

Plasmid modeling and bacterial transformation

Focus question	How does genetic modification work? How does DNA work in bacteria? How might we use that to aid in genetic modification?
Learning target	Students will model the process of plasmid creation and uptake by bacteria. Students will genetically modify (transform) bacteria by inserting a plasmid and examine the results of the transformation.
Vocabulary	Restriction enzymes, plasmid vectors, nucleotides, DNA ligase

This lesson uses Bio-Rad's pGLO Bacterial Transformation Kit.

LS1: From Molecules to Organisms: Structures and Processes

Performance expectation HS-LS1-1	Classroom connection: Students create a model of a plasmid that contains genes to synthesize a specific protein for a desired trait. Classroom connection: Students genetically modify <i>E. coli</i> bacteria to glow in the presence of arabinose under a UV light. They make observations about which bacteria grow best in which condition determined by the plasmid DNA that is contained within the bacteria.
Performance expectation HS-LS3-1	Classroom connection: Students ask questions and explain the results in each plate related to the role of DNA in coding the differences in the treated vs untreated <i>E. coli</i> .
Performance expectation Extension: HS-LS3-3	Classroom connection: Students determine the transformation efficiency of their experiment.
Performance expectation Extension: HS-ESS3-4	Classroom connection: Students learn one technique to genetically modify an organism (<i>E. coli</i>), which is an example of a technological solution, then evaluate whether genetic modification reduces impacts of human activities on natural systems.

Science and engineering practices (Part 1)

Developing and Using Models Construct an Explanation	Classroom connection: Student models will show the process of finding a gene of interest, cutting DNA with restriction enzymes, inserting the gene of interest into a plasmid, and the process of DNA repair to add that gene to a plasmid. Students will then <i>construct an explanation</i> to show how that plasmid will be “inserted” into a bacterium and how the bacteria will express those genes.
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Science and engineering practices (Part 2)

Asking Questions and Defining Problems	Classroom connection: Students analyze their results to determine which bacteria showed change and why.
Analyzing and Interpreting Data	Classroom connection: Students use their data to calculate transformation efficiency in their bacteria.
Constructing Explanations and Designing Solutions	Classroom connection: Students obtain information about the process they used to transform bacteria and find information from other sources to explain how the protein the bacteria are now producing results in glowing, and to communicate that with support from evidence. Classroom connection: Students will be able to explain why selective breeding would not have created this bacterial trait. Classroom connection: Students evaluate and refine a solution to determine if genetic modification results in reduced impacts of human activities on natural systems.

Disciplinary core ideas (Part 1)

LS1.A: Structure and Function	Classroom connection: Plasmids are circular pieces of DNA; students will insert a gene of interest to show how the plasmid can be engineered. Students will design a solution to overcome the barriers of bacteria to successfully insert the plasmid.
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Disciplinary core ideas (Part 2)

LS3.B: Variation of Traits	Classroom connection: Transformed bacteria will grow on plates that non-transformed bacteria will not.
LS3.A: Inheritance of Traits	Classroom connection: The bacteria that incorporated the plasmid will express genes that control glowing in UV light (make GFP), while those that did not incorporate the plasmid will not.

Cross-cutting concepts (Parts 1 and 2)

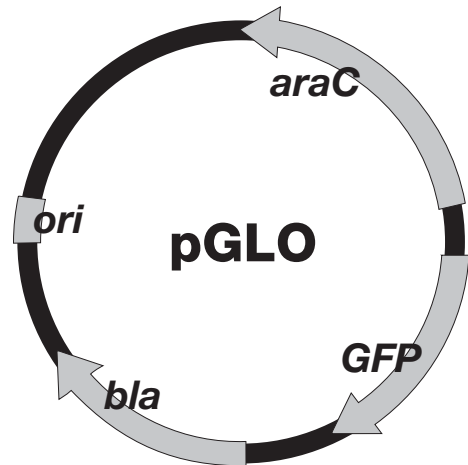
Structure and Function	Classroom connection: Enzymes have various functions; restriction enzymes can be used to remove DNA or insert DNA into plasmids. Genetic modification is possible as a result of various discoveries about enzymes that can cut DNA at specific points and pastes DNA back together again. These methods use the physical and chemical properties of several structures in a cell in order to accomplish the transformation.
Cause and Effect Stability and Change	Classroom connection: These methods use the physical and chemical properties of several structures in a cell in order to accomplish the transformation. Genetic modification is possible as a result of various discoveries about specific enzymes, particularly restriction enzymes that can be used to remove DNA or insert DNA into plasmids.

Background

Part 1: Plasmid modeling

Genetic modification uses plasmid DNA from bacteria to move specific genes from one organism to another. As Lesson 5 shows, there are multiple steps in transferring DNA from one species to another.

This lesson breaks down the process even more and provides a way for students to model the creation of a plasmid with desired genes for a trait that may be used to modify another organism. This will demonstrate the process that was used to make the plasmid in the Bio-Rad pGLO™ Bacterial Transformation Kit which is part 2 of this lesson.



Part 2: Bacterial transformation

This activity was designed to demonstrate in one lab period how bacteria can be genetically modified. (The previous lessons were designed to be used to prepare students for this lab.) Genetic modification is a powerful tool wherein DNA from one organism is added to the DNA of a different species. The results of genetic modification in agriculture have resulted in plants that are resistant to diseases, can make their own pesticides, and can resist herbicides. In this lesson, green fluorescent protein (GFP) is inserted into non-virulent *E. coli* bacteria in the form of a plasmid.

The source of GFP is a jellyfish, *Aequorea victoria*. GFP allows the jellyfish to fluoresce and glow in UV light. The mechanism to transform the bacteria uses a plasmid. The kit comes with a plasmid that contains the gene for GFP, a gene for antibiotic resistance, and a gene regulation system that turns on the production of the protein in the presence of arabinose, a monosaccharide. The plasmid is added to the bacteria in solution, then conditions are created to encourage the bacteria to uptake the plasmid. The bacteria is then grown overnight on various media that act as controls and/or that select for transformed cells.

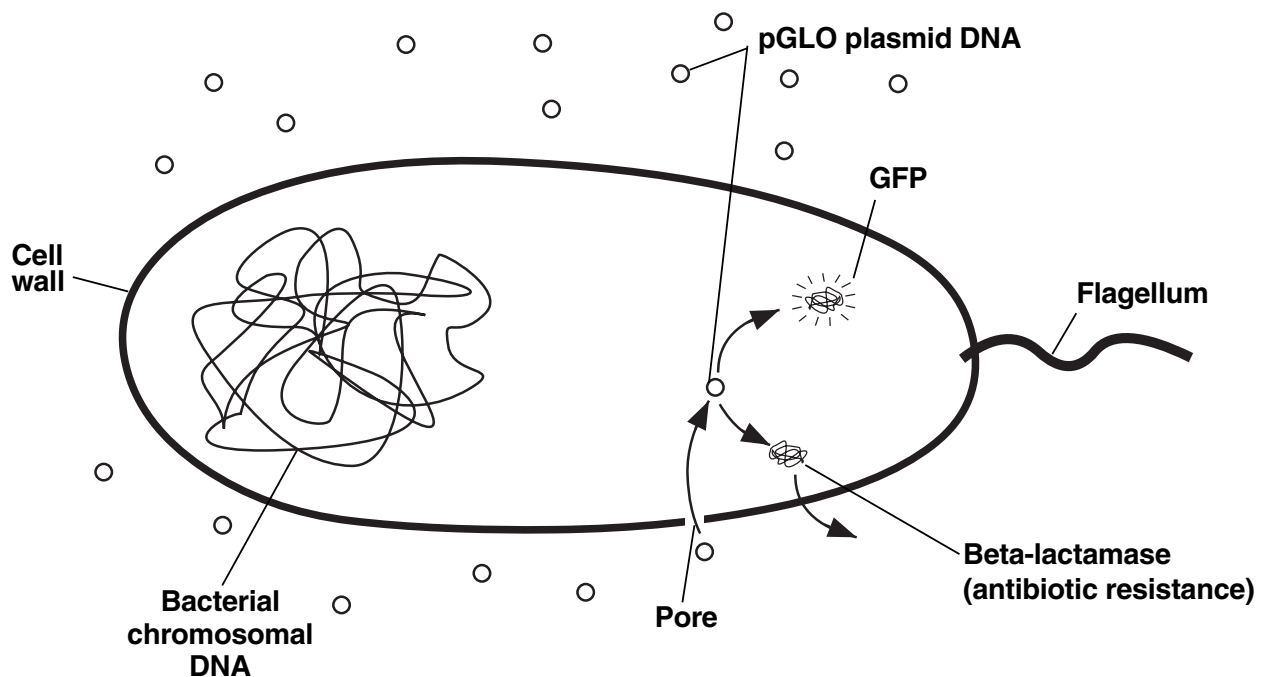
As always when using bacteria in the lab, sterile technique should be used when transferring

materials by sterile pipette or sterile loops. The condition for uptake of the plasmid by the bacteria is critical for success in this lab. The process, called heat shock, can be compared to how skin pores close in extreme cold, open in warmth, and close again quickly when exposed to cold once more. Bacterial membranes also have pore spaces that will be closed in the cold, opened in the heat, and closed again tightly to hold in the plasmid if it crossed the membrane. The transformation solution used with the bacteria also sets up a charge differential across the membrane that attracts the plasmid to cross the membrane. The colonies in suspension will be held on ice, then placed in a water bath at 42° C for 50 seconds, then immediately put back on ice.

Teachers are encouraged to read the manual for the kit before using this activity and during preparation for the lab. *This teacher document is intended to supplement the manual.* The teacher must visit this page to download the copy of the manual: bio-rad.com/en-us/sku/1660033EDU-pglo-bacterial-transformation-kit-instruction-manual?ID=1660033EDU (one download per customer).

Prior knowledge

If students have completed the *Moving genes* lesson, they should have a beginning conception of the steps in the process of genetic modification. This activity asks students to model the creation of the plasmid and describes how to illustrate a potential method of uptake by the plasmid by bacteria. Students need to know about the structures of bacteria: chromosomal DNA and plasmid DNA, the composition of the cell membrane, and how heat may affect it.



Materials

- Pop beads
- Mini marshmallows and toothpicks
- Blocks
- Or other similar materials for making sets of nucleotides and a strand of DNA
- Bio-Rad pGLO bacterial transformation kit 1660003EDU

Teacher preparation

Part 1: Plasmid modeling

Gather materials for student use to make models. Provide students with time to research plasmids and how restriction enzymes, DNA ligase and vectors apply to plasmids.

Allow students to work in groups of 2-4 to design their plasmid models.

It is preferred that students do not get the requirements of the model before beginning construction, but that through teacher questioning and explanation of their models to other groups, they will see that some models are more complete than others. Allow students to make modifications of their models. As an assessment, students can orally (or in writing) describe their model with limitations to you or the class before moving on to Part 2.

Student models should include:

- the gene or genes of interest from the original species within the DNA strand;
- a gene to allow for the selection of the transformed cells to be replicated (often this is an antibiotic resistance gene);
- the action of the restriction enzyme to “cut” out the gene(s) of interest;
- the addition of the gene or genes of interest into the existing DNA of the organism you are trying to modify; and
- the action of the DNA ligase that “attaches” the DNA nucleotides together in place.

This diagram shows the bacteria and what is happening during the transformation. This helps students visualize how a plasmid may enter a bacterium and how many plasmids there are inside their vials during the experiment.

Part 2: Bacterial transformation

1. Review safety procedures and lab skills

Safety procedures (see pages 5-6 in the lab manual)

The *Escherichia coli* bacteria HB101 K-12 strain contained in this kit is not a pathogenic organism like the *E. coli* strain O157 H7 that has sometimes been implicated in food poisoning. HB101 K-12 has been genetically modified to prevent its growth unless grown on an enriched medium. However, handling of the *E. coli* K-12 strain requires the use of standard Microbiological Practices. These practices include, but are not limited to, the following: Work surfaces are decontaminated once a day and after any spill of viable material. All contaminated liquid or solid wastes are decontaminated before disposal. All persons must wash their hands: (i) after they handle material containing bacteria, and (ii) before exiting the laboratory. All procedures are performed carefully to minimize the creation of aerosols. Mechanical pipetting devices are used; mouth pipetting is prohibited; eating, drinking, smoking, and applying cosmetics are not permitted in the work area; wearing protective eyewear and gloves is strongly recommended.

If an autoclave is not available, all solutions and components (loops and pipettes) that have come in contact with bacteria can be placed in a fresh 10% bleach solution for at least 20 min for sterilization. A shallow pan of this solution should be placed at every lab station. No matter what you choose, all used loops and pipettes should be collected for sterilization. Sterilize petri dishes by covering the agar with 10% bleach solution. Let the plate stand for 1 hr or more, then pour excess plate liquid down the drain. Once sterilized, the agar plates can be double-bagged and treated as normal trash. Safety glasses are recommended when using bleach solutions.

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. Ampicillin is a member of the penicillin family of antibiotics. Those with allergies to penicillin or to any other member of the penicillin family of antibiotics should avoid contact with ampicillin.

Lab skills (See pages 6–7 in the lab manual)

- Sterile technique
- Use of the pipette
- Decontamination and disposal
- Incubation

2. **Prepare agar plates** at least 5 days before planning to do the lab in class, the plates should be prepared. **See pages 11–17 in the lab manual for this preparation procedure.** Total preparation time for “cooking” the agar and pouring the plates is about 2 hours.

Materials for agar preparation

- 1000 mL flask
- 500 mL distilled water
- Hot plate with magnetic stir plate
- Magnetic stir bar
- Long thermometer
- 1 package of LB agar (included in kit)
- 1 vial ampicillin (included in kit)
- 1 vial arabinose (included in kit)
- Transformation solution (included in kit)
- Sterile pipettes (included in kit)
- Sterile petri dishes (included in kit)

Tips for agar preparation

- Boiling the water and agar is easiest on a hot plate with a magnetic stir bar added, even though the procedure suggested is using a microwave.
- It is important to attend to the flask, as once boiling it can easily boil over.
- Simmer the agar and water for 5–10 minutes after boiling to sterilize.
- Once the agar has boiled, remove it from heat as it needs to cool to 55° C. This gives enough time to re-hydrate the ampicillin and arabinose.
- Once you have added the transformation solution to the ampicillin and arabinose vials, begin to label the bottom of the plates with a permanent marker LB, LB/AMP and LB/AMP/ARA. The number needed for 8 lab groups is 8 LB, 16 LB/AMP and 8 LB/AMP/ARA. The manual calls for a starter LB plate for each lab group, but that is not really necessary. Two to four starter plates should give enough colonies for all groups to use. The teacher or a lab assistant can distribute the colonies for groups.
- Begin to pour plates once agar is cooled adequately (you can safely handle the flask). The agar will go far if you pour just enough in each plate to cover the bottom of the dish. Quickly replace the lid.
Note: Stretching materials for 10 groups (2 classes) is doable. You will need to provide additional sterile petri dishes for the starter plates if you choose to do this, but you should have enough agar if the plates are all the small size that come with the kit.
- Store the agar plates on the benchtop for a full day, then invert (bottom side up) to reduce the amount of condensation. Plates should be stored in the refrigerator until use.

3. Streak the starter plates

At least 24–36 hours before the lab, re-hydrate the *E. coli* according to the directions on page 16 of the lab manual. Streak the starter plates (LB). Incubate the streaked plates at 37° C overnight or on a countertop for 2–3 days.

4. Prepare lab solutions (4 students per station)
 - Fill one microtube per station with 1mL of transformation solution.
 - Fill one microtube per station with 1mL of LB broth.
 - Refrigerate solutions until lab day.

5. Prepare lab stations

On the day of the lab, prepare each lab station with the following materials:

 - 1 foam microtube holder/float with the following 4 microtubes:
 - 1 empty microtube labeled +
 - 1 empty microtube labeled -
 - 1 microtube with 1mL of transformation solution labeled TS
 - 1 microtube with 1mL of LB broth labeled LB
 - 1 permanent marker
 - Package of sterile pipettes
 - Package of sterile loops
 - Gloves (optional)
 - 4 agar plates:
 - 1 LB
 - 2 LB/Amp
 - 1 LB/Amp/Ara
 - Cup of crushed ice
 - For the class:
 - LB starter plates with bacterial colonies
 - Remaining transformation solution and LB broth
 - Water bath set to 42° C

The student portion of the lab manual begins on **page 32** with Lesson 1 and contains several pieces that are not included in this unit. However, teachers may use these to assess student understanding. Detailed step-by-step instructions for the lab are included if preferred over the quick guide.

Once students complete the procedure, they should complete the table with their predictions about bacterial growth and fluorescence. If students show interest in further study, the GFP Purification Kit from Bio-Rad is included in Nourish the Future's *Advanced biotech* topic area.

Student handout

Plasmid uptake

Now that you have your model, how might we get the bacteria to uptake the plasmid you made?

1. If a plastic bottle or balloon acts as the bacteria model, how might we get the genes inside the bottle?

Allow students to discuss in their lab group, then ask for ideas. If students are struggling, remind them that they might need to use the physical properties of the cell materials (membrane, cytoplasm) and they might need to be reminded of the processes they have learned (osmosis, diffusion, charge differential, etc).

2. What will happen to the cell membrane if we heat the bacteria a bit? (Think of yourself in a hot tub or sauna.) What will happen to the cell membrane if we put it in ice water? (Think of yourself on a chilly day without a coat on.)

Although this may seem like a silly analogy, this is exactly what scientists are doing to the bacteria: warming it to allow the pore spaces to open, then chilling it to encourage them to close.

3. How could the conditions in question 2 help us get the plasmid in the bottle or balloon?

Help students visualize what might happen to the pore spaces when warm and then when cold.

Differentiation

Other ways to connect with students with various needs:

- **Local community:** Students may research what biotech labs may be located in their community or nearby. A virtual tour of a biomanufacturing plant may be of interest. There are several Virtual visits available online. The county extension service or land grant university in your area may also offer speakers or programs to help consumers understand genetic modification.
- **Students with special needs (language/reading/auditory/visual):** Since this lesson is primarily tactile and/or kinesthetic, it could be modified to have students draw the model in a series of comic book frames. Or one student could verbally describe the action while another creates the model and takes it through the steps of the process. Students in cooperative groups can rotate tasks and utilize all students' strengths.
- **Extra support:** Students may watch Genetic Engineering at: youtu.be/nfC689E1UVk and Plasmid Rap at: youtu.be/QFfUFyUvP-w. Watch Identification and Isolation of the Gene of Interest at: youtu.be/aYhf-FXpoYs
- **Extensions:**
 - Students may complete additional lessons from the Transformation kit: Lesson 3 on Data Collection and Lesson 4 Determining the transformation efficiency. This efficiency may be influenced by the procedure used during the lab.
 - New technology is available that will change the way genetic modification works. The technique is called CRISPR and there are various videos and articles about it. To learn the science behind CRISPR, visit HHMI Biointeractive: Click and Learn CRISPR-Cas 9: Mechanisms and Applications at hhmi.org/biointeractive/crispr-cas-9-mechanism-applications or watch What is CRISPR-Cas? at youtu.be/52j0EPzhpzc

Assessments

Rubric for assessment (Part 1)

Skill	Developing	Satisfactory	Exemplary
Apply scientific ideas, principles, and/or evidence to provide an explanation of phenomena and solve design problems, taking into account possible unanticipated effects.	Explanation for how the model represents the plasmid contains only some of the parts to be transferred to a bacteria to make the proteins of interest.	Explanation for how the model represents the plasmid contains all the parts to be transferred to a bacteria to make the proteins of interest.	Explanation for how the model represents the plasmid contains all the parts to be transferred to a bacteria to make the proteins of interest and the reason why those proteins are of interest.
Apply scientific ideas, principles, and/or evidence to provide an explanation of phenomena and solve design problems, taking into account possible unanticipated effects.	Explanation for how the model represents the plasmid contains only some of the parts to be transferred to a bacteria to make the proteins of interest.	Explanation for how the model represents the plasmid contains all the parts to be transferred to a bacteria to make the proteins of interest.	Explanation for how the model represents the plasmid contains all the parts to be transferred to a bacteria to make the proteins of interest and the reason why those proteins are of interest.

Rubric for assessment (Part 2)

Skill	Developing	Satisfactory	Exemplary
Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells.	Students completed the lab procedure but have no glowing colonies or they have contamination on their agar plates.	Students genetically modify <i>E. coli</i> bacteria to glow in the presence of arabinose under a UV light. They make observations about which bacteria grow best in which condition determined by the plasmid DNA that is contained within the bacteria.	Students genetically modify <i>E. coli</i> bacteria to glow in the presence of arabinose under a UV light. They make observations about which bacteria grow best in which condition determined by the plasmid DNA that is contained within the bacteria and explain their observations.
Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.	Students ask questions but are unable to explain the results in each plate related to the role of DNA in coding the differences in the treated vs untreated <i>E. coli</i> .	Students ask questions and explain the results in each plate related to the role of DNA in coding the differences in the treated vs untreated <i>E. coli</i> .	Students ask questions and explain the results in each plate related to the role of DNA in coding the differences in the treated vs untreated <i>E. coli</i> . Students can suggest a use for the GFP protein in a mobile organism.
Extension: Apply concepts of statistics and probability to explain the variation and distribution of expressed traits in a population.		Students determine the transformation efficiency of their experiment.	
Extension: Evaluate or refine a technological solution that reduces impacts of human activities on natural systems.		Students learn one technique to genetically modify an organism (<i>E. coli</i>), which is an example of a technological solution, then evaluate whether genetic modification reduces impacts of human activities on natural systems.	
Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.	Students ask questions but are unable to explain the results in each plate related to the role of DNA in coding the differences in the treated vs untreated <i>E. coli</i> .	Students ask questions and explain the results in each plate related to the role of DNA in coding the differences in the treated vs untreated <i>E. coli</i> .	Students ask questions and explain the results in each plate related to the role of DNA in coding the differences in the treated vs untreated <i>E. coli</i> . Students can suggest a use for the GFP protein in a mobile organism.

Rubric for self-assessment

Skill		Yes	No	Unsure
Part 1	My model thoroughly explained the creation of a plasmid.			
	I was able to explain how my model operated in writing or orally to my classmates.			
Part 2	My group completed the lab and had glowing colonies.			
	I can explain the pattern of growth that we observed in our plates.			
	I can explain the process used in this lab to transform bacteria.			
	Extension: I can calculate the transformation efficiency we obtained during this lab.			