditions, one cell multiples to become millions of genetically identical cells in just 24 hours. There are millions of individual bacteria in a single millimeter of a bacterial colony.

a. Insert a sterile inoculation loop straight into the vial of rehydrated bacterial culture. Remove the loop and streak the plates, as illustrated in Figure 1. Streaking takes place sequentially in four quadrants. The first streak spreads out the cells. Go back and forth with the loop about a dozen times in each of the small areas shown. In subsequent quadrants, the cells become more and more dilute, thus increasing the likelihood of producing single colonies.

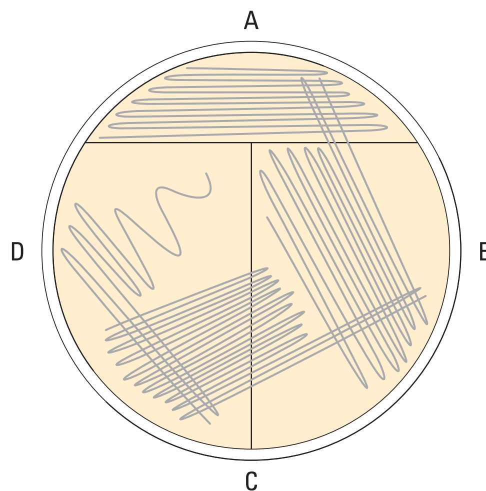


Figure 1. Streaking Starter Plates with *E. coli*

b. For subsequent streaks, use as much of the surface area of the plate as possible. After the initial streak, rotate the plate approximately 45 degrees and start the second streak. **Do not dip into the rehydrated bacteria a second time!** Go into the previous streak about two times and then back and forth as shown for a total of about 10 times.

c. Rotate the plate again and repeat streaking.

d. Rotate the plate for the final time and make the final streak. Repeat steps a–c with the remaining LB plates for each student workstation. Although you can use the same inoculation loop for all starter plates, it is recommended that you use a new, sterile loop for each plate if you have enough. When you are finished with each plate, cover it immediately to avoid contamination.

e. Place the plates upside down inside the incubator overnight at 37°C or at room temperature for 2–3 days if an incubator is unavailable. Use for transformation within 24–36 hours because bacteria must be actively growing to achieve high transformation efficiency. (Remember, bacterial growth is exponential.) **Do not refrigerate before use. This will slow bacterial growth.**

f. *E. coli* forms off-white colonies that are uniformly circular with smooth edges. Avoid using plates with contaminant colonies such as mold.

3. Prepare plasmid.
The quantity of DNA is so small that the vial may appear empty. Tap the vial or spin it in a microcentrifuge to ensure that the DNA is not sticking to the cap. If the plasmid is not hydrated, refer to instructions that come with the sample. Store the vial of hydrated DNA in a refrigerator. Rehydrated plasmid should be used within 24 hours.

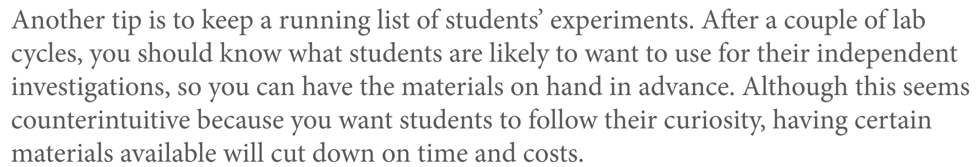
Advance Preparation for Step 3:
Immediately Before Transformation Investigation

1. Aliquot solutions.
Each student workstation will need 1 mL of transformation solution and 1 mL of LB nutrient broth. You might have to aliquot these solutions into separate color-coded 2 mL microtubes. If the LB nutrient broth is aliquoted one day prior to the lab, it should be refrigerated. Make sure to label the tubes with permanent marker.

2. Set up student and common workstations.

See the list of materials to be supplied at each workstation.

Some leftover materials can be combined and stored for future use. For example, extra salt solutions ($CaCl_2$ in the case of this lab), solutions of DNA, and buffers can be stored in a refrigerator freezer. Where possible, standardize materials for use in multiple labs. This allows you to keep fewer items but larger quantities, giving some leeway for making extra as needed. However, if the plasmid goes through multiple freeze-thaw cycles in a frost-free freezer, the DNA in the plasmid can degrade. It is recommended that you check the shelf life of materials with the commercial vendor.



Another tip is to keep a running list of students' experiments. After a couple of lab cycles, you should know what students are likely to want to use for their independent investigations, so you can have the materials on hand in advance. Although this seems counterintuitive because you want students to follow their curiosity, having certain materials available will cut down on time and costs.

Timing and Length of Lab

Consider this investigation to be a learning module, not a typical teacher-directed "cookbook lab." The investigation provides students myriad opportunities to develop biotech laboratory skills; as they work through the background information and answer questions, they are exploring concepts more deeply.

Day 1

Allow approximately one class period (45–60 minutes) to preview the lab and let students work through the background information and prelab questions interspersed in the Getting Started section of the investigation. Alternatively, you can assign this material for homework.

Day 2

Allow one class period (45–60 minutes) for students to transform cells and spread plates.

Day 3

It may take longer than 24 hours for students to be able to observe transformed cells. You will have to monitor the incubation conditions and bacterial growth/transformation.

Allow approximately one class period (45–60 minutes) for students to observe transformants and controls, analyze and interpret results, and calculate transformation efficiency. One option is to assign postlab assessment questions for homework, although student collaboration is recommended.

Day 4+

Allow one or two class periods for students to design and conduct an independent investigation. Time will be needed for post experimental observation and data analysis. In addition, students should be given time to present their results to peers.

If students have performed colony transformation experiments before, they may review Procedure and proceed to the independent investigation(s). However, it is recommended that all students read the background information and work through Getting Started.

Safety and Houskeeping

- Students must apply basic sterile technique when working with and culturing bacteria. Although the strain of *E. coli* and the DNA plasmid used in this investigation are not pathogenic, their handling requires appropriate microbiological procedures.
- Remind students to wash their hands when entering or leaving the lab area. They should not eat, drink, apply cosmetics, or use personal electronic devices in the work area.

- Work surfaces should be decontaminated with a 10% household bleach solution at least once a day and after any spill of viable material.
- All contaminated liquid or solid wastes are decontaminated before disposal. This can be done in an autoclave (20 minutes at 121°C) or in a 10% bleach solution (soaked for 20 minutes).
- Ampicillin may cause allergic reactions or irritation to the eyes, respiratory system, and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing. Since ampicillin is a member of the penicillin family of antibiotics, students (or teachers) with allergies to penicillin or to any other member of the penicillin family of antibiotics should avoid contact with ampicillin.

ALIGNMENT TO THE AP BIOLOGY CURRICULUM FRAMEWORK

This investigation can be conducted during the study of concepts pertaining to the storage, retrieval, and transmission of genetic information (big idea 3), with a connection to evolution and natural selection (big idea 1). As always, it is important to make connections between big ideas and enduring understandings, regardless of where in the curriculum the lab is taught. The concepts align with the enduring understandings and learning objectives from the AP Biology Curriculum Framework, as indicated below.

Enduring Understandings

- 1A2: Natural selection acts on phenotypic variations in populations.
- 1C3: Populations of organisms continue to evolve.
- 3A1: DNA, and in some cases RNA, is the primary source of heritable information.
- 3B1: Gene regulation results in differential gene expression.
- 3C1: Changes in genotype can result in changes in phenotype.
- 3C2: Biological systems have multiple processes that increase genetic variation.

Learning Objectives

- The student is able to connect evolutionary changes in a population over time to a change in the environment (1A2 & SP 7.1).
- The student is able to evaluate given data sets that illustrate evolution as an ongoing process (1C3 & SP 5.3).
- The student can justify the claim that humans can manipulate heritable information by identifying at least two commonly used technologies (3A1 & SP 6.4).
- The student can predict how a change in a specific DNA or RNA sequence can result in changes in gene expression (3A1 & SP 6.4).
- The student is able to pose questions about ethical, social, or medical issues surrounding human genetic disorders [an application of genetic engineering] (3A3 & SP 3.1).

- The student can use representations to describe how gene regulation influences cell products and function (3B1 & SP 1.4).
- The student is able to predict how a change in genotype, when expressed as a phenotype, provides a variation that can be subject to natural selection (3C1 & SP 6.4, SP 7.2).
- The student is able to construct an explanation of the multiple processes that increase variation within a population (3C2 & SP 6.2).

ARE STUDENTS READY TO COMPLETE A SUCCESSFUL INQUIRY-BASED, STUDENT-DIRECTED INVESTIGATION?

This investigation reinforces the following skills:

- Using pipettes (plastic bulb-type or other volumetric measuring devices)
- Measuring temperature (°C)
- Applying metric system
- Applying quantitative skills

Skills Development

Students will develop the following skills:

- Using sterile technique
- Disposing properly of materials and solutions that come in contact with bacteria
- Transferring bacterial colonies from agar plates to microtubes
- Transforming bacterial cells with plasmid DNA
- Delivering transformed cultures to agar plates
- Applying mathematics to quantify transformation efficiency

Potential Challenges

With any type of microbiology technique, including working with and culturing bacteria, it is important not to introduce contaminating microorganisms into the experiment. When students are working with the inoculation loops, pipettes, and agar plates, you should stress that the round circle at the end of the loop, the tip of the pipette, and the surface of the agar plate should not be touched or placed onto contaminating surfaces, such as bench tops. While some contamination will likely not ruin the investigation, students should practice sterile technique. You might consider having students do a dry run of the procedures to practice sterile technique before working with bacteria.

Best results are obtained if starter plates are fresh (24–36 hr growth), with bacterial colonies measuring about 1–1.5 mm in diameter. **Refrigeration of cultured plates will significantly lower transformation efficiency.** The optimum temperature for growing *E. coli* is 37°C.

Students often have difficulty reading the graduations (markings) on the plastic pipette. (If students are using automatic pipetting devices, you should provide instruction on how to load and dispel minute samples.) The 100 μL, 250 μL, and 1 mL marks will be used as units of measurement. (You might need to remind students that “μl” and “μL” are alternative symbols for the same volumetric measurement.)

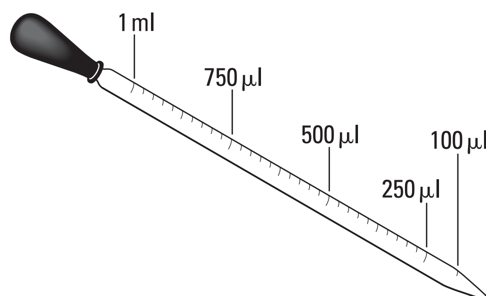


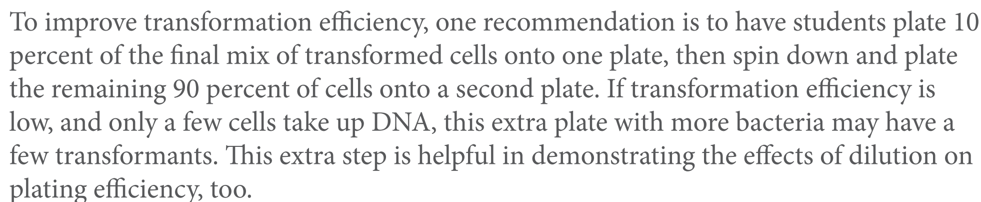
Figure 2. Measuring Volume with a Pipette

Another challenge area for students is transferring bacterial colonies from agar plates to microtubes. Students are tempted to scrape more bacterial colonies off the starter plate than are necessary. A single colony that is 1 mm in diameter contains millions of bacterial cells. To increase transformation efficiency, students should select 2–4 “fat” colonies that are 1–1.5 mm in diameter. Students should select *individual* colonies rather than a swab of bacteria from the dense portion of the plate. Remind students that “less is more.”

The transfer of plasmid DNA from its stock tube to the transformation suspension is crucial. Unless you are confident that students can make this transfer successfully, consider adding the plasmid to the transformation suspensions yourself. Look carefully at the loop to see if there is a film of plasmid solution across the ring, similar to seeing a soapy film across a ring for blowing soap bubbles. Do not add more plasmid than is recommended in the procedure — unless students want to do a little independent investigating about the relationship between the amount of plasmid and the efficiency of transformation of *E. coli*. Over-saturating the cell solution with DNA decreases the transformation efficiency.

Impatient students often skip steps in the procedure or fail to read instructions carefully. In this investigation, they must adhere to the instructions unless they are conducting an independent experiment on the effect(s) of varying the transformation procedure. The “heat shock” procedure increases the bacterial uptake of foreign DNA, and the rapid temperature change and the duration of the heat shock are critical. For optimal results, the tubes containing the cell suspensions must be taken directly from ice, placed into the water bath at 42°C (have a student monitor the temperature) for 50 seconds, and returned immediately to the ice. The absence of the heat shock will result in a 10-fold decrease in transformants; 90 seconds of heat shock will give about half as many transformants as will 50 seconds of heat shock.

About one percent of bacterial cells can be transformed under laboratory conditions. Factors affecting transformation efficiency include the size of the bacterial colony used, the amount of plasmid used, technique, and incubation times. Some *E. coli* strains are more susceptible to transformation than others due to the composition of the cell wall.



To improve transformation efficiency, one recommendation is to have students plate 10 percent of the final mix of transformed cells onto one plate, then spin down and plate the remaining 90 percent of cells onto a second plate. If transformation efficiency is low, and only a few cells take up DNA, this extra plate with more bacteria may have a few transformants. This extra step is helpful in demonstrating the effects of dilution on plating efficiency, too.

Despite all efforts, sometimes transformation is unsuccessful. If this happens, one suggestion is to use a random number generator to come up with imaginary colony counts for the purpose of working through the transformation efficiency calculations.

The last area of challenge for students is spreading transformants and controls to the agar plates. Delivering an excess of transformed culture to the plates is counterproductive because the plates may not absorb the additional liquid and spreading will be uneven. Transferring bacterial suspensions from the microtubes is tricky; the bacteria will settle at the bottom, but students can hold the top of a closed tube between the index finger and thumb of one hand and gently flick the bottom of the tube with the index finger of the other hand. (You should demonstrate this technique.) After transferring bacteria to an agar plate, the students should cover the plates with lids immediately after pipetting in the transformation culture and spreading the cells. They should spread the suspension evenly around the surface of the agar by quickly skating the flat surface of a new sterile loop back and forth across the plate surface. An alternative method is to use small sterile glass beads to spread the suspensions by gently rocking the beads across the surface of the agar.

Remind students to store their plates in the incubator *upside down* to prevent any condensation from dripping onto the agar.

THE INVESTIGATIONS

Getting Started: Prelab Assessment

The Getting Started section of the investigation contains two sets of questions for preparing students and encourages them to ask their own questions about transformation and explore answers. You may assign the background material and prelab questions for homework; as a think, pair/group, share activity, in which pairs or small groups of students brainstorm ideas and then share them with other groups; or as a whole-class discussion.

Before beginning this investigation, students should have a solid understanding of the structure and function of DNA. You might want to take them through the discovery of transformation in 1928 by Frederick Griffith. Griffith was studying the bacterium *Streptococcus pneumoniae,* which causes pneumonia, the leading cause of death in the Western Hemisphere at the time of his research. Ask students to investigate Griffith's experiments and their significance in identifying the "transforming principle," later identified as DNA. Students can present their findings in the form of a poster or diagram with annotation.

As students work through the introductory material, several questions will emerge about transformation and the use of plasmids to transfer genetic information. One strategy for prelab assessment is to join student groups, encourage them to ask questions beyond those listed in the investigation, listen to their answers, and then ask more probing questions.

Using Plasmids with Colored Marker Genes

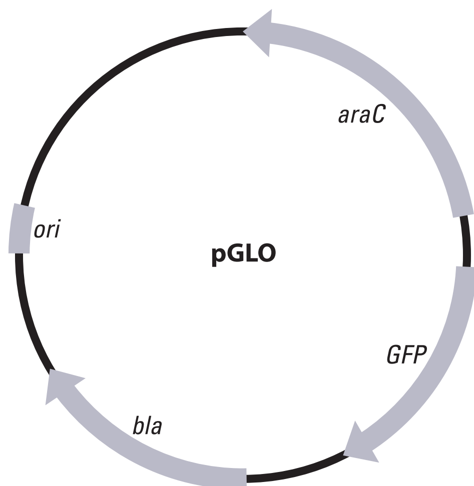
If you choose a plasmid system that includes colored marker genes like beta-GAL and fluorescent markers like GFP and its cousins, you might want to consider taking students through a more in-depth prelab activity. This activity is also appropriate for students who are familiar with transformation experiments performed in a previous biology class. Using pGLO plasmid to transform bacteria, students observe the expression of green fluorescent protein (GFP). Students can work through the activity for homework or as a group.

Spark students' interest in GFP by having them do a little online investigation about jellyfish that glow in the dark. What makes bioluminescent jellyfish, *Aequorea victoria*, easy to spot in deep, dark water is the expression of green fluorescent protein (GFP). The GFP gene can be transferred into bacteria, and if transformation is successful, the bacteria will express their newly acquired jellyfish gene and glow brilliant green under ultraviolet (UV) light.

Ask students to discuss the following question:

Suppose you have a plasmid that contains both the gene for GFP (pGLO) and a gene for resistance to ampicillin (pAMP). How will you be able to tell if bacterial cells have been transformed using the plasmid containing genes for GFP and ampicillin resistance?

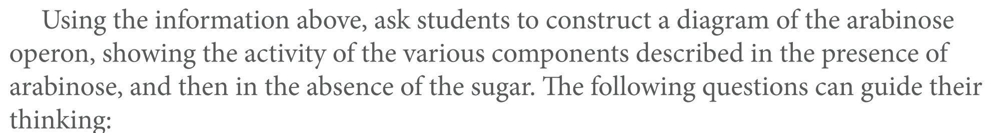
Take this a step further by having students examine the plasmid in Figure 3 and the corresponding caption.



GFP (the Aequorea victoria *jellyfish gene) codes for green fluorescent protein, and araC is the gene that codes for the protein that regulates transcription of GFP. Bla is the gene that codes for beta-lactamase, an enzyme that confers resistance to ampicillin by disabling ampicillin molecules. "Ori" is the plasmid's origin of replication, and the arrows indicate the direction of transcription and translation.*

Figure 3. pGLO Plasmid

In addition to genes for green fluorescent protein and resistance to ampicillin, the pGLO plasmid has a special gene regulation system that switches on GFP production if the sugar arabinose is present in the nutrient medium, and the bacteria glow when exposed to UV light. This system is an example of an inducible operon.



Using the information above, ask students to construct a diagram of the arabinose operon, showing the activity of the various components described in the presence of arabinose, and then in the absence of the sugar. The following questions can guide their thinking:

- What evidence will indicate whether your attempts at performing a genetic transformation are successful?
- What will agar plates containing arabinose look like if they contain transformed cells? Without arabinose?

Designing and Conducting Independent Investigations

There are several directions in which students can go with their own investigations.

1. Students can determine whether any satellite colonies have been transformed. Do not tell them this in advance, but the majority of satellite colonies form when transformed cells release beta-lactamase (the enzyme encoded by the plasmid that degrades ampicillin) into the surrounding medium. Nontransformed bacteria can then survive and grow.
2. Students can vary the transformation process by altering the amount of DNA, ratio of transformation solutions, time for heat shock, or growth stage of bacteria.
3. Students can investigate the effects of mutations on gene expression and whether mutations affect plasmids. However, you must make sure that any mutagens students choose to explore are safe. There are several postulated or proven mutagens that students likely could handle safely, including the following:
   - Dilute hydrogen peroxide
   - Caffeine
   - UV light source (The bacteria must be kept in the dark to prevent DNA repair, and students must wear UV goggles.)
   - Potassium nitrate (used in food preservation)
4. Can bacteria take up two different plasmids? This is an advanced investigation that requires two different plasmids. However, it can lead to very interesting outcomes because some pairs of plasmids are compatible, while others are not.
5. Does having this plasmid give the bacteria an advantage other than antibiotic resistance? Mix equal amounts of transformed bacteria with untransformed bacteria, and plate them together on one plate. Which colonies are bigger after 24 hours? Which colonies are more numerous? This investigation would tie nicely into labs on interspecific competition or natural selection.

Summative Assessment

Students observe the results they obtained and record their observations in their lab notebook. The Analyzing Results section of the lab in the Student Manual provides several questions for consideration, but encourage students to come up with some of their own questions.

The following are suggested guidelines to assess students' understanding of the concepts presented in the investigation, but you are encouraged to develop your own methods of postlab assessment. Some of the tasks can be assigned for homework following the completion of the investigation.

1. Have students record all their data, results, and conclusions in a lab notebook, formal paper, or mini-posters. Based on the students' product, do you think students have met the learning objectives of the investigation?
2. As you visited the different lab groups, were they able to work through the various activities interspersed throughout the investigation without difficulty? What additional questions did students raise? Did they have ideas for how they could explore answers to their questions?
3. Did students have sufficient mathematical skills to calculate transformation efficiency?
4. What technical challenges did students have using the equipment required for the investigation? Have students list their challenge areas and discuss solutions.

Where Can Students Go from Here?

The background to this investigation asks students to think about several applications of genetic transformation, including genetically modified food and possible ethical, social, or medical issues raised by the manipulation of DNA by biotechnology. Ask students to discuss why these issues are "issues." What questions are posed by genetic engineering? Students also can respond to the quote from Michael Crichton's novel and film *Jurassic Park:* "Just because science can do something doesn't mean that it should."

SUPPLEMENTAL RESOURCES

Background Information/Prelab Activities

http://biology.arizona.edu
The University of Arizona Biology Project is an online interactive resource for learning biology, with an extensive molecular biology/biotechnology module.

Curriculum Module (Professional Development), AP Biology: *From Gene to Protein—A Historical Perspective,* College Board, 2010.
This set of instructional strategies developed by AP Biology teachers takes students on an inquiry-based journey as they explore key discoveries that allowed scientists to identify DNA as *the* molecule of heredity and how it is able to store, retrieve, and transmit information necessary for living systems. Drawing their own conclusions, students explore the contributions of notable scientists, including Frederick Griffith, Hershey and Chase, Watson and Crick, and Meselson and Stahl. The instructional activities are examples of how teachers can engage students by accommodating their different learning styles, knowledge bases, and abilities and, at the same time, provide depth of content and skills.

http://dnalc.org. Dolan DNA Learning Center, Cold Spring Harbor.
This resource provides myriad interactive activities for students to prepare students for conducting investigations using biotechnology practices, including DNA Subway and iPlant Collaborative.

Griffith, AJ, *Natural plasmids of filamentous fungi,* Microbiol. Rev. 1995 December 59(4), **http://www.ncbi.nlm.nih.gov/pubmed/8531891**

Johnson, A. Daniel, *40 Inquiry Exercises for the College Biology Lab,* NSTA Press, Arlington, VA, 2009.
This information provides great insight into developing student-directed, inquiry-based laboratory investigations for advanced students, while also providing strategies on how teachers can adapt their more teacher-directed labs into opportunities for independent exploration. Unit 3 in the manual, "DNA Isolation and Analysis," provides exercises for more advanced students to use bioinformatics programs to study and manipulate DNA sequences.

http://phschool.com/science/biology_place
Developed by Pearson Education, this interactive and informative resource allows students to visualize and apply their understanding of biological concepts. Designed for AP Biology students, Lab Bench connects laboratory procedures to key concepts.

Procedural Resources

Bio-Rad Biotechnology Explorer™ pGLO Bacterial Transformation Kit, Catalog #166-003EDU, ***www.explorer.bio-rad.com***
This guided inquiry-based curriculum module developed by Bio-Rad Laboratories is a source from which this investigation can be modified. Using pGLO plasmid to transform bacteria, students observe the expression of green fluorescent protein.

Rapoza, M., and H. Kruezer, *Transformations: A Teacher's Manual*, publication from Carolina Biological Supply Company, Burlington, NC, 2004.
http://www.carolina.com
This resource, developed in cooperation with the Dolan DNA Learning Center of Cold Spring Harbor Laboratory, provides extensive background and procedural information for multiple transformation laboratory exercises. All of the plasmids described in the resource contain the gene for ampicillin resistance, and all of the experimental procedures use ampicillin to select transformed cells. Several of the plasmids contain an additional marker gene that causes the transformed cell to be colored, including pVIB, pGREEN, and pBLU.

Resources for Extensions of Investigation

Plasmid isolation and purification are fairly simple processes that students might want to try. Chemicals, bacterial strains, culture media, and other supplies can be purchased from several commercial companies, including Carolina Biological (***http://www.carolina.com***) and Bio-Rad (***http://explorer.bio-rad.com***). Students can isolate specific plasmids of your choice and use them to transform bacteria that do not naturally contain the plasmid(s). Using the skills and knowledge obtained from this investigation, students can design an experiment to investigate whether or not their transformation was successful.

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INVESTIGATION 8

BIOTECHNOLOGY: BACTERIAL TRANSFORMATION*

How can we use genetic engineering techniques to manipulate heritable information?

BACKGROUND

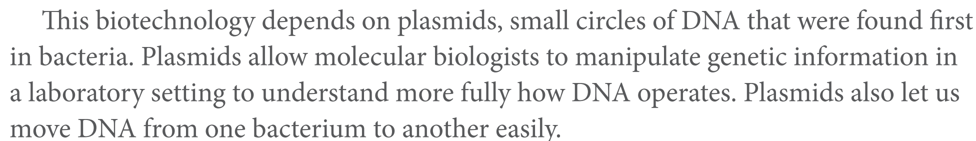
Are genetically modified foods safe? There is ongoing debate about whether it is safe to eat fruit and vegetables that are genetically modified to contain toxins that ward off pests. For instance, biotechnologists have succeeded in inserting a gene (Bt) from the bacterium *Bacillus thuringiensis* into the corn genome. When expressed, the Bt toxin kills caterpillars and controls earworms that damage corn, but is the corn safe for human consumption?

Genetic information passed from parent to offspring via DNA provides for continuity of life. In order for information in DNA to direct cellular activities, it must be transcribed into RNA. Some of the RNAs are used immediately for ribosomes or to control other cellular processes. Other RNAs are translated into proteins that have important roles in determining metabolism and development, i.e., cellular activities and phenotypes (traits). When the DNA of a cell changes, the RNAs and proteins they produce often change, which in turn changes how that cell functions.

DNA inside a cell can change several ways. It can be mutated, either spontaneously or after the DNA replication machinery makes an error. Biotechnologists may cause an intentional mutation in a cell's own DNA as a way to change how that cell behaves. The most powerful tool biotechnologists have, though, is the ability to transfer DNA from one organism to another and make it function there. With this tool, they can make cells produce novel protein products the cells did not make previously.

Examples of this powerful tool are all around us. Insulin that people take to control their blood sugar levels is often made from engineered bacteria. Some vaccines, as well as enzymes used for manufacturing denim jeans, are also made using engineered cells. In the near future, engineered bacteria and other cells being developed could help clean up spilled oil or chemicals, produce fuel for cars and trucks, and even store excess carbon dioxide to help slow global climate change. Can you think of other possible applications of genetic engineering? However, biotechnology and human manipulation of DNA raise several ethical, social, and medical issues, such as the safety of genetically modified foods. Can you think of other issues to consider?

* Transitioned from the *AP Biology Lab Manual* (2001)



This biotechnology depends on plasmids, small circles of DNA that were found first in bacteria. Plasmids allow molecular biologists to manipulate genetic information in a laboratory setting to understand more fully how DNA operates. Plasmids also let us move DNA from one bacterium to another easily.

In this investigation, you will learn how to transform *Escherichia coli (E. coli)* bacteria with DNA it has not possessed before so that it expresses new genetic information. Bacterial cells that are able to take up exogenous (external) genetic material are said to be "competent" and are capable of being transformed. You also will calculate transformation efficiency to find out how well the *E. coli* took up the "foreign" DNA. Using these techniques, you will have the opportunity to explore the field of biotechnology further. You might want to explore the following questions:

- What causes mutations in bacteria? Can mutations affect plasmids?
- What is the function of plasmids in bacteria?
- Do cells take up more plasmids in some conditions and less in others?

By learning and applying these fundamental skills, you will acquire the tools to conduct more sophisticated biotechnology investigations, including designing your own experiments to manipulate DNA.

This investigation also provides you with the opportunity to review, connect, and apply concepts that you have studied previously, including cell structure of bacteria; structure and function of cell membranes, enzymes, and DNA and RNA; transcription and translation; the operon model of the regulation of gene expression; evolution and natural selection; and interactions between organisms and their environment.

Interspersed within each investigation are supplemental activities designed to keep you on track and to provide opportunities for you to take a deeper dive into the concepts. Your teacher may assign these activities for homework or ask that you do them as you work through each investigation.

Learning Objectives

- To demonstrate the universality of DNA and its expression
- To explore the concept of phenotype expression in organisms
- To explore how genetic information can be transferred from one organism to another
- To investigate how horizontal gene transfer is a mechanism by which genetic variation is increased in organisms
- To explore the relationship between environmental factors and gene expression
- To investigate the connection between the regulation of gene expression and observed differences between individuals in a population of organisms

General Safety Precautions

Basic Sterile Technique

When working with and culturing bacteria, it is important not to introduce contaminating bacteria or fungi into the experiment. Because these microorganisms are ubiquitous, i.e., you can find them everywhere — on fingertips, bench tops, lab tables, etc. — you must avoid these contaminating surfaces. When working with inoculation loops, bulb pipettes, micropipettes, and agar plates, do not touch the tips of them (or, in the case of agar, the surface itself) or place them directly onto contaminating surfaces. Be sure to wash your hands before beginning the procedure and after — and cover your sneezes. Do not eat, drink, apply cosmetics, or use personal electronic devices in the work area.

Working with *E. coli*

The host *E. coli* used in this investigation, plasmids, and the subsequent transformants created by their combination are *not* pathogenic (disease-causing) bacteria like the *E. coli* O157:H7 strain that has been in the news. However, handling *E. coli* requires appropriate microbiological and safety procedures. Your teacher will provide instructions, but these practices include, but are not limited to, the following:

- Decontaminating work surfaces once a day and after any spill of viable material with a 10% household bleach solution
- Decontaminating all contaminated liquid or solid wastes before disposal [This can be done in an autoclave (20 minutes at 121°C) or in a 10% bleach solution (soaked for 20 minutes).]
- Washing your hands after handling organisms containing recombinant DNA and before leaving the lab
- Wearing protective eyewear and disposable gloves
- Not eating, drinking, applying cosmetics, or using personal electronic devices, such as iPods and cell phones, in the work area

THE INVESTIGATIONS

Getting Started

DNA provides the instructions necessary for the survival, growth, and reproduction of an organism. When genetic information changes, either through natural processes or genetic engineering, the results may be observable in the organism. These changes may be advantageous for the long-term survival and evolution of a species, but it also may be disadvantageous to the individuals who possess the different genetic information.

In bacteria, genetic variation does not happen by mutation alone. It also can be introduced through the lateral (horizontal) transfer of genetic material between cells. Some bacteria undergo conjugation, which is direct cell-to-cell transfer. Other bacteria acquire DNA by transduction (viral transmission of genetic information). The third route is transformation, which is uptake of "naked" DNA from the environment outside the cell.

(You may have previously studied transformation in a different context. In an experiment conducted in 1928, Frederick Griffith, seeking a vaccine against a virulent strain of pneumonia, suggested that bacteria are capable of transferring genetic information through transformation. Little did Griffith know that his work would provide a foundation for genetic engineering and recombinant DNA technology in the 21st century.)

The concept of cell transformation raises the following questions, among others:

- To transform an organism to express new genetic information, do you need to insert the new gene into every cell in a multicellular organism or just one? Which organism is best suited for total genetic transformation — one composed of many cells or one composed of a single cell?
- Can a genetically transformed organism pass its new traits on to its offspring? To get this information, which would be a better candidate for your investigation — an organism in which each new generation develops and reproduces quickly or one that does this more slowly?
- Based on how you answered the first two sets of questions, what organism would be a good choice for investigating genetic transformation — a bacterium, earthworm, fish, or mouse?

If your answer to the last question is "bacterium," you are on the right track. Genetic transformation of bacteria most often occurs when bacteria take up plasmids from their environment. Plasmids are not part of the main DNA of a bacterium. They are small, circular pieces of DNA that usually contain genes for one or more traits that may be beneficial to survival. Many plasmids contain genes that code for resistance to antibiotics like ampicillin and tetracycline. [Antibiotic-resistant bacteria are responsible for a number of human health concerns, such as methicillin-resistant *Staphylococcus aureas* (MRSA) infections.] Other plasmids code for an enzyme, toxin, or other protein that gives bacteria with that plasmid some survival advantage. In nature, bacteria may swap these beneficial plasmids from time to time. This process increases the variation

between bacteria — variation that natural selection can act on. In the laboratory, scientists use plasmids to insert "genes of interest" into an organism to change the organism's phenotype, thus "transforming" the recipient cell. Using restriction enzymes, genes can be cut out of human, animal, or plant DNA and, using plasmids as vectors (carriers of genetic information), inserted into bacteria. If transformation is successful, the recipient bacteria will express the newly acquired genetic information in its phenotype (Figure 1).

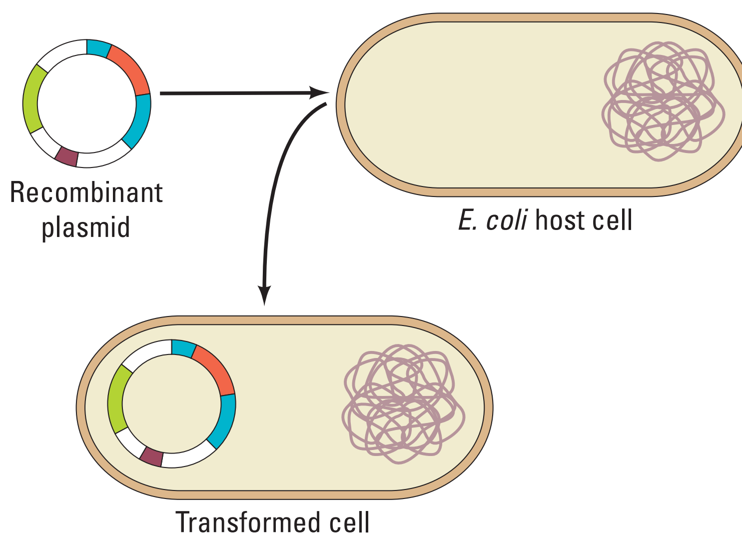


Figure 1. Transformation of Bacteria

In nature, the efficiency of transformation is low and limited to relatively few bacterial strains. Also, bacteria can take up DNA only at the end of logarithmic growth; at this time, the cells are said to be "competent." In the lab, you have discovered several ways to increase the rate of transformation. Now, rather than just a few bacteria taking up a plasmid you want them to use, millions of bacteria can be transformed. The number of bacteria that take up a plasmid successfully is called the "transformation efficiency." This is one of the values you will calculate in this lab unit.

In this investigation, you will use a predefined procedure to transform *E. coli* bacteria with a plasmid carrying a foreign gene. There are several different plasmids your instructor can choose from; you will be instructed about which one to work with for this unit.

E. coli is an ideal organism for the molecular geneticist to manipulate because it naturally inhabits the human colon and easily can be grown in a nutrient medium such as LB broth.

But what is *E. coli's* natural or pre-transformation phenotype?

- Observe the colonies of *E. coli* grown on the starter LB/agar plate provided by your teacher to glean some information before you determine if any genetic transformation has occurred. What traits do you observe in pre-transformed bacteria? Record your observations in your laboratory notebook.

- Some bacteria are naturally resistant to antibiotics, but others are not. How could you use two LB/agar plates, some *E. coli*, and some ampicillin (an antibiotic) to determine how *E. coli* cells are affected by ampicillin?
- What would you expect your experimental results to indicate about the effect of ampicillin on the *E. coli* cells? Do you think that exposure to ampicillin will cause the *E. coli* cells to evolve resistance to ampicillin? Why or why not?
- How will you be able to tell if host *E. coli* cells have been genetically transformed? (**Hint**: You will need some information from your teacher about the plasmid you will be using.)

Procedure

Your teacher will provide you with a plasmid containing one or more genes. The plasmid likely will contain the gene for resistance to ampicillin (pAMP), an antibiotic that is lethal to many bacteria, including *E. coli* cells. This transformation procedure involves the following three main steps to introduce the plasmid DNA into the *E. coli* cells and to provide an environment for the cells to express their newly acquired genes:

1. Adding $CaCl_2$
2. "Heat shocking" the cells
3. Incubating the cells in nutrient broth for a short time before plating them on agar

Materials

Your Workstation

- *E. coli* starter plate prepared by your teacher
- Poured agar plates prepared by your teacher, most likely the following:
  - 2 LB agar plates
  - 2 LB/amp agar (LB agar containing ampicillin) plates
- Transformation solution ($CaCl_2$, pH 6.1) kept *ice cold*
- LB nutrient broth
- Sterile inoculation loops
- 100–1000 μL sterile bulb pipettes
- 1–10 μL micropipettes with sterile tips
- Microcentrifuge tubes
- Microcentrifuge tube holder/float
- Container full of crushed ice
- Marking pen

Common Workstation

- DNA plasmid (most likely pAMP at 0.005 μg/μL)
- 42°C water bath and thermometer
- 37°C incubator
- 20 μL adjustable-volume micropipettes and tips (optional)
- 10% household bleach
- Biohazardous waste disposal bags
- Masking or lab tape

In your lab notebook, record data, answers to questions, and any questions that arise during this part of the activity.

Step 1 Form lab teams, as instructed by your teacher. Familiarize yourself with sterile technique, materials and lab equipment, and safety procedures for handling bacteria and decontaminating the work area.

Step 2 Label one closed microcentrifuge tube (micro test tube) "+ plasmid" and one tube "-plasmid." (What do the "+" and "-" symbols mean?) Label both tubes with your group's number (e.g., G2), and place them in the microcentrifuge tube holder/float.

Step 3 Carefully open the tubes and, using a 100–1000 µL bulb pipette with a sterile tip, transfer 250 µL of the ice cold transformation solution ($CaCl_2$) into each tube. (Note that "µl" and "µL" are alternative symbols for the same volumetric measurement.)

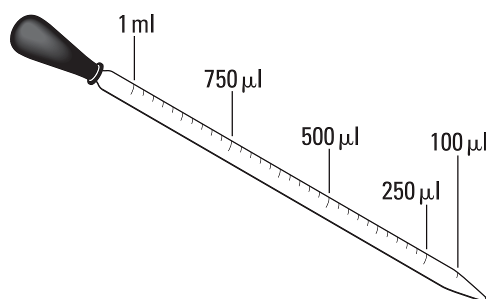


Figure 2. Measuring Volume with a Pipette

Step 4 Place both tubes on (into) the ice.

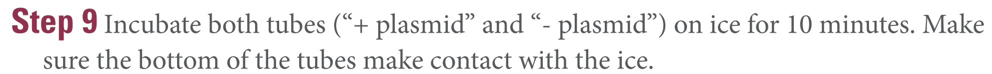
Step 5 Use a sterile inoculation loop to pick up a single colony of bacteria from your starter plate. Be careful not to scrape off any agar from the plate. Pick up the "+ plasmid" tube and immerse the loop into the $CaCl_2$ solution (transforming solution) at the bottom of the tube. Spin the loop between your index finger and thumb until the entire colony is dispersed in the solution. Appropriately discard the loop.

Step 6 Use a new sterile 100–1,000 µL micropipette to repeatedly pulse the cells in solution to thoroughly resuspend the cells. (Note that the clear transformation solution will become cloudy as the *E. coli* cells are suspended.) Place the tube back on the ice.

Step 7 Using a new sterile inoculation loop, repeat Steps 5 and 6 for the "- plasmid" tube.

CAUTION: Keep your nose and mouth away from the tip end when pipetting suspension culture to avoid inhaling any aerosol!

Step 8 Using a 1–10 µL micropipette with a sterile tip, transfer 10 µL of the plasmid solution *directly into the* E. coli *suspension* in the "+ plasmid" tube. Tap tube with a finger to mix, but avoid making bubbles in the suspension or splashing the suspension up the sides of the tube. Do not add the plasmid solution into the "- plasmid" tube! (Why not?)



Step 9 Incubate both tubes ("+ plasmid" and "- plasmid") on ice for 10 minutes. Make sure the bottom of the tubes make contact with the ice.

Step 10 While the tubes are sitting on ice, label each of your agar plates on the bottom (not the lid) as directed by your teacher.

Step 11 Following the 10-minute incubation at 0°C, remove the tubes from the ice and "heat shock" the cells in the tubes. It is critical that the cells receive a sharp and distinct shock! Make sure the tubes are closed tightly! Place the tubes into a test tube holder/float, and dunk the tubes into the water bath, set at 42°C, for exactly 50 seconds. Make sure to push the tubes all the way down in the holder so that the bottom of the tubes with the suspension makes contact with the warm water.

Step 12 When the 50 seconds have passed, place both tubes back on ice. For best transformation results, the change from 0°C to 42°C and then back to 0°C must be rapid. Incubate the tubes on ice for an additional two minutes.

Step 13 Remove the holder containing the tubes from the ice and place on the lab counter. Using a 100–1,000 µL micropipette with sterile tip, transfer 250 µL of LB nutrient broth to the "+ plasmid" tube. Close the tube and gently tap with your finger to mix. Repeat with a new sterile micropipette for the "- plasmid" tube.

Step 14 Incubate each tube for 10 minutes at room temperature.

Step 15 Use a 10–1,000 µL micropipette with sterile tip to transfer 100 µL of the transformation ("+ plasmid") and control ("- plasmid") suspensions onto the appropriate LB and LB/Amp plates. Be sure to use a separate pipette for each of the four transfers.

Step 16 Using a new sterile inoculation loop for each plate, spread the suspensions evenly around the surface of the agar by quickly "skating" the flat surface of the sterile loop back and forth across the plate surface (Figure 3). Do not poke or make gashes in the agar! Your teacher might suggest that you use small sterile glass beads to spread the suspensions by gently rocking the beads across the surface of the agar. Allow the plates to set for 10 minutes.

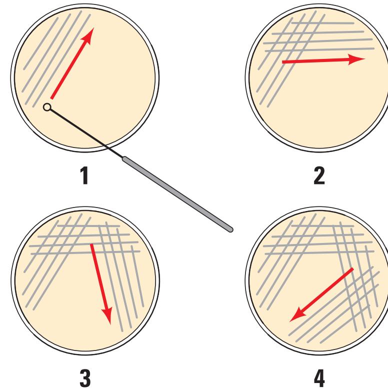


Figure 3. Technique for Plating Bacteria on Agar

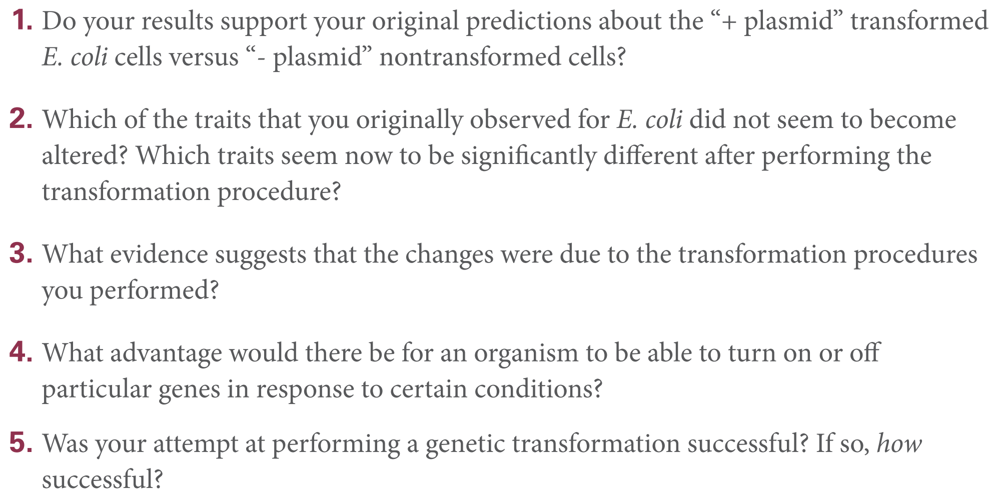
Step 17 Stack your plates and tape them together. Place the stack upside down in the 37°C incubator for 24 hours or as per instructed by your teacher.

Analyzing Results

Think about these questions *before* collecting data and analyzing your results. Be sure to record your answers in your laboratory notebook.

1. On which of the plates would you expect to find bacteria most like the original non-transformed *E. coli* colonies you initially observed? Why?
2. If there are any genetically transformed bacterial cells, on which plate(s) would they most likely be located? Again, why?
3. Which plates should be compared to determine if any genetic transformation has occurred? Why?
4. What barriers might hinder the acquisition of plasmids?
5. How can the procedures described above (addition of CI_2 and "heat shocking") help facilitate the introduction of plasmids into the *E. coli* cells?

Consider the amount of bacterial growth you see on each plate. What color are the colonies? How many bacterial colonies are on each plate? Additional questions you might want to consider include the following:



1. Do your results support your original predictions about the "+ plasmid" transformed *E. coli* cells versus "- plasmid" nontransformed cells?
2. Which of the traits that you originally observed for *E. coli* did not seem to become altered? Which traits seem now to be significantly different after performing the transformation procedure?
3. What evidence suggests that the changes were due to the transformation procedures you performed?
4. What advantage would there be for an organism to be able to turn on or off particular genes in response to certain conditions?
5. Was your attempt at performing a genetic transformation successful? If so, *how* successful?

By calculating transformation efficiency, you can measure the success of your transformation quantitatively.

Calculating Transformation Efficiency

Your next task is to learn how to determine the extent to which you genetically transformed *E. coli* cells. This quantitative measurement is referred to as the transformation efficiency. What is the importance of quantifying how many cells have been transformed? In many applications, it is important to transform as many cells as possible. For example, in some forms of gene therapy, cells are collected from the patient, transformed in the laboratory, and then put back into the patient. The more cells that are transformed to produce the needed protein, the more likely the therapy will work.

Calculating transformation efficiency gives you an indication of how effective you were in getting plasmids carrying new information into host bacterial cells. In this example, transformation efficiency is a number that represents the total number of bacterial cells that express the gene for ampicillin resistance divided by the amount of DNA plasmid used in the experiment. The transformation efficiency is calculated using the following formula.

$$\text{Transformation efficiency} = \frac{\text{Total number of colonies growing on the agar plate}}{\text{Amount of DNA spread on the agar plate (in } \mu\text{g)}}$$

What two pieces of information will you need to calculate the efficiency of your transformation? Be sure to record all calculations.

1. Calculate the total number of transformed cells.

Observe the number of colonies visible on your LB/amp plate. Do not open the plate! Each colony on the plate can be assumed to be derived from a single cell. As individual cells reproduce, more and more cells are formed and develop into what is termed a colony. Thus, the most direct way to determine the total number of bacteria that were transformed with the plasmid is to count the colonies on the plate.

2. Calculate the amount of plasmid DNA in the bacterial cells spread on the LB/amp plate.

You need two pieces of information to find out the amount of plasmid DNA in the bacterial cells spread on the LB/amp plate: a) the total amount of DNA with which you began the experiment and b) the fraction of the DNA in the bacteria that actually got spread onto the LB/amp plate.

Once you determine this information, you will multiply the total amount of plasmid DNA used in the transformation times the fraction of DNA you spread on the LB/amp plate.

a. Calculate the total amount (mass) of plasmid DNA.

The total amount (mass) of DNA with which you began the experiment is equal to the product of the concentration and the total volume used, or

DNA in μg = (concentration of DNA of μg/μL) x (volume of DNA in μL)

In this example, assume you used 10 μL of plasmid at a concentration of 0.005 pAMP μg/μL.

- Calculate the amount (mass) of plasmid DNA (pAMP) in μg per 1 μL of solution.
- Calculate the total amount of DNA used in this experiment.

How will you use this information?

b. Calculate the fraction of plasmid DNA that actually got spread onto the LB/amp plate.

Since not all the DNA you added to the bacterial cells will be transferred to the agar plate, you need to calculate what fraction of the DNA was actually spread onto the LB/amp plate.

$$\text{Fraction of DNA used} = \frac{\text{Volume spread on the LB/amp plate } (\mu L)}{\text{Total sample volume in test tube } (\mu L)}$$

Calculate the fraction of plasmid DNA you spread on the LB/amp plate.

(Hint: Refer to the procedure and your notes. How many microliters of cells containing DNA did you spread onto the plate? What was the total volume of solution in the test tube? Did you add *all* the volumes?)

c. Calculate the micrograms of plasmid DNA that you spread on the LB/amp plate.

To answer this question, you multiply the total mass of plasmid DNA used times the fraction of plasmid DNA you spread on the LB/amp plate.

DNA spread in µg = Total amount of DNA used in µg x fraction of DNA used

What does this number tell you?

3. Calculate transformation efficiency.

Look at your calculations. Fill in the blanks with the correct numbers.

Number of colonies on the LB/amp plate: ____________________

Micrograms of plasmid DNA spread on the plate: ____________________

Now calculate the efficiency of the transformation.

$$\text{Transformation efficiency} = \frac{\text{Total number of colonies growing on the agar plate}}{\text{Amount of DNA spread on the LB/amp plate (in µg)}}$$

4. What does this mean?

Transformation efficiency calculations result in very large, and very small, numbers. For both very large and very small numbers, scientists often use a mathematical shorthand referred to as scientific notation. For example, if the calculated transformation efficiency is 1,000 bacteria/µg of DNA, they often report this as 10^3 transformants/µg.

How would scientists report 10,000 transformants/µg in scientific notation?

Suppose scientists calculated an efficiency of 5,000 bacteria/µg of DNA. How would they report this in scientific notation?

a. Report your calculated transformation efficiency in scientific notation.

b. What does your calculation of transformation efficiency mean?

c. Biotechnologists generally agree that the transformation protocol that you have just completed has a transformation efficiency of between 8.0×10^2 and 7.0×10^3 transformants per microgram of DNA. How does your transformation efficiency compare? What factors could explain a transformation efficiency that was greater or less than predicted?

Evaluating Results

1. What are some challenges you had in performing your investigation? Did you make any incorrect assumptions?
2. What are some possible sources of error in the transformation procedure? If you had to repeat the procedure, what are ways to minimize potential sources of error?
3. Were you able to perform without difficulty the mathematical routines required to calculate transformation efficiency? Which calculations, if any, were challenging or required help from your classmates or teacher?
4. Can you suggest other preliminary activities that would have better prepared you to tackle the investigation?
5. Does a bacterial cell take in a plasmid with genes the cell already possesses? If so, would this affect your calculations?

Designing and Conducting Your Investigation

Think about these questions again for a minute.

- What causes mutations in bacteria? Can mutations affect plasmids? How would you be able to tell if any observed changes in phenotypes are due to the expression of genes carried on plasmids and are not attributed to a possible mutagen?
- Do bacteria take up more in plasmid in some conditions and less in others? What conditions favor uptake, and which ones inhibit it?
- What other questions do you have about plasmids and transformation?

You can either design an investigation focusing on the information below OR design one based on a question(s) or observation you had as you worked through the genetic transformation you just conducted. Be sure that your experiment applies the science skills you acquired as you worked through this investigation. Make sure that your teacher approves your plan.

You should have noted satellites around the transformed colonies. (Satellites are smaller colonies that grow around the larger transformed colony.) What observations can you make about the satellites? Do they look like transformed bacteria? How can you tell if the satellites contain the plasmid? Design and conduct an experiment to determine if the *E. coli* satellite colonies from your genetic transformation experiment are transformed, too. Available to you are the same chemicals, supplies, and equipment you used in the previous investigation.

Where Can You Go from Here?

The background to this investigation asks you to think about several applications of genetic transformation, including genetically modified food and possible ethical, social, or medical issues raised by the manipulation of DNA by biotechnology. Why are these "issues"? What questions are posed by genetic engineering? In terms of what you have learned about biotechnology, how would you respond to the quote from Michael Crichton's novel and film *Jurassic Park*: "Just because science can do something doesn't mean that it should"?