FUTURELAB+

BIOMED

*Nucleic Acids and Proteins: Disease Treatment Innovations*

# Genetic Engineering for Protein Production

Laboratory Investigation

Developed in partnership with:

**Bay Area Bioscience Education Community**

# In this Lesson Plan:

Print the **Teacher Section** →

Print the **Student Section** →



Teachers [T] *and* Student Resources [S] *can be printed independently. Select the appropriate printer icon above to print either section in its entirety.*

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**Cover Image**
This is an illustration of a protein.

BIOMED / NUCLEIC ACIDS AND PROTEINS: DISEASE TREATMENT INNOVATIONS

# Lab: Genetic Engineering for Protein Production

ACTIVITY DURATION

Six class sessions
(45 minutes each)

DRIVING QUESTION

*How can we modify the DNA of an organism to create and isolate a useful protein product?*

ESSENTIAL QUESTIONS

***How can we genetically modify a microorganism such as bacteria by introducing a foreign gene?***

***How can we isolate a protein product, such as Green Fluorescent Protein, from genetically modified bacteria?***

***Why are bacterial transformation and protein purification useful biotechnology techniques for making therapeutics?***

OVERVIEW

Many essential therapeutics, such as insulin used to treat Type 1 diabetes, are proteins. In order to make these drugs, we can modify DNA from organisms, such as bacteria, with the corresponding gene and later isolate and purify the protein product.

In Part 1 of this lab, students genetically engineer *E. coli* bacteria to produce a fluorescent green protein, resulting in glowing bacterial colonies. This is done via a process called bacterial transformation to introduce a GFP (Green Fluorescent Protein) gene originally from jellyfish into the bacteria. This gene is inducible, meaning that it can be "turned on or off," illustrating the role of gene regulation in protein expression. In Part 2 of this lab, students isolate GFP from successfully transformed *E. coli* colonies using nickel affinity chromatography.

BACKGROUND INFORMATION

Micropipetting is a necessary skill for this lab. The previous lab: *Using ELISA in Vaccine Trials* includes micropipetting reference materials as well as opportunities for student practice that can be helpful to review at any point before this lesson. Familiarity with the structure and function of DNA and the central dogma of molecular biology (relationship between genes and the proteins they code for) is helpful for students to understand so they know what goes on inside a transformed bacterial cell to produce a new trait. It is also helpful for students to have a basic understanding of bacteria as a useful model organism in science and the basic role of antibiotics.

# Have you ever wondered…

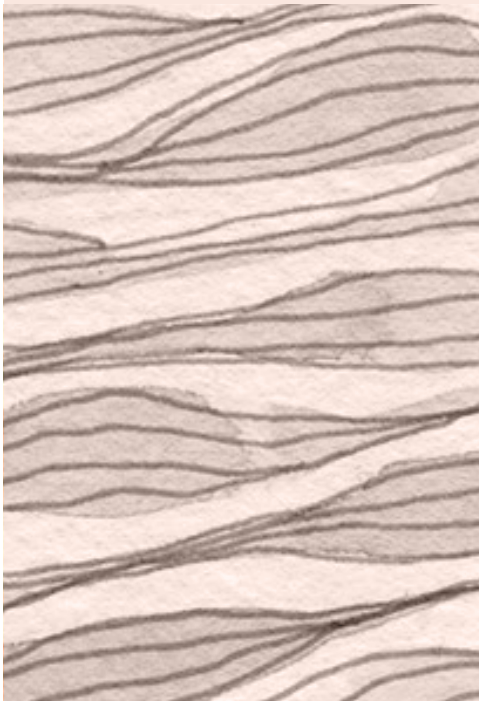
***How medicines are made?***

Many essential therapeutics, such as insulin to treat Type 1 diabetes, are proteins. In order to make these drugs, we can modify DNA from organisms, such as bacteria, with the corresponding gene and later isolate and purify the protein product.

# MAKE CONNECTIONS!

### *How does this connect to the larger unit storyline?*

DNA modification and protein isolation/purification are essential in developing new pharmaceutical drugs. Bacterial transformation and nickel affinity chromatography are simple techniques that demonstrate this.



### *How does this connect to careers?*

***Molecular biologists*** design and perform experiments utilizing molecular biology techniques such as DNA modification and protein purification. They play a key role in determining the best process for developing a new drug.

***Lab technicians*** use basic lab techniques like micropipetting and bacterial cell culture. They follow written protocols as they collect samples and perform tests to analyze body fluids, tissues and other substances. They maintain clear records of their findings.

***Biomanufacturing technicians*** assist scientists in laboratories by settting up, operating, and maintaining instruments and equipment. They help monitor experiments, make observations, and calculate and record results. Often work is completed in a sterile clean-room for the preservation of product quality. Ultimately these technicians help create compounds and chemical products that are used in many other industries.

### *How does this relate to the product development life cycle?*

When developing a new product that is a protein, an important step is to determine how best to modify a microorganism to produce it and then how to isolate and purify it.

# Pedagogical Framing

***Instructional materials are designed to meet national education and industry standards to focus on in-demand skills needed across the full product development life cycle—from molecule to medicine—which will also expose students and educators to the breadth of education and career pathways across biotechnology.***

***Through this collection, educators are equipped with strategies to engage students from diverse racial, ethnic, and cultural groups, providing them with quality, equitable, and liberating educational experiences that validate and affirm student identity.***

***Units are designed to be problem-based and focus on workforce skill development to empower students with the knowledge and tools to be the change in reducing health disparities in communities.***

## SOCIAL-EMOTIONAL LEARNING

Students work cooperatively with a partner and a group of four throughout the lab, helping them develop relationship skills. They also build social awareness by providing feedback to each others' scientific models and incorporating peer feedback at the end of the lesson.

## CULTURALLY AND LINGUISTICALLY RESPONSIVE INSTRUCTION

This lesson is grounded in the real-world topic of making pharmaceuticals via genetically modified organisms (GMOs). The bacterial transformation and protein purification protocols students complete are techniques used in actual academic and industry labs, helping students build transferable biotech skills. Many student work products in this lesson, including the summative assessment, honor verbal as well as nonverbal communication modalities. Students have the opportunity to demonstrate their understanding through drawing as well as through writing.

## COMPUTATIONAL THINKING PRACTICES

After transforming bacterial cells with new DNA, students will find patterns in the resulting bacterial colonies to determine the success of the protocol. Students will then explain the observed patterns using information about genes and their corresponding traits, as well as the role of different components of the bacterial growth media.

## OBJECTIVES

*Students will be able to:*

**Describe** how bacterial transformation followed by nickel-affinity chromatography can be used to genetically modify an organism and isolate the resulting protein using scientific text.

**Transform** *E. coli* bacteria with a Green Fluorescent Protein (GFP) gene from a jellyfish using scientific protocols.

**Isolate** Green Fluorescent Protein from successfully transformed *E. coli* bacteria using nickel-affinity chromatography.

**Create** a scientific model to explain what happens inside a transformed bacteria cell to produce a new protein and how to isolate it using experimental results.

## Materials

| *Documents* |
| --- |
| **Lab Preparation (for teacher)** |
| **Phenomenon Stations (2 of each)** |
| **Background Reading: Bacterial Transformation with Gene Regulation (1 per student)** |
| **Background Reading: Protein Purification (1 per student)** |
| **Vocabulary Tool: Bacterial Transformation with Gene Regulation (1 per student)** |
| **Vocabulary Tool: Protein Purification (1 per student)** |
| **Student Protocol, Part 1: Bacterial Transformation (1 per group of 4)** |
| **Student Protocol, Part 2: Protein Purification (2 per group of 4)** |
| **Student Guide (1 per student)** |

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## Materials

### *Reagents*

**Student Protocol, Part 1: Bacterial Transformation**

- E. coli *culture plate*
- *10 ng/µL pQuince plasmid (250 µL)*
- *50 mM $CaCl_2$ (10 mL)*
- *LB nutrient broth, sterilized (10 mL)*
- *LB agar plates (1 per group plus 2–4 per class section)*
- *LB/ampicillin agar plates (2 per group)*
- *LB/ampicillin/arabinose agar plates (1 per group)*

**Student Protocol, Part 2: Protein Purification**

- E.coli *transformed with pQuince (2 colonies per group of 4)*
- *Nickel bead slurry (2.5 mL)*
- *PBS tablet + 100 mL water*
- *25 mg/mL lysozyme (2.5 mL)*
- *Elution Buffer (5 mL)*
- *LB/ampicillin/arabinose agar plates (2 per group of 4)*

### *Equipment and Consumables*

**Student Protocol, Part 1: Bacterial Transformation**

- *P1000 micropipettes (2 per group)*
- *P1000 tips (1 box per group)*
- *P20 micropipettes (1 per group)*
- *P20 tips (1 box per group)*
- *Inoculation loops, 10 µL size (2 per group)*
- *1.5 mL microtubes (2 per group)*
- *Microtube rack (1 per group)*
- *Foam cup of ice (1 per group)*
- *Foam microtube floater (1 per group)*
- *Permanent markers (2 per group)*
- *Waste bucket (1 per group)*
- *Timer (1 per group)*
- *Water bath*
- *10% bleach*
- *Incubator*
- *UV light source*
- *UV safety goggles*

**Student Protocol, Part 2: Protein Purification**

- *P1000 micropipettes (2 per group)*
- *P1000 tips (1 box per group)*
- *P200 micropipettes (2 per group)*
- *P200 tips (1 box per group)*
- *Inoculation loops, 10 µL size (4 per group)*
- *1.5 mL microtubes (4 per group)*
- *Microtube rack (1 per group)*
- *15 mL tube/beaker (2 per group)*
- *Permanent markers (2 per group)*
- *Waste container (1 per group)*
- *10% bleach*
- *Incubator*
- *Centrifuge*
- *UV light source*
- *UV safety goggles*

# Day 1

## Procedure

### LEARNING OUTCOMES

*Students will be able to:*

**Ask** questions and make observations about different products made by genetic modification using images.

**Transform** *E. coli* bacteria with a Green Fluorescent Protein (GFP) gene from a jellyfish using scientific protocols.

**Describe** how bacterial transformation can be used to genetically modify an organism to express Green Fluorescent Protein using scientific text.

**Teacher Note >** *If students require more background information or are not able to participate in the laboratory investigations (such as students who are absent or remote learners) digital substitutes are described in Lab Preparation.*

**Teacher Note >** *At least one week before the lab, refer to Lab Preparation for instructions on aliquoting reagents, preparing agar plates and* E.coli *starter plates, and setting up lab stations. Before class today, ensure that the* E.coli *starter plates you streaked 24 hours prior have grown. Each group needs all lab materials today except agar plates.*

**Small Group (8 minutes)**

1. Set up *Phenomenon Stations* around the room like an art gallery (two of each station). Ask students to visit each and record two observations and two questions about what they see on question #1 in the *Student Guide, Part 1: Pre-Lab*. Possible sentence starters include: "I notice… Reminds me of…I wonder…Could it be…".

   Stations:

   1. Impossible burger (made using plant-based heme protein—leghemoglobin—isolated from genetically modified yeast)
   2. Insulin (made from isolating insulin from genetically modified bacteria)
   3. Glowing animals (made by genetically modifying them with jellyfish GFP protein)

2. Have students share responses with their groups and add a new observation or question they hear from a peer to each station. Prompt students to complete the table in the *Student Guide, Part 1: Pre-Lab* by considering what the stations have in common. Encourage students to brainstorm additional phenomena they have observed that may relate to this topic.

3. Ask students to choose one of the stations and draw a scientific model that explains how the images in the stations are related to each other and response on Question #2 in the *Student Guide, Part 1: Pre-Lab.* This drawing serves as a pre-assessment of students' understanding of how protein products are made from genetically modified organisms. Students will build on this knowledge throughout the lab and make a detailed scientific model that explains the process of bacterial transformation and protein isolation.

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# Day 1

*Continued*

## LEARNING OUTCOMES

*Students will be able to:*

**Obtain** and communicate how vaccines elicit the immune response and the production of antibodies, using scientific text.

**Construct** an explanation of how an indirect ELISA is used to detect the amount of a certain protein in a sample using scientific text.

# Procedure

**Whole Group (2 minutes)**

1 Introduce the lab to students by explaining that they will perform a procedure that is similar to the one that resulted in the products they just observed. Share that students will be working with bacteria (a non-pathogenic strain of *E. coli*) and that it is important to follow these safety guidelines:

- *Wash your hands before and after the lab.*
- *Do not eat or drink during the lab.*
- *Keep your lab station clean and clear of clutter.*

**Whole Group (5 minutes)**

1 Search for a 'what is a plasmid' video to introduce students to plasmids and their natural relationship with bacteria. Start the video at 0:22 and end at 1:55.

2 Review with students that plasmids are small bits of DNA that are naturally taken up by bacteria and these genes in the plasmid can be expressed as proteins by the bacteria cell's natural machinery. Remind them that the video indicated that plasmids can be "easily manipulated…".

3 Ask students to brainstorm how bioengineers might use the natural relationship between bacteria and plasmids in the creation of the products they viewed in the Phenomenon stations. Allow students to share their ideas with the class.

**Small Group (25 minutes)**

1 Break students into lab groups and pass out the *Student Protocol, Part 1: Bacterial Transformation* and *Background Reading: Bacterial Transformation with Gene Regulation*.

2 Ask students complete Steps #1–10 in the *Student Protocol, Part 1: Bacterial Transformation* and start the reading during waiting steps.

**Individual (5 minutes)**

1 Exit Ticket: Ask students to return to their drawings in the *Student Guide, Part 1: Pre-Lab* and add one thing based on what they learned today.

2 Homework: Ask students to write their own sentences for each of the words in *Vocabulary Tool: Bacterial Transformation with Gene Regulation.*

# Day 2

## LEARNING OUTCOMES

*Students will be able to:*

**Predict** the type of *E. coli* bacterial growth on various media using their understanding of bacterial transformation and gene regulation.

**Plate** *E. coli* bacteria on various media to determine if they have been successfully transformed using scientific protocols.

# Procedure

**Prepare in Advance**

1 Before class, remove the tubes of bacteria from the incubator and set up lab stations using *Lab Preparation*.

2 The only lab materials each group of 4 students needs today are:

- 1 LB plate
- 2 LB/amp plates
- 1 LB/amp/ara plate
- 2 permanent markers

**Small Group (10 minutes)**

1 Instruct students to discuss possible experimental outcomes with their lab groups and record their predictions on Question #3 in the *Student Guide, Part 1: Pre-Lab*.

2 Circulate as students work and ask questions to prompt them to elaborate on their thinking, such as "What makes you think that?"

**Whole Group (10 minutes)**

1 Explain to the class that the diagram on Question #1 in the *Student Guide, Part 2: Lab* provides students with an overview of the major lab steps and read each one together. Explain the purpose of each step as listed below and have students record this information. This understanding will be helpful when students are considering possible sources of error.

**a.** Add bacteria and plasmid to $CaCl_2$ → Makes cell walls permeable.

**b.** Ice bath/heat shock → Increases holes in cell membrane so pQuince DNA can get in.

**c.** Feed cells with a nutrient broth (LB) → Helps stressed-out cells recover and provides the necessary nutrients and environment for optimal replication.

**d.** Plate bacteria → Grow bacteria and determine if transformation was successful.

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## Day 2

*Continued*

# Procedure

**Small Group (10 minutes)**

1 Have a student from each lab group retrieve the bacteria for his or her group from the incubator.

2 Remind students that because they are working with bacteria, it is important to follow the safety guidelines:

- Wash your hands before and after the lab.
- Do not eat or drink during the lab.
- Keep your lab station clean and clear of clutter.

3 Ask students to complete Steps #11–15 of *Student Protocol, Part 1: Bacterial Transformation*.

**Small Group (10 minutes)**

1 Instruct students to finish reading and answering the questions on *Background Reading: Bacterial Transformation with Gene Regulation*, if they have not already done so.

2 Have students check their answers using the key and clarify any questions that arise. Students may also take time to compare the sentences they wrote in the *Vocabulary Tool: Bacterial Transformation* activity with one another for feedback and to check for understanding.

**Individual (5 minutes)**

1 Exit Ticket: Return to your answer to the question "What do you think these three stations have in common?" in Question #1 in the *Student Guide, Part 1: Pre-Lab*. What can you add based on what you have learned today?

# Day 3

## LEARNING OUTCOMES

*Students will be able to:*

**Explain** whether or not their predictions were supported and consider possible sources of error using experimental results.

**Create** a scientific model to explain what happens inside a transformed bacterial cell to produce a new protein using experimental results.

# Procedure

**Teacher Note >** *If there is no UV shield on your transilluminator or other UV light source, make sure students are wearing UV goggles when observing results.*

| **Whole Group (10 minutes)** | |
|---|---|
| 1 | Prompt students to reflect on their initial questions generated from looking at the *Phenomenon Stations* to see which of their questions have been answered and whether they have new ones. |
| 2 | Watch the following video and discuss the impact GFP has had on research: *How glow-in-the-dark jellyfish inspired a scientific revolution.* |

| **Small Group (15 minutes)** | |
|---|---|
| 1 | Instruct lab groups to quickly view plates using a transilluminator and document their results* in Question #2 in the *Student Guide, Part 2: Lab*. A cell phone can capture an image quickly. (Students can share with one another rather than each one taking a photo to speed up the process.) |
| 2 | Have students work together to answer questions #1–3 in the *Student Guide, Part 3: Data Analysis*. Prompt students to review the four main steps of the lab (Question #1 in the Student Guide, Part 2: Lab) and brainstorm what type of errors could occur in each step to lead to unexpected results. This can be difficult for students to come up with on their own, so it may be best to do it together as a class. |

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## Day 3

*Continued*

# Procedure

**Small Group (15 minutes)**

1 Ask students to synthesize what they learned by drawing a scientific model that explains what happens inside transformed bacteria to produce a new protein. Share the following features of a scientific model:

- Represents a system or a phenomenon
- Context-rich and specific
- Pictorial (drawings and pictures) and written (text, labels, equations, etc.)
- Observable and unobservable features (make the "invisible" visible!)
- Revisable over time

2 With their partners or whole lab group, ask students to start Question #1 of the *Student Guide, Part 4: Making a Model*—draw a scientific model. Suggest having students draw on large whiteboards or poster paper.

3 As students work, circulate and ask questions to elicit deeper student thinking and make thinking visible *(e.g., "Why did you draw___in that way?" "How is___connected to___?" "How could you explain ___ using pictures instead of words___ ")*

**Teacher note** > *Not all groups will be able to use a transilluminator at the same time. Groups that are not capturing images should review their vocabulary sentences with their groups. Circulate to clarify any confusion and remind students that they will be synthesizing their understanding of the phenomena using some of this vocabulary at the end of the lesson.*

**Individual (5 minutes)**

1 Exit Ticket: Aside from GFP, what is an example of another protein you might want bacteria to make? Why might this protein be useful or helpful for humans?

# Day 4

## LEARNING OUTCOMES

*Students will be able to:*

**Provide** and receive feedback on a scientific model to explain what happens inside a transformed bacterial cell to produce a new protein using experimental results.

**Plate** successfully transformed *E. coli* bacteria to prepare for protein isolation using scientific protocols.

# Procedure

**Prepare in Advance**

1 Before class, check that there is at least one successfully transformed (glowing) bacteria colony for each pair of students and set up lab stations using *Lab Preparation*. They cannot begin *Lab Part 2: Protein Purification* without transformed bacteria.

2 Set up the following lab materials per each group of four students:

- 2 successfully transformed (glowing) bacteria colonies
- 2 LB/amp/ara plates
- 2 permanent markers
- 2 loops

3 If students do not have experience with streaking plates, it may be helpful to make a few "practice plates" (with only agar) in advance for students to practice on before transferring bacteria. This may help prevent errors with streaking, such as digging too deep into the agar.

**Small Group (10 minutes)**

1 Ask students to look back at the observations they made in the phenomenon stations in *Student Guide, Part 1: Pre-Lab* and identity observations they could explain in the models they began creating in *Student Guide, Part 4: Making a Model*.

2 Ask students to complete drawing the models they began in *Student Guide, Part 4: Making a Model*.

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## Day 4

*Continued*

# Procedure

**Small Group (20 minutes)**

1 Ask students to switch models with another pair or group and provide feedback:

- What aspect of the model is the most helpful in understanding the science behind the lab?
- What aspect of the model is confusing or could be clearer?
- What suggestions do you have for improving the model?

2 Ask students to modify their models to incorporate at least one piece of feedback they received.

3 Save student models because they will add to them after *Student Protocol, Part 2: Protein Purification* on Day 6.

**Small Group (10 minutes)**

1 Ask students to form their lab pairs and retrieve their plates of transformed bacteria from the incubator and remind them of the safety guidelines:

- Wash your hands before and after the lab.
- Do not eat or drink during the lab.
- Keep your lab station clean and clear of clutter.

2 Ask students to complete Steps #1–5 of *Student Protocol, Part 2: Protein Purification*.

**Individual (5 minutes)**

1 Exit Ticket: What was one way you changed your model after receiving feedback? How did this change improve your model?

# Day 5

## Procedure

### LEARNING OUTCOMES

*Students will be able to:*

**Lyse** successfully transformed *E. coli* bacteria cells to prepare for protein isolation using scientific protocols.

**Describe** how nickel-affinity chromatography can be used to purify a protein from a cell using scientific text.

**Prepare in Advance**

1 Before class, check that the transformed bacteria each pair of students plated on Day 4 has grown and set up lab stations using *Lab Preparation*. They cannot continue with the protocol until there is a clear lawn of glowing bacteria on their plate.

2 Set up the following lab materials per each group of four students:

- 2 P1000 micropipettes plus tips
- 2 P200 micropipettes plus tips
- 2 loops
- 220 µL Lysozyme
- 2.2 mL PBS
- 2 empty 1.5 mL microtubes
- 1 Waste bucket
- 2 Permanent markers

**Whole Group (10 minutes)**

1 Warm-up: "The insulin that is used to treat diabetes is an example of a pharmaceutical made by isolating a protein from genetically modified microorganisms (the human gene for insulin is introduced into an organism, such as yeast). Explain or draw how you think this isolation process might occur."

2 Ask students to share with a partner and add one thing to the explanation or drawing based on what their partners said.

3 Remind students that yesterday they grew a plate of transformed bacteria and share that today they will start the process of isolating the protein responsible for the glowing trait (Green Fluorescent Protein). This is analogous to isolating a pharmaceutical, such as insulin from a genetically modified microorganism.

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## Day 5

*Continued*

# Procedure

**Small Group (20 minutes)**

1 Ask students to form their lab pairs and retrieve their plates of transformed bacteria from the incubator and remind them of the safety guidelines:

- Wash your hands before and after the labs.
- Do not eat or drink during the lab.
- Keep your lab station clean and clear of clutter.

2 Ask students to complete Steps #6–12 of *Student Protocol, Part 2: Protein Purification.*

3 Store all tubes of bacterial lysate in the freezer until the next class.

**Individual (15 minutes)**

1 Ask students to complete *Background Reading: Protein Purification* and check their answers with the key.

2 Ask students to write their own sentence for each of the words in *Vocabulary Tool: Protein Purification* and complete for homework.

3 Exit Ticket: Return to your answer to Question #1 in the *Student Guide, Part 1: Pre-Lab* "What do you think these three stations have in common?" What can you add based on what you have learned today?

# Day 6

## Procedure

### LEARNING OUTCOMES

*Students will be able to:*

**Isolate** Green Fluorescent Protein from successfully transformed *E. coli* bacterial cell lysate using nickel-affinity chromatography.

**Create** a scientific model to explain how to isolate a protein from genetically modified bacteria using experimental results.

**Prepare in Advance**

1 Before class, remove the bacterial cell lysates from the freezer and allow to thaw at room temperature. Set up lab stations using *Lab Preparation*.

2 Set up the following lab materials per each group of four students:

- P1000 micropipettes plus tips
- P200 micropipettes plus tips
- 2.2 mL PBS
- 220 µL Elution buffer
- 2 microtubes with 100 µL nickel bead slurry
- 2 empty 1.5 mL microtubes
- 1 Waste bucket
- 2 Permanent markers

**Teacher note >** *If there is no UV shield on your transilluminator or other UV light source, make sure students are wearing UV goggles when observing results.*

**Whole Group (5 minutes)**

1 Warm-Up: "If you could modify bacteria to produce any protein you wanted and then isolate that protein—what would it be and why?"

2 Ask students to share with a partner and randomly call on a few students to share with the class.

3 Remind students that yesterday they prepared bacterial cell lysate (broken open transformed bacteria cells) and share that today they will complete the process of isolating the protein responsible for the glowing trait (Green Fluorescent Protein).

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## Day 6

*Continued*

# Procedure

**Small Group (20 minutes)**

1 Ask students to form their lab pairs and retrieve their plates of transformed bacteria from the incubator and remind them of the safety guidelines:

- Wash your hands before and after the lab.
- Do not eat or drink during the lab.
- Keep your lab station clean and clear of clutter.

2 Ask students to complete Steps #13–22 of *Student Protocol, Part 2: Protein Purification*.

**Small Group (15 minutes)**

1 Have students work together to answer Question #4 in the *Student Guide, Part 3: Data Analysis*.

2 Pass back scientific models and ask students to add to them to explain how the GFP produced by the transformed bacteria can be isolated by nickel affinity chromatography on Question #2 of the *Student Guide, Part 4: Making a Model*.

3 Optional: If time allows, ask students to switch models with another pair or group and provide feedback:

- What aspect of the model is the most helpful to understanding the science behind the lab?
- What aspect of the model is confusing or could be clearer?
- What suggestions do you have for improving the model?

4 Ask students to answer Questions #3–4 in the *Student Guide, Part 4: Making a Model* to consider limitations of their model and how it could be used to make predictions.

**Individual (15 minutes)**

1 Look back at your responses to the Phenomenon Stations in *Student Guide, Part 1: Pre-Lab*. What is a question you wrote down that you can now answer? What is an observation you made that you can now explain?

## Extension

# Procedure

**Individual or Small Group**

To allow students or student groups to show their understanding of how they used the properties of DNA, genes, protein synthesis, bacteria, plasmids, and proteins to complete this lab. The instructor may ask students to create a short informational video explaining what was happening at the major steps in the lab, or one part of the lab (transforming bacteria or isolating proteins). Students should use their finished models as a visual aide to help them explain to the viewer how this technique works to move genes from one organism to another and be able to “harvest” these proteins for human use.

# National Standards

| | |
|---|---|
| **Next Generation Science Standards** | **LS1.A: Structure and Function**<br>All cells contain genetic information in the form of DNA molecules. Genes are regions in the DNA that contain the instructions that code for the formation of proteins, which carry out most of the work of cells. |
| | **LS3.A: Inheritance of Traits**<br>Each chromosome consists of a single very long DNA molecule, and each gene on the chromosome is a particular segment of that DNA. The instructions for forming species' characteristics are carried in DNA. All cells in an organism have the same genetic content, but the genes used (expressed) by the cell may be regulated in different ways. Not all DNA codes for a protein; some segments of DNA are involved in regulatory or structural functions, and some have no as-yet known function. |
| | **Developing and Using Models**<br>Develop, revise, and/or use a model based on evidence to illustrate and/or predict the relationships between systems or between components of a system. |
| | **Systems and System Models**<br>Models can be used to simulate the flow of energy, matter, and interactions within and between systems at different scales. |
| **Math** | **MP.2 Reason abstractly and quantitatively.**<br>Consider the relative sizes of genes, proteins, cells, and bacteria colonies. |

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# National Standards

*Continued*

| | |
|---|---|
| **Career and Technical Education (CTE)** | **A1.2**<br>Describe the use of model organisms in biotechnology research and manufacturing. |
| | **A3.1**<br>Define and describe the structure and function of DNA ribonucleic acid (RN) and proteins, explain the consequences of DNA mutations on proteins. |
| | **A3.3**<br>Employ standard techniques of DNA extraction, purification, restriction digests, bacterial cell culture, and agarose gel electrophoresis and document and evaluate results. |
| | **A3.5**<br>Predict outcomes of DNA and protein separation protocols. |
| | **A4.2**<br>Describe conditions that promote cell growth under aseptic conditions in the laboratory and workplace. |
| | **A8.1**<br>Follow written protocols and oral directions to perform a variety of laboratory and technical tasks. |
| | **A8.6**<br>Properly and safely use and monitor a variety of scientific equipment, including pH meters, microscopes, spectrophotometers, pipettes, micropipettes, and balances. |
| | **A8.7**<br>Determine which equipment is appropriate to use for a given task and the units of measurement used. |
| | **A9.2**<br>Identify several products obtained through recombinant DNA technology. |
| | **A9.3**<br>Outline the steps in production and delivery of a product made through recombinant DNA technology. |

Lab

# Preparation

KEY

*When the preparation task should take place in relationship to the lab*

*The amount of time necessary to complete the preparation task*

## Quick Tips

1 Before continuing, check the *Materials List* to make sure you have all the necessary equipment and reagents for the lab.

2 *Student Protocol, Part 1: Bacterial Transformation* and *Student Protocol, Part 2: Protein Purification* are written in a continuous five-day sequence, but you can break them into two three-day sequences with up to 1 month in between.

3 We recommend having students complete this lab in **groups of four**:

- Student Protocol, Part 1: Each pair is responsible for one reaction tube (with plasmid DNA or without plasmid DNA) and shares one set of results.
- Student Protocol, Part 2: Each pair streaks a plate of transformed bacteria and isolates GFP.

4 Before students can perform *Student Protocol, Part 2: Protein Purification*, they must have successfully transformed bacteria from *Student Protocol, Part 1: Bacterial Transformation*. Each pair of students needs one colony of transformed bacteria.

5 *Virtual Learning Options* for this lab, including digital-only resources, are provided.

## Preparation

| | | |
|---|---|---|
| **1** | Up to four weeks before lab | 2+ hrs |
| | *Student Protocol, Part 1: Bacterial Transformation* | |
| ☐ | Aliquot LB broth and $CaCl_2$ into one tube each with ~750 µL per group and leave at room temperature or refrigerate until ready for use. | |
| ☐ | Prepare one LB, two LB/amp, and one LB/amp/ara plate per group plus two-four LB plates per class section and store upside down in the refrigerator.<br>— View *BABEC How-To Videos: How to Pour Plates*<br>— See instructions *Preparing LB Agar Plates* | |

*Continues next page >*

## Lab

*Continued*

# Preparation

*Student Protocol , Lab Part 2: Protein Purification*

☐ Dissolve Phosphate Buffered Saline (PBS) tablet in 100 mL water.

☐ Aliquot reagents as follows and leave at room temperature or refrigerate until ready for use.

| Reagent | Factor in # of student pair groups | Final volume for each group |
|---|---|---|
| PBS | 2 pairs x 2 mLs x 1.1 (overage) | 4.4 mL |
| Lysozyme | 2 pairs x 100 µL x 1.1 (overage) | 220 µL |
| Elution Buffer | 2 pairs x 100 µL x 1.1 (overage) | 220 µL |
| Nickel Resin (beads) Slurry | 2 pairs x 1 tube of 100 µL | 2 tubes of 100 µl |

☐ Prepare two LB/amp/ara plates per group, seal with parafilm and store upside down in the refrigerator.

— View *BABEC How-To Videos: How to Pour Plates*

— See instructions *Preparing LB Agar Plates*

*Continues next page >*

## Lab

*Continued*

# Preparation

| | | |
|---|---|---|
| **2** | 24 hours before lab | 30 min |
| | *Student Protocol, Part 1: Bacterial Transformation* | |
| ☐ | Fill the water bath and set it to 42˚C. | |
| ☐ | Set the incubator to 37˚C. | |
| ☐ | Take agar plates out of the fridge so they come to room temperature before the lab. | |
| ☐ | *Streak two-four starter plates per class section on LB agar plates and place at 37˚C.* Bacteria should be 24 hours old and warm for the lab.<br>**Note >** *See Streaking Starter Plates for the procedure to follow to streak the start plates.* | |
| | *Student Protocol, Part 2: Protein Purification* | |
| ☐ | Set the incubator to 37˚C. | |
| ☐ | Take agar plates out of the fridge so they come to room temperature before the lab. | |

*Continues next page >*

## Lab

*Continued*

# Preparation

**3a** | Up to one day before each part of the lab | 30 min

☐ Set up lab stations (one per group of four students)

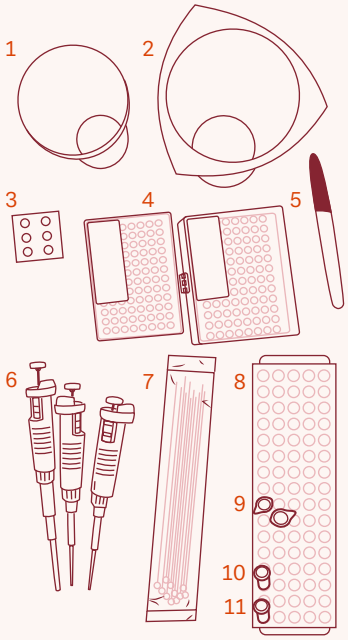
**Note >** *Prepare ice cups (crushed works best) immediately before use.*

☐ *Student Protocol, Part 1: Bacterial Transformation*

**Note >** *May be completed in one 90-minute period or two 45-minute periods. If breaking up the protocol into two periods, all materials are needed for Day 1 except the four agar plates.*

☐ Give to each group of four students:

| | |
|---|---|
| 1 | Foam cup of crushed ice or ice bath |
| 2 | Waste bucket |
| 3 | Foam microtube floater |
| 4 | Micropipette tips |
| 5 | Permanent marker |
| 6 | Micropipettes (P20, P200, P1000) |
| 7 | Loops |
| 8 | Microtube rack |
| 9 | Two empty 1.5 mL microtubes |
| 10 | One tube $CaCl_2$ (~750 µL) |
| 11 | One tube LB broth (~750 µL) |

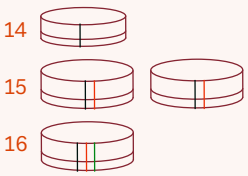



☐ Keep with you and bring to each group when ready:

| | |
|---|---|
| 12 | Two–four starter plates of *E. coli* |
| 13 | Plasmid DNA |


☐ Distribute to each goup when ready (Day 2):

| | |
|---|---|
| 14 | One LB plate |
| 15 | Two LB/Amp plates |
| 16 | One LB/Amp/Ara plate |




*Continues next page >*

## Lab

*Continued*

# Preparation

**3b** Up to one day before each part of the lab — 30 min

☐ Set up lab stations (one per group of four students)

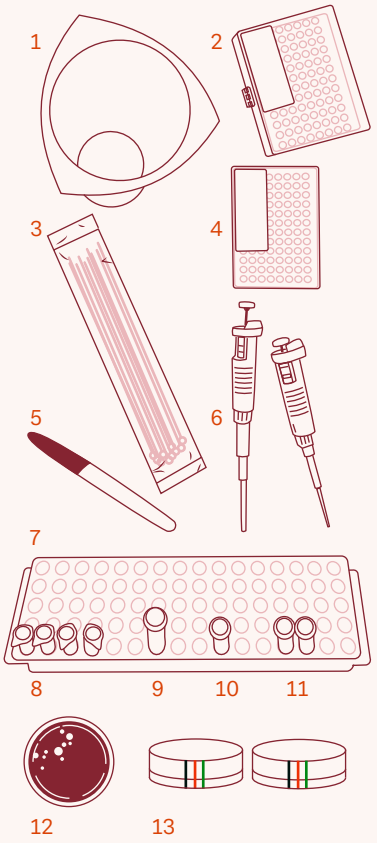
**Note** > *Prepare ice cups (crushed works best) immediately before use.*

☐ *Student Protocol, Part 2: Protein Purification*

**Note** > *Must be completed over at least three days. Refer to the Procedure for which materials are needed for each day.*

☐ Give to each group of four students:

| | |
|---|---|
| 1 | Waste bucket |
| 2 | Blue tips for micropipettes P1000 |
| 3 | 4 loops |
| 4 | Yellow tips for micropipettes P200 |
| 5 | Permanent marker |
| 6 | Micropipettes (P200, P1000) |
| 7 | Microtube rack |
| 8 | 4 empty 1.5 mL microtubes |
| 9 | 4.4 mL PBS |
| 10 | 220 µL lysozyme and elution buffer |
| 11 | 2 tubes with 100 µL nickle bead slurry |
| 12 | 2 colonies of transformed bacteria |
| 13 | 2 LB/Amp/Ara plates |




**4** After the lab — 30 min

☐ Properly dispose of lab supplies

**Note** > *See Lab Safety.*

Lab

# Virtual Learning Options

| 1 | Anytime | 30 min |
|---|---|---|

☐ Use the same Background Readings and Student Guide.

☐ Replace the Student Protocols with your choice of the following videos and simulations and show students the sample results to analyze.

*Student Protocol, Part 1: Bacterial Transformation*

☐

| Bacterial Transformation Videos | Bacterial Transformation Simulations |
|---|---|
| *BABEC* | *LabXChange* |
| *DNALC* | *DNALC* |

☐ Sample results (expected):

Under UV Light

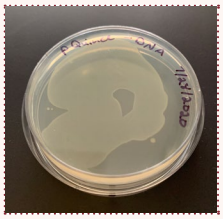
LB (~DNA)

LB/Amp (~DNA)

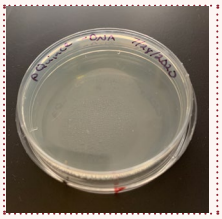
LB/Amp (+DNA)

LB/Amp/Ara (+DNA)

Normal Light

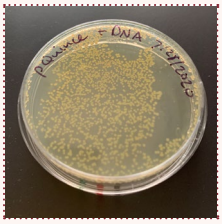


LB (~DNA)



LB/Amp (~DNA)

LB/Amp (+DNA)



LB/Amp/Ara (+DNA)

*Student Protocol, Part 2: Protein Purification*

☐ Simulation: *Purifying Protein by Column Chromatography* (LabXChange)

☐ Sample results (expected):

## Skills

# Lab Safety

**Your school's** specific safety and disposal policies should always take precedence.

**Make** sure you are **familiar** with the procedures at your site.

**To disinfect consumables and used plates**

1 Select your cleaning agent: You can use the following easily available solutions for killing bacteria on classroom surfaces, agar plates, plastic consumables:

- 10% bleach solution
- Lysol or similar disinfectant spray
- Rubbing alcohol (isopropanol or ethanol, at least 70% concentration)

2 Disinfect agar plates with live bacteria:

- Use a pail, bucket, or other large liquid storage unit and fill with your cleaning agent of choice.
- Put all the bacterial plates into the cleaning solution, making sure that each plate has contact with the solution.
- Let soak for 10–20 minutes.
- Remove plates from the bleach solution and put into a trash bag.
- Trash can be disposed as usual and the cleaning solution can be disposed of in the sink with running water.

3 Disinfect plastic consumables:
For inoculating loops, transfer pipettes, pipette tips, and any other disposable materials that have come into contact with live bacteria, follow the same procedures as listed above.

**Note >** ***If you have an autoclave****, you can sterilize any used LB agar plates and plastic consumables following the manufacturer's directions. Autoclaved waste can then be disposed of in the regular waste.*

*Continues next page >*

## Skills

*Continued*

# Lab Safety

| The *E.coli* used in this lab | |
|---|---|
| 1 | *E. coli* is a bacteria found everywhere in our environment. The strain we use for this lab and in many research scientific labs are harmless to humans and are NOT pathogenic. They have been specially engineered to help scientists with their work. |
| 2 | If you touch the bacteria with your hands, simply wash with soap and water. If you get some bacteria in your eyes, simply flush with water. As always, use safety precautions when working in the laboratory. |

| Aseptic technique | |
|---|---|
| 1 | When growing bacteria in culture, it is important to prevent the growth of unwanted microorganisms in the nutrient-rich media. |
| 2 | Aseptic technique is a series of methods that are used to minimize the chances of contamination. |
| 3 | Examples include use of sterile tubes and pipettes, sterilized solutions, cleaning the work area with disinfectants, use of Bunsen burners, and keeping the caps of tubes, plates, and pipette boxes closed. |

| UV safety | |
|---|---|
| 1 | Ultraviolet (UV) radiation can cause damage to eyes and skin. |
| 2 | Use UV rated safety glasses or goggles if looking directly at UV light. |

Skills

# Preparing LB Agar Plates

| Prepare in Advance | |
|---|---|
| 1 | Set up the following materials:<br>• LB Agar, 300 mL bottles<br>• 100 X Ampicillin (10 mg/mL), 3 mL tube<br>• 100 X Arabinose (200 mg/mL), 3 mL tube<br>• Microwave (to heat LB agar before pouring)<br>• Oven mitt (to handle heated LB agar)<br>• Permanent markers (black, red, green)<br>• 40–120 of 60 x 15 mm Petri dishes (small plates) |

| Procedure | |
|---|---|
| 1 | Loosen the cap of a bottle of 300 mL LB agar. *A single bottle will pour 25–35 plates (or 1+ sleeves).* |
| 2 | Microwave the bottle for 60–90 seconds. |
| 3 | Use caution and oven mitts as you swirl the mixture. *Caution: solid agar may superheat so agitate the bottle by pushing on it gently in the microwave so it can bubble and release pressure before you pick it up.* |
| 4 | Repeat Steps 2–3 until the LB agar is liquefied. |
| 5 | Cool each bottle to approximately 50–55˚C.<br>*Do not use a thermometer to check.*<br>*It should be comfortable to the touch, but has not yet solidified.*<br>While waiting for it to cool, continue to the next step. |

*Continues next page >*

## Skills

*Continued*

# Preparing LB Agar Plates

6 Label your Petri dishes (25–35) with a stripe of permanent marker as specifiedbelow.

| Plate Type | Symbol | |
|---|---|---|
| LB | One line:<br>One black line |  |
| LB + Amp | Two lines:<br>One black and one red line |  |
| LB +<br>Amp + Ara | Three lines:<br>one black, one red and one green. |  |

7 When the bottle is cool to the touch, add the following depending on which type of plate you are pouring:

- LB plates: do not add anything
- LB/Amp plates: add 3 mL of ampicillin and swirl to mix (final concentration of ampicillin = 100 µg/mL)
- LB/Amp/Ara plates: add 3 mL of ampicillin and 3 mL arabinose and swirl to mix (final concentration of ampicillin = 100 µg/mL and final concentration of arabinose = 2%)

**Note >** *Once ampicillin is added, DO NOT reheat the agar! Ampicillin will break down at high temperatures.*

8 Stack plates 3–5 high *(depending on how many you can comfortably pick up with one hand).*

9 Holding the whole stack in your hand, start by lifting up the lid of the plate on the bottom of the stack. *(Hold the lid open just enough for you to pour the LB Agar from the bottle).*

*Continues next page >*

## Skills

*Continued*

# Preparing LB Agar Plates

| | |
|---|---|
| 10 | Fill the plate about 1/3 full of liquid agar and replace the lid.<br><br>If you notice air bubbles or that the liquid agar has not evenly spread throughout the plate, gently swirl the plate, avoiding splashing agar on the lid of the dish. (For longer storage of plates, particularly at room temperature, pour thicker plates—about ½ full.) |
| 11 | Continue pouring from the bottom plate up.<br>*Work quickly as the agar may solidify.* |

**Note >** *Once you start pouring a bottle of LB agar, the whole bottle should be poured. Extra plates are useful for student practice.*

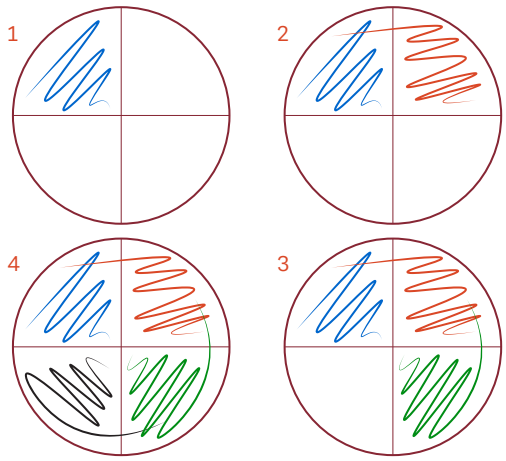
| | |
|---|---|
| 12 | Plates should be left out (cured) at room temperature for one to two days after pouring. Curing evaporates off the excess moisture from the condensation accumulated on the plates. |
| 13 | Tape sleeves closed to prevent accidental opening. |
| 14 | For storage, refrigerate as a stack in their original plastic sleeves with the plates *upside down* (the lid on the bottom). |

Skills

# Streaking Starter Plates

**Teacher Note >** *Starter plates are needed to produce bacterial colonies of* E. coli *on agar plates. LB agar plates should be streaked to produce single colonies and incubated at 37°C for 24 hours before the transformation begins. Under favorable conditions, one cell multiplies to become millions of genetically identical cells in just 24 hours. There will be millions of individual bacteria in a single millimeter of a bacterial colony. Depending on time, you may prefer your students to learn how to streak their own plates for individual colonies.*

| Procedure | |
|---|---|
| 1 | Draw quadrants on the underside of the Petri dish. Using a sterile inoculation loop or sterile pipette tip, pick up one bacterial colony from a live *E. coli* culture plate. |
| 2 | Using a back and forth motion, gently spread the colony into one quadrant of the LB starter plate. Keep the lid slightly tilted open—only as much as necessary. Be careful not to puncture the agar. |
| 3 | Rotate the plate one-quarter of a turn. Go into the previous streak about two times and then back and forth as shown for a total of about five to ten times. |
| 4 | Again, rotate the plate one-quarter of a turn and pass over a previous streak from the previous quadrant several times with the loop. |
| 5 | Repeat Step 3, but this time, drag out the loop to form a tail not touching any previous streaks. Close your plate to avoid further contamination. |

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## Skills

*Continued*

# Streaking Starter Plates

6 Place the used loop (or tip) in a disinfectant solution waste cup. Follow this procedure for the remaining starter plates. Once starter plates are inoculated, *incubate them upside down (lid on the bottom)* in a 37˚C incubator for 24 hours.

7 The next day you should see individual bacterial colonies in Quadrant 4, and very dense bacterial growth in Quadrant 1. Quadrants 2 and 3 will have bacterial density somewhere in between, similar to what is seen below:

## Bacterial Transformation with Gene Regulation Questions

**ANSWER KEY** **Do not share with students**

**Directions**
*Answer the questions below after closely reading the background material.*

1. Describe the process and goal of bacterial transformation in simple terms.

Answers will vary. Example: Bacterial transformation is the process of introducing foreign DNA into a cell so that it creates a desired protein product.

2. What is one example of how genetic engineering is used to help people?

Genetic engineering is used to produce insulin for people with diabetes.

3. Where is GFP found in nature?

Green Fluorescent Protein (GFP) was discovered in the bioluminescent jellyfish called Aequorea victoria, a jellyfish that fluoresces and glows in the dark.

4. How has GFP been used in scientific research?

The gene that codes for GFP was isolated in 1994 and was quickly used in laboratories as a way to brightly label proteins in a living cell (the DNA sequence for GFP was added to the DNA sequence of the protein of interest). This "tagging" of proteins allowed researchers to observe and track specific proteins inside cells to learn more about what they do inside the body.

5. What is a plasmid?

Plasmids are circular pieces of DNA found in bacteria that can replicate (make copies of themselves) independently of the large bacterial chromosome, and can transfer easily between cells.

6. To what antibiotic will the transformed bacteria be resistant? What does antibiotic resistant mean?

The transformed bacteria will be resistant to ampicillin, meaning that the bacteria will not be killed by it. They will be able to grow in the presence of the antibiotic because they have the ampr gene, which encodes the beta-lactamase protein, which breaks down ampicillin.

7. Why is the process of gene regulation often compared to a light switch?

Gene regulation is the process of turning genes on and off, like a light switch turns the lights on and off. When a gene is "on" it is said to be "expressed" (transcribed into mRNA that is then translated into protein). Transcription factors are the types of proteins that control gene expression.

8. AraC is a transcription factor that controls expression of GFP. What happens to the GFP gene when arabinose IS NOT present? What happens when arabinose IS present?

When arabinose IS NOT present, AraC binds DNA forming a loop and blocking RNA polymerase, so the GFP gene is OFF. It is not transcribed into mRNA so the protein is not made. When arabinose IS present, arabinose binds to araC breaking the DNA loop and allowing RNA polymerase to transcribe GFP gene. Arabinose switches the GFP gene ON.

## Protein Purification Questions

**ANSWER KEY** **Do not share with students**

**Directions**
*Answer the questions below after closely reading the background material.*

1. Describe one reason a company might want to purify a protein.

A company might want to purify a protein, such as insulin to use as medication for those with diabetes. Other possible answers: for food production (i.e., rennet → cheese) to study the protein function.

2. Why were six histidine amino acids added to the beginning of the GFP protein that you will be purifying?

The six histidine amino acids serve as a 'tag' on the GFP protein that can be used in nickel affinity chromatography to separate it from the rest of the cell lysate.

3. How do nickel-coated beads help separate the GFP from the rest of the proteins in the cell lysate? Include a brief description of the steps required to accomplish this isolation of GFP in your answer.

Histidine has an affinity for nickel so we can use nickel-coated beads to bind to the histidine along with GFP, centrifuge to separate from the rest of the proteins in cell lysate, perform a wash step, and then finally use elution buffer (containing imidazole) to release GFP from the beads.

## Student Guide, Part 1: Pre-Lab

**ANSWER KEY**

**Directions**

*In this lab, you will play the role of a molecular biologist exploring how to modify the DNA of an organism to create and isolate a useful protein product. To begin, carefully examine each station provided by your teacher and record two observations and two questions about what you see.*

1. Phenomenon Stations

Any observations and questions are relevant—the purpose is to spark curiosity and elicit prior knowledge.

2. Choose one of the three stations and draw a scientific model that explains how the images in the stations are related to each other. Consider the following questions to help you draw your model: What is going on inside the organisms? How was the product made?

This drawing serves as a pre-assessment of students' understanding of how protein products are made from genetically modified organisms. Students will build on this knowledge throughout the lab and make a detailed scientific model that explains the process of bacterial transformation and protein isolation.

3. Predictions: Below is a diagram of how your experiment will be set up in *Student Protocol, Part 1: Bacterial Transformation*. Use the information in the diagram and your understanding of bacterial transformation from the background reading to make predictions in the tables on the next page.

*Continues next page >*

## Student Guide, Part 1: Pre-Lab

**ANSWER KEY** **Do not share with students**

*Continued*

Example of possible predictions:

### *E. coli* without pQuince (–DNA)

| Type of LB Agar Plate | Bacteria growth? | Glowing green? | Explain Your Prediction<br>*The bacteria will/will not… because…* |
|---|---|---|---|
| LB | ■ Yes<br>☐ No | ☐ Yes<br>■ No | The bacteria will grow because there is no antibiotic in the plate that could kill the bacteria. The bacteria will not glow green because they do not have the GFP, which does not have the pKiwi plasmid DNA. |
| LB + Amp | ☐ Yes<br>■ No | ☐ Yes<br>■ No | The bacteria will not grow in the plate with antibiotics because the bacteria does not have the pQuince plasmid DNA, which carries the gene that makes the bacteria resistant to antibiotics. |

### *E. coli* with pQuince (+DNA)

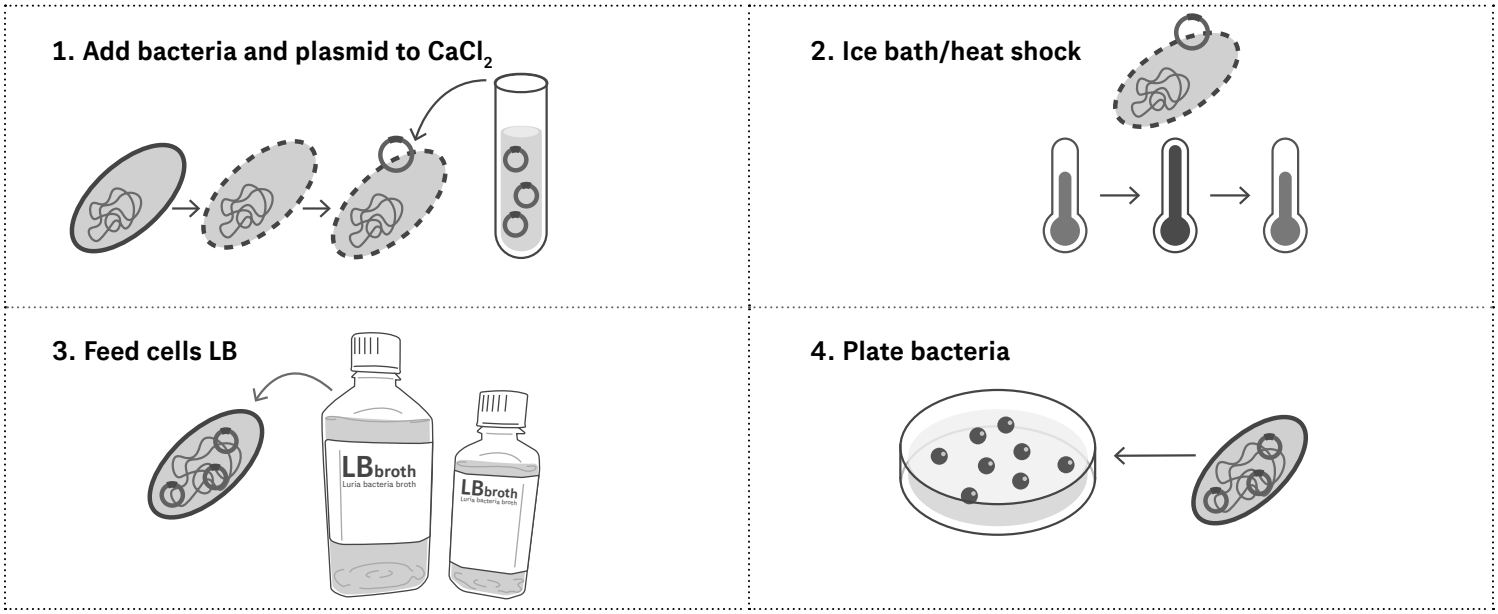
| Type of LB Agar Plate | Bacteria growth? | Glowing green? | Explain Your Prediction<br>*The bacteria will/will not… because…* |
|---|---|---|---|
| LB + Amp | ■ Yes<br>☐ No | ☐ Yes<br>■ No | The bacteria will grow because they have the antibiotic resistance gene. The bacteria will not glow green even though they have the pQuince plasmid because there is no arabinose present to bind to the AraC promoter and 'turn on' or express the GFP gene. |
| LB + Amp + Ara | ■ Yes<br>☐ No | ■ Yes<br>☐ No | The bacteria will grow because they have the antibiotic resistance gene. The bacteria will also glow green because there is arabinose present to bind to the AraC promoter and 'turn on' or express the GFP gene. |

## Student Guide, Part 2: Lab

**ANSWER KEY** — **Do not share with students**

**Directions**
*Answer the questions below to prepare for the lab and record your data after completing the lab.*

1. The image below provides an overview of the steps you will perform to transform your bacteria. Record the purpose of each step as your teacher explains:




1.

Adding bacterial and plasmid to $CaCl_2$ → Makes cell walls permeable.

2.

Ice bath/heat shock → Increases holes in cell membrane so pQuince DNA can get in.

3.

Feed cells with a nutrient broth (LB) → Helps stressed-out cells recover.

4.

Plate bacteria → Grow bacteria and determine if transformation was successful.

*Continues next page >*



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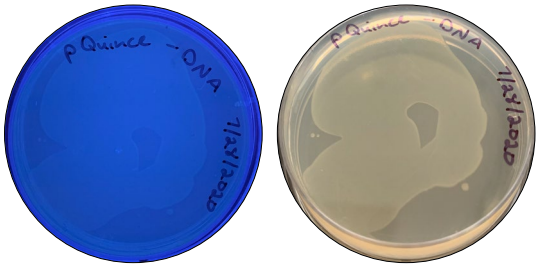
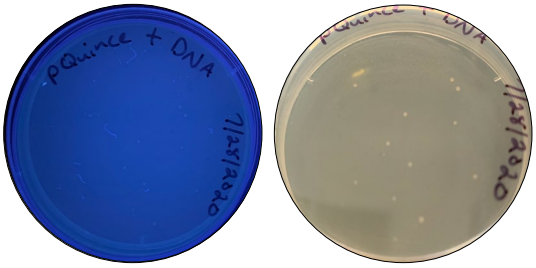
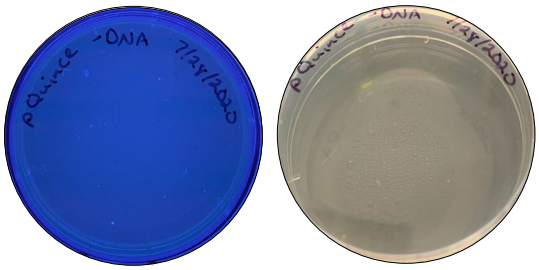
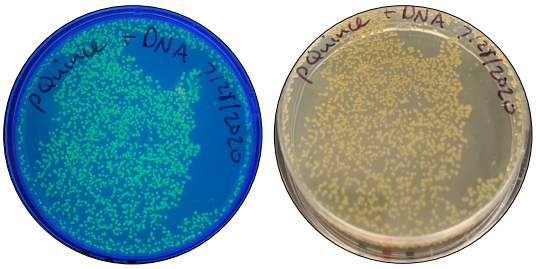
## Student Guide, Part 2: Lab

**ANSWER KEY** **Do not share with students**

*Continued*

2. Record the number of colony forming units (CFU) that grew and sketch your observations for each plate in the chart below. (If there are too many bacteria colonies to count, write "lawn".)

Expected results below in table with results seen under the transilluminator and natural light

| Plate | ***E. coli* without pQuince** (–DNA) | Plate | ***E. coli* with pQuince** (+DNA) |
|---|---|---|---|
| LB |  <br># colonies Yes, lawn<br>glowing green? No | LB + Amp |  <br># colonies 11 CFU<br>glowing green? No |
| LB + Amp |  <br># colonies None<br>glowing green? No | LB + Amp + Ara |  <br># colonies >500 CFU<br>glowing green? Yes |

*Continues next page >*

## Student Guide, Part 2: Lab

**ANSWER KEY** **Do not share with students**

*Continued*

3. *Consider your results for Student Protocol, Part 2: Protein Purification:* Did you successfully isolate GFP from the transformed *E. coli* bacteria? What evidence supports your answer?

Answers will vary but students should include some sort of empirical evidence from their lab results. Example: Yes, I successfully isolated GFP from the transformed *E. Coli* bacteria because I can see a substance collected at the bottom of my microtube that is glowing green.

## Student Guide, Part 3: Data Analysis

**ANSWER KEY** — **Do not share with students**

**Directions**

*Analyze your results from the lab by answering the questions below.*

1. Which results were the same as your predictions? What does this mean? *A result that supported my prediction was ….This means that…*

Answers will vary but students should include in their explanation a comparison of their prediction for each plate and the actual results.

2. Which results were different from your predictions? What could have caused this? *A result that refuted my prediction was…This could have happened because…*

Answers will vary but students should include in their explanation a comparison of their prediction for each plate and the actual results.

3. Describe two potential sources of error in *Student Protocol, Part 1: Bacterial Transformation* (mistakes that lead to unexpected results) and how they may affect the results. Errors include: systematic/procedural, random, and human.

| Source of error | How it may affect results |
|---|---|
| Not enough plasmid DNA added to bacteria in the beginning of the lab. *human* | Either all of the bacteria will die on the LB/ampicillin and LB/Amp/Ara plates or there will be a very low number of colonies because there are not enough bacteria transformed with ampicillin resistance. |
| Heat shock period too short. *human* | Bacteria do not have enough time to take in the plasmid DNA. This means that not a lot of bacteria would be transformed and no growth would be seen on either of the +DNA plates since the bacteria are not resistant to ampicillin. |
| Transfer of bacteria from ice to hot water bath took too long. *human* | The bacteria could heat up if the transfer took too long, and the bacteria will not be as shocked as expected. This means that the surfaces of the bacteria would not become porous enough to efficiently take in the plasmid DNA. Either all of the bacteria will die on the LB/ampicillin and LB/Amp/Ara plates or there will be a very low number of colonies. |

4. Consider your results for *Student Protocol Part 2: Protein Purification:* Why is it useful for scientists to be able to isolate a protein product from a genetically modified organism? Describe one example.

It is useful for scientists to be able to isolate a protein product from a GMO to be used as medicine or to make food.

Students may need to conduct research to learn about other applications, such as rennet (enzyme to curdle milk for cheese) and heme (Impossible Burger). Example below: Humulin is a drug produced by genetic engineering techniques. It is made by inserting human genes responsible for insulin production into *E. Coli* bacteria, thus stimulating the bacteria to synthesize insulin.

## Student Guide, Part 4: Making a Model

**ANSWER KEY** — **Do not share with students**

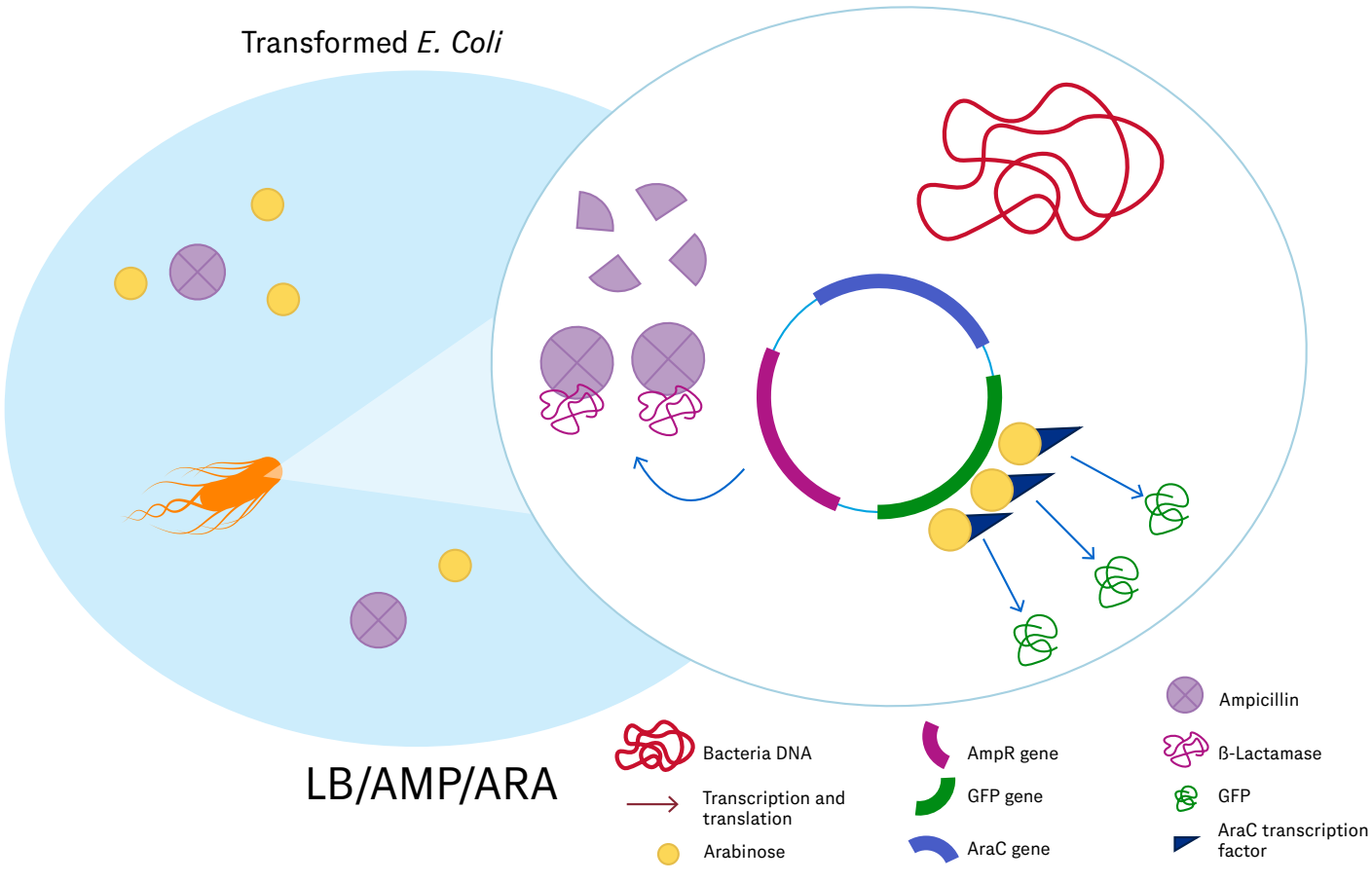
**Directions**

*Review the scientific model you drew in Question #2 of the Student Guide, Part 1: Pre-Lab and consider what changes you could make based on what you have learned. Then, follow the steps below to create a new model.*

1. A scientific model represents the observable (visible) and unobservable (invisible) features of a phenomenon. Draw a model that explains what occurs inside transformed bacterial cells to produce a new protein.

*Fill in the blanks below to help plan your model.*

| | |
|---|---|
| Organism being modified: | *E. coli* bacteria |
| Names of new genes: | Green fluorescent protein (GFP), AmpR, AraC |
| Proteins for which new genes code: | GFP, Beta-Lactamase, AraC |
| Traits to which the new genes lead: | Green fluorescence, antibiotic resistance, expression of GFP |
| Organism from which GFP came: | Jellyfish |




*Continues next page >*

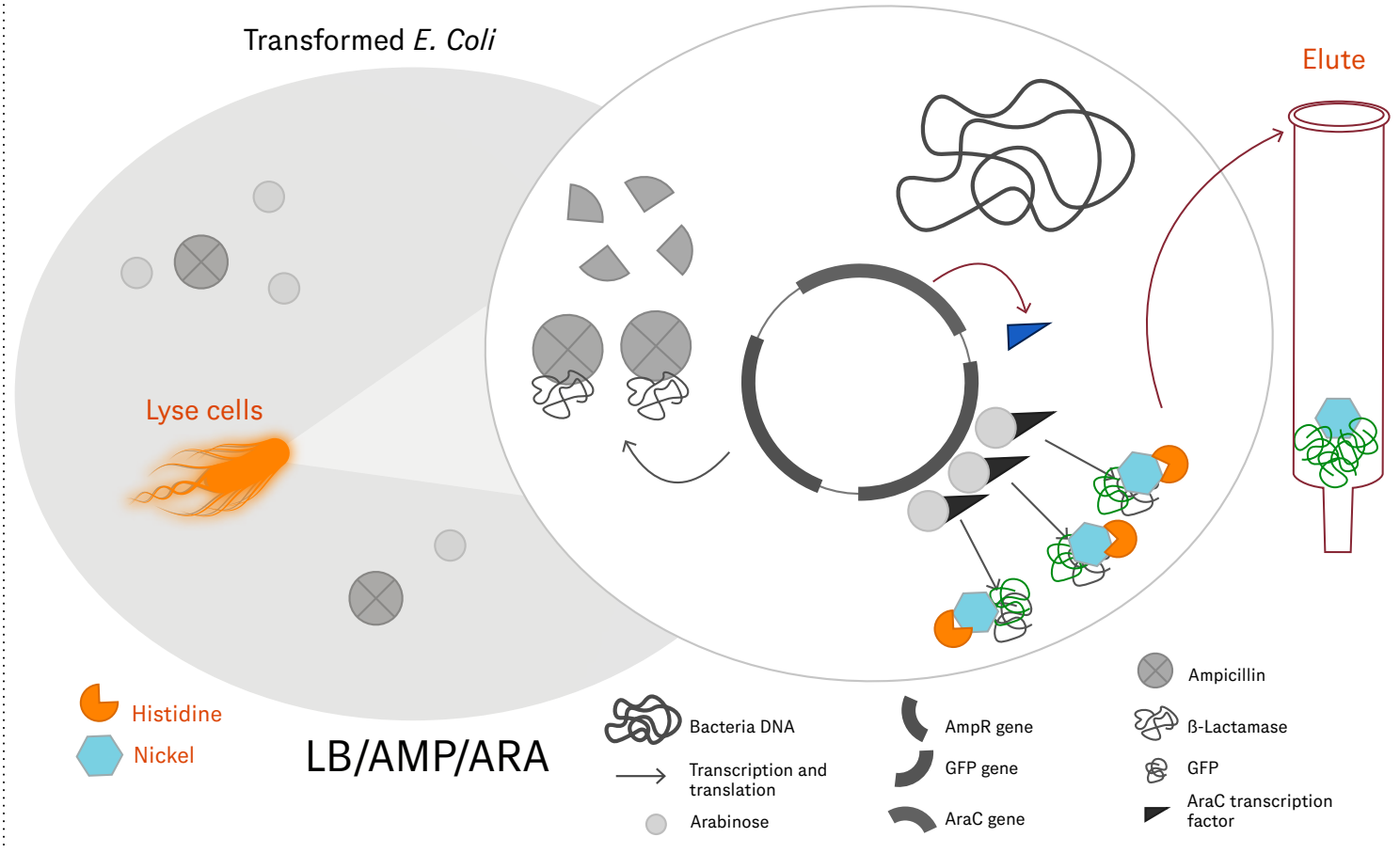
## Student Guide, Part 4: Making a Model

**ANSWER KEY** **Do not share with students**

*Continued*

2. Work with your group to add on to your model to explain how the GFP produced by the transformed bacteria can be isolated by nickel affinity chromatography. Use the following keywords in your diagram:

— Histidine
— Nickel
— Elute
— Lyse




*Continues next page >*

## Student Guide, Part 4: Making a Model

**ANSWER KEY** **Do not share with students**

*Continued*

3. After viewing other models and receiving feedback on your model, describe three limitations of your model. Consider the following questions:
   a. What parts of the bacterial transformation and protein purification system are not shown in the model?
   b. What is an assumption in your model and how does it impact the reliability of the model?
   c. What is estimated, rather than observed directly, in your model?

Examples of limitations include:

- Precise molecular structures of DNA, RNA, and proteins and how these structures relate to their functions.
- A sense of quantity and scale (e.g., How many plasmid molecules are added to one bacteria cell? How many GFP proteins are made from one GFP gene? How do the sizes of the molecules compare?)
- Model assumes other cellular processes happening inside the bacteria cell are not impacting production of the introduced proteins.
- Model assumes that every gene introduced in the bacteria leads to a complete, functional protein.
- Cannot directly observe the processes of transcription and translation.

4. Consider how you could use your model in further investigations. Could you use your model to reliably *predict* how to modify the DNA of any organism to create and isolate a useful protein product? Explain why or why not and provide an example.

Potential ideas:

- The model can be useful for getting a general impression of how a new gene introduced into an organism could possibly lead to a new protein that can then be isolated.
- It may reliably predict how to isolate a different his-tagged protein (maybe insulin) from transformed *E. coli* bacteria.
- It may not reliably predict how to modify DNA in different organisms (e.g., a plant may require a different process of introducing foreign DNA than *E. coli* bacteria).
- This model specifically shows how to isolate a his-tagged protein product using affinity chromatography. If the protein has a different molecular tag or no tag, the model cannot reliably predict how it could be isolated.

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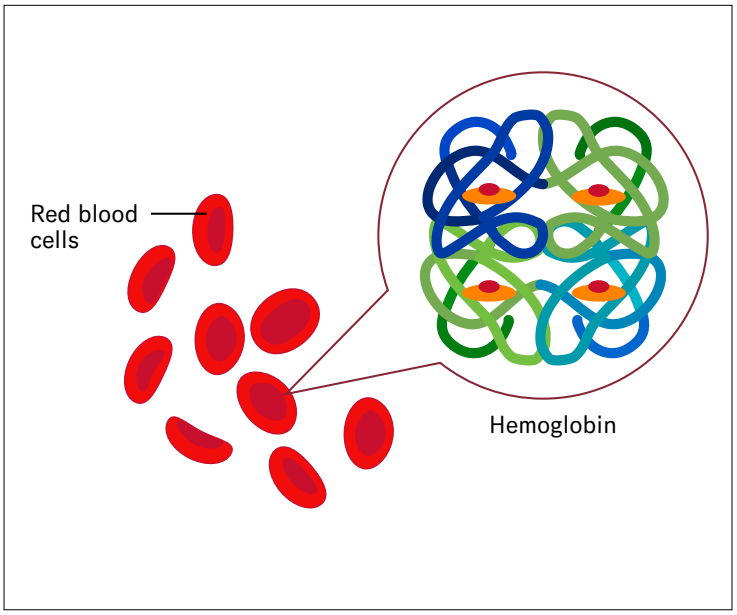
## Phenomenon Station 1

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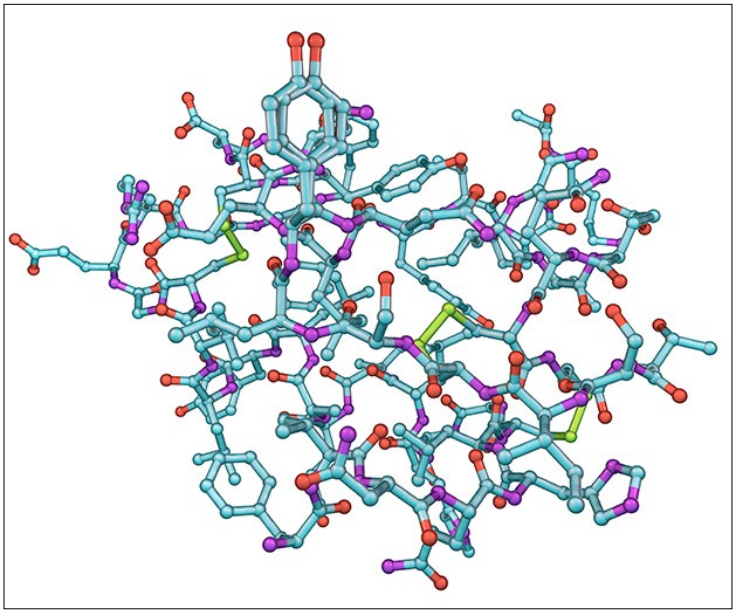
## Phenomenon Station 2

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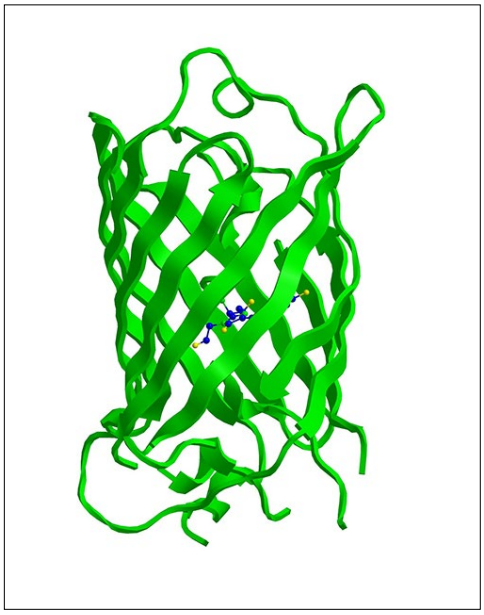
## Phenomenon Station 3



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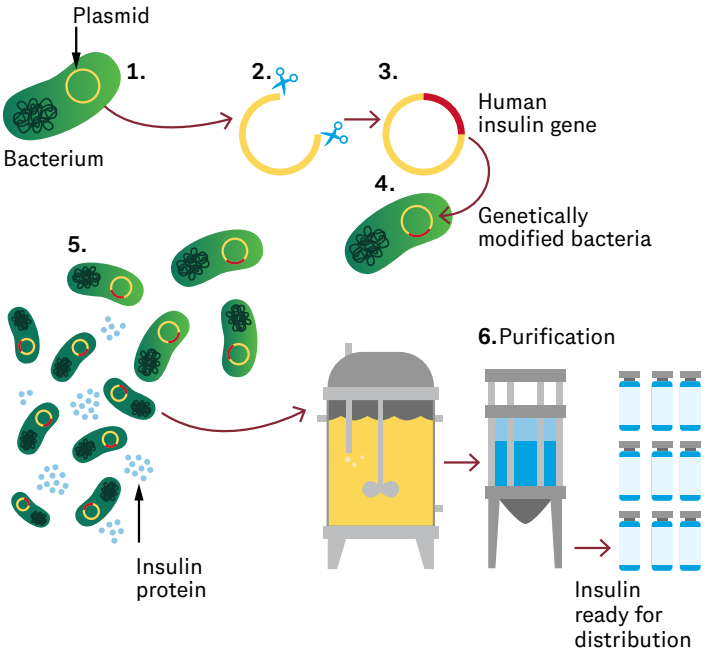
## Background Reading: Bacterial Transformation with Gene Regulation

### Bacterial Transformation

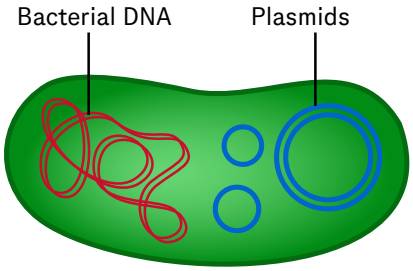
Bacterial transformation is a widely used tool in genetic engineering in which bacteria take in a piece of DNA from a different organism. As a result, the bacteria build the new proteins and express the new traits that were encoded in the foreign piece of DNA. This process has many important biotechnology uses in areas such as biofuels and medicine. For example, many people with diabetes need insulin made from bacteria that have been transformed with the human insulin gene.

### Insulin production from genetically engineered bacteria




Bacteria are commonly used for genetic transformation experiments because they are simple, single-celled organisms that grow and reproduce very quickly. Bacterial cells store their DNA on one large, circular chromosome. They may also contain one or more small circular pieces of DNA called plasmids. Plasmids replicate (make copies of themselves) independently of the large bacterial chromosome, and can transfer easily between cells. As a result, plasmid DNA allows for the addition of new traits into a cell. Humans have taken advantage of bacteria's natural process of transferring plasmids to introduce new DNA (and therefore new proteins and traits) into organisms.

### Two Forms of DNA in Bacteria

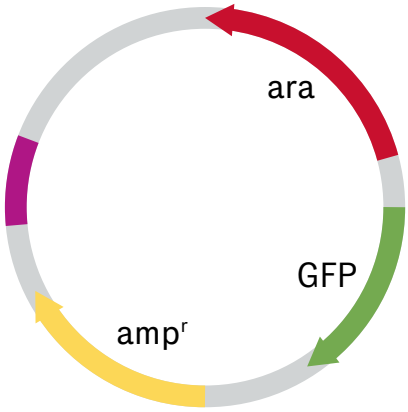



### pQuince

In this lab, you will genetically modify *E. coli* bacteria. You may have heard of certain strains of *E. coli* making someone sick, but this strain is non-pathogenic and is used widely in labs. We will transform it by adding a plasmid called pQuince that contains three important genes:

- Green Fluorescent Protein Gene *(GFP)*: Codes for Green Fluorescent Protein *(GFP)*, which causes the bacteria to glow green under UV light.
- Ampicillin Resistance Gene *(amp$^r$)*: Codes for ß–Lactamase protein, which breaks down the deadly antibiotic ampicillin, preventing it from killing the bacteria.
- AraC Gene *(araC)*: Codes for AraC protein, which controls expression of the GFP gene.

### pQuince plasmid

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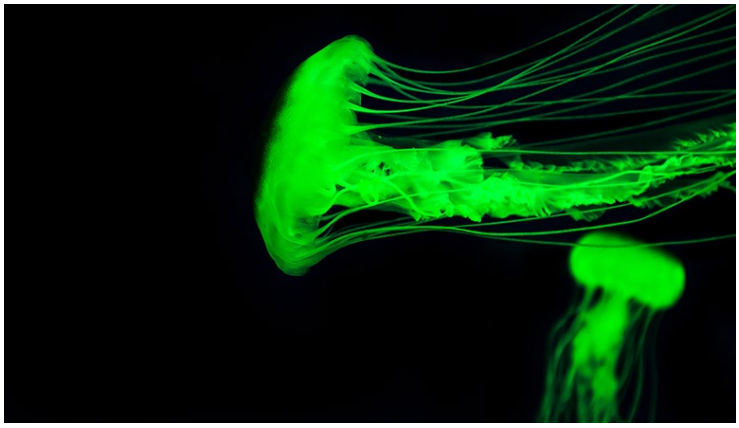
## Background Reading: Bacterial Transformation with Gene Regulation

*Continued*

### Green Fluorescent Protein

Green Fluorescent Protein (GFP) was discovered in the bioluminescent jellyfish called *Aequorea victoria*, a jellyfish that fluoresces and glows in the dark. The gene that codes for GFP was isolated in 1994 and was quickly used in laboratories as a way to brightly label proteins in a living cell (the DNA sequence for GFP was added to the DNA sequence of the protein of interest). This "tagging" of proteins allowed researchers to observe and track specific proteins inside cells to learn more about what they do inside the body. The discovery of GFP proved to be so important that the Nobel Prize in Chemistry was awarded to Osamu Shimomura, Marty Chalfie, and Roger Tsien in 2008 for their work. Since then, Roger Tsien's laboratory at University of California San Diego (UCSD) has altered the GFP gene to make a full rainbow of proteins.

### Natural Expression of GFP in Some Jellyfish



### Bacteria expressing fluorescent proteins of different colors

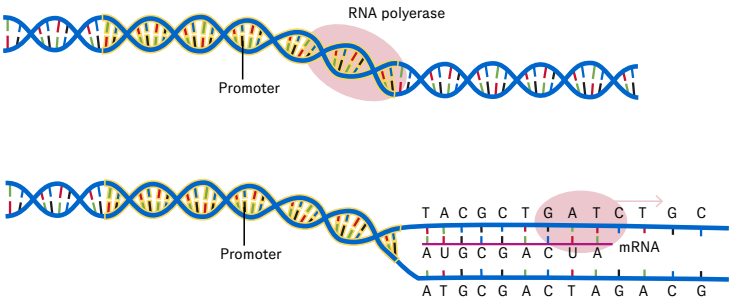
### Antibiotic Resistance

The plasmid we are using contains an ampicillin-resistance gene (*amp*$^r$) in addition to the GFP gene. Ampicillin is a type of antibiotic, which means it kills bacteria. When *amp*$^r$ is expressed, it makes an enzyme called ß–Lactamase that breaks down ampicillin so that bacteria can survive in the presence of ampicillin. Including this gene in the plasmid is important because it allows us to isolate only the *E. coli* cells that have been successfully transformed. When introduced to media that contain ampicillin, the bacteria that took up the plasmid will survive while all other bacteria will not.

### Gene Regulation

The pQuince plasmid also contains the araC gene, which controls the expression of the GFP gene. In fact, there are specific processes for controlling which genes are turned "on" and which are turned "off" in every single cell of your body. When a gene is expressed or 'turned on', it means the information stored in its sequence of DNA is used to make a gene product (usually a protein). In other words, when a gene is expressed, it is transcribed into mRNA that is then translated into protein. However, not all genes are expressed in every cell. This is why your brain cells and muscle cells are very different even though they have all the same genes. For example, brain cells express receptor proteins that allow them to receive messages from neurotransmitters and muscle cells express motor proteins that allow them to contract.

### A gene being transcribed

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## Background Reading: Bacterial Transformation with Gene Regulation

*Continued*

Gene regulation refers to all the different cellular processes that control when and where genes are expressed in every cell of your body. Proteins called transcription factors are used by cells to turn transcription on or off depending on the conditions inside the cell, kind of like a light switch. Transcription factors bind to the promoter of a gene, a region of DNA that initiates transcription of a particular gene. When the transcription factor binds to this section of DNA, it recruits the RNA Polymerase to the gene and transcription begins (turning the gene "on"). Once RNA polymerase transcribes the DNA into mRNA, the gene is then said to be "expressed." Once the mRNA transcript is made, it can be translated into protein.

### AraC and Arabinose

In pQuince, GFP gene expression requires three main materials: AraC transcription factor protein, RNA polymerase, and arabinose (a sugar). In a growth media that does not contain arabinose (LB or LB/Amp), only two of those are present: AraC and RNA polymerase. In this case, AraC binds to a region of DNA that causes it to fold in on itself and make a loop. This loop physically prevents RNA polymerase from binding to the promoter and transcribing the GFP gene. Therefore, in normal growth media, the GFP gene inside the bacteria is 'off' (i.e., not expressed).

However, when arabinose is present in growth media, it binds to AraC. This AraC+arabinose complex breaks the DNA loop, allowing RNA polymerase to bind to the promoter of the GFP gene and begin transcription. Therefore, in growth media with arabinose, the GFP gene inside the bacteria is switched 'on', resulting in production of the glowing green protein.

Sources:

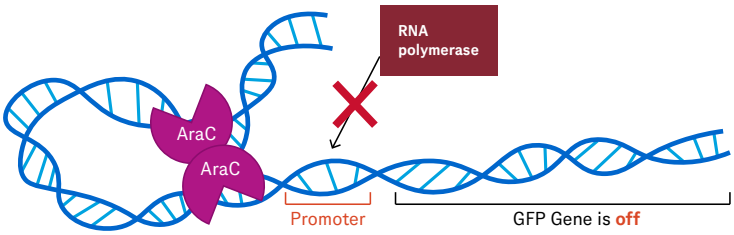
*Protocol—Bacterial Transformation*

*Genetic Transformation*

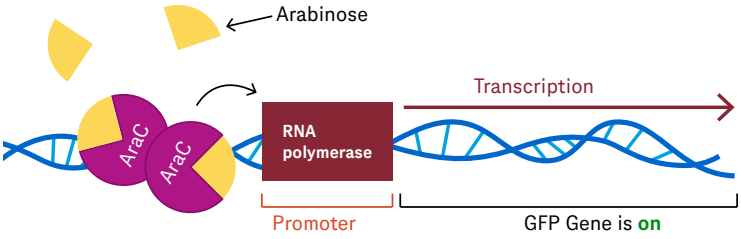
*GFP History*

*Gene Expression*

### Absence of arabinose, GFP gene 'off'




### Presence of arabinose, GFP gene 'on'



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## Bacterial Transformation with Gene Regulation Questions

**Directions**
*Answer the questions below after closely reading the background material.*

1. Describe the process and goal of bacterial transformation in simple terms.

2. What is one example of how genetic engineering is used to help people?

3. Where is GFP found in nature?

4. How has GFP been used in scientific research?

5. What is a plasmid?

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## Bacterial Transformation with Gene Regulation Questions

*Continued*

6. To what antibiotic will the transformed bacteria be resistant? What does antibiotic resistant mean?

7. Why is the process of gene regulation often compared to a light switch?

8. AraC is a transcription factor that controls expression of GFP. What happens to the GFP gene when arabinose IS NOT present? What happens when arabinose IS present?

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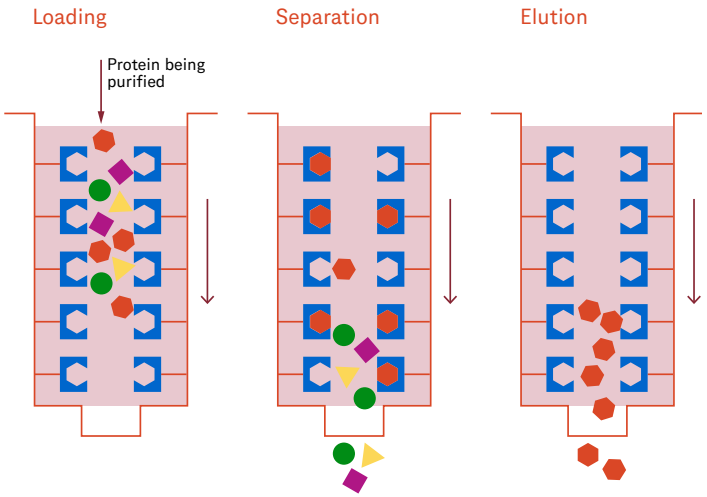
## Background Reading: Protein Purification

### Why purify a protein?

Obtaining protein that is purified (separated from all other molecules in a cell) is an important process for many companies and research labs. Pharmaceutical companies manufacture proteins, such as insulin, for use as medication. Rennet is an example of a protein that is purified by food production companies to curdle milk to make cheese. Researchers and biotech companies often wish to study the function of proteins in biochemical assays, for example to figure out the enzyme activity of a protein. To this end, many purification techniques have been developed to isolate the protein of interest from the multitude of other proteins in the cell.

Chromatography is a lab technique for separating the components of a mixture. In this lab, we will use chromatography to isolate Green Fluorescent Protein from the *E. coli* bacteria you transformed. First, we must lyse or break open the cells. Then, the cell lysate (the contents of a cell after it has been lysed or burst) is mixed with a binding substance that will interact only with the type of protein we want to isolate. Usually, the binding substance is packed into a column through which the lysate is passed. The proteins that bind to the column are separated from the rest of the lysate and can be subsequently collected in a now more concentrated solution (see below):

### Column Chromatography

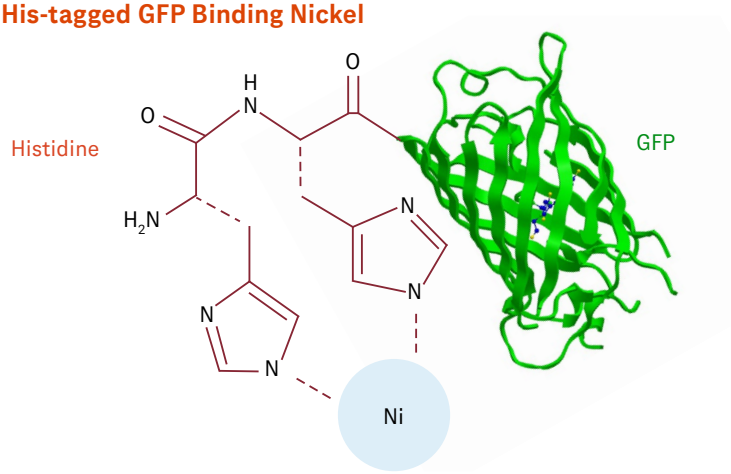



### Nickel Affinity Chromatography

In the purification you will be performing in the lab, the protein of interest has been modified through genetic engineering. DNA that codes for six extra histidine (his) amino acids have been added to the beginning of the DNA sequence for the Green Fluorescent Protein (GFP) gene. As a result, once the DNA has been transcribed into mRNA and translated into protein, GFP includes a new "tag" made of histidines. Because only this protein will have this unique tag, the purification process specifically separates GFP from the other proteins in the cell lysate.

In this procedure, instead of passing the cell lysate through a column, affinity beads that are coated in nickel (Ni) are added to the cell lysate. Nickel-coated beads are used because nickel has an affinity for (will bind to) the histidine tag on GFP. The side chain of histidine, known as imidazole, is polar and will have an affinity for the positively-charged Ni atoms. The protein-bound bead can then be separated from the rest of the proteins in the cell lysate through centrifugation (spinning).

### His-tagged GFP Binding Nickel




Following a wash step, the protein can be released from the beads by adding an elution buffer. The elution buffer contains an excessive amount of imidazole, which will outcompete the 6-his-tagged GFP, causing it to release from the bead. The released protein is now in the flow-through (known as the effluent). Thus, the protein can be freed (or eluted) from the nickel beads and collected in the effluent. The resulting purified green fluorescent protein is now ready to be studied in further biochemical assays.

Sources: *Purification of Proteins Using Polyhistidine Affinity Tags*

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## Protein Purification Questions

**Directions**
*Answer the questions below after closely reading the background material.*

1. Describe one reason a company might want to purify a protein.

2. Why were six histidine amino acids added to the beginning of the GFP protein that you will be purifying?

3. How do nickel-coated beads help separate the GFP from the rest of the proteins in the cell lysate? Include a brief description of the steps required to accomplish this isolation of GFP in your answer.

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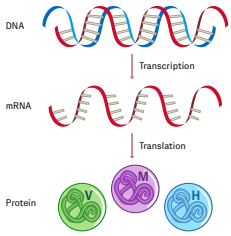
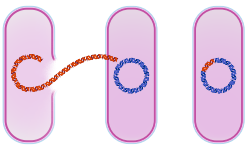
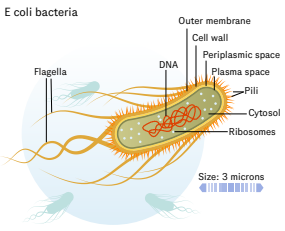
## Vocabulary Tool: Bacterial Transformation

**Directions**
*For each vocabulary word, write a new sentence that helps you practice using it.*

| Word | Image | Definition | Example Sentence | My Sentence |
|---|---|---|---|---|
| **Genetic Engineering** |  | The process of changing an organism's genes using biotechnology to make something useful | *Genetic engineering* is used to make pest-resistant crops, such as Bt corn. | |
| **Gene Expression** |  | When information stored in a gene's DNA sequence is used to make a product (usually a protein) | When the GFP *gene is expressed*, it is transcribed into mRNA, and then translated into a protein. | |
| **Gene Regulation** |  | The mechanisms that control whether or not a gene is expressed ("on" or "off") | Even though all of your cells contain all of your DNA, they look different from each other because *gene regulation* controls which genes are expressed in which cells. | |
| **Bacterial Transformation** |  | A process during which a bacterial cell takes in a new gene from a different organism; this results in the bacteria having a new trait. | One method of genetic engineering is *bacterial transformation*. | |
| ***E. Coli*** |  | Bacteria naturally found inside human intestines. Some *E. coli* are harmless (such as the strain used in bacterial transformation), but others can make you sick. | The type of bacteria we are transforming is called *E. Coli*. | |

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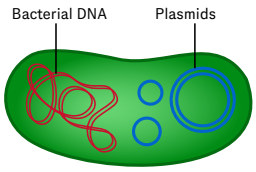
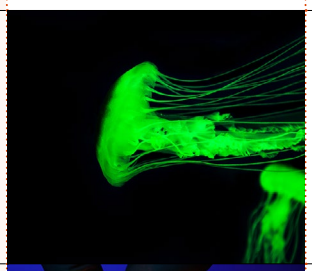
## Vocabulary Tool: Bacterial Transformation

*Continued*

| Word | Image | Definition | Example Sentence | My Sentence |
|---|---|---|---|---|
| **Plasmid** |  | A small, circular piece of DNA separate from chromosomes | We are transforming bacteria with a *plasmid* called pQuince. | |
| **GFP** *Green Fluorescent Protein* |  | A protein from a jellyfish that makes organisms glow green | The mice are glowing because they have *GFP*. | |
| **Fluorescence** |  | The emission of light (glowing) | Some jellyfish display *fluorescence* because they express GFP. | |
| **Bacteria Culture** |  | A method through which bacteria are grown in a Petri dish using a nutrient-rich medium. | We will grow *bacteria cultures* to see if we successfully transformed the *E. coli*. | |
| **Agar Medium** |  | A nutrient-rich, jelly-like solid used to grow bacterial cultures | You will grow bacteria on an *agar medium.* | |

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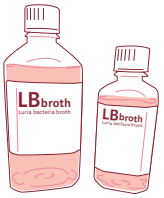
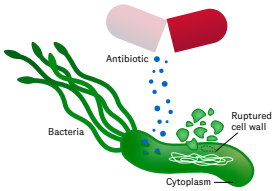
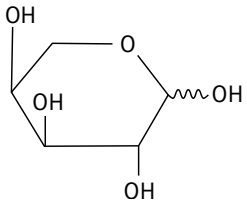
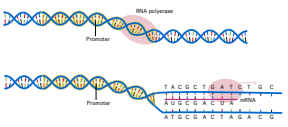
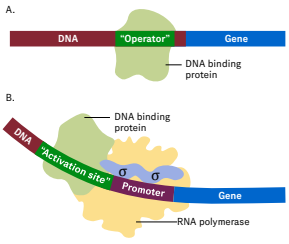
## Vocabulary Tool: Bacterial Transformation

*Continued*

| Word | Image | Definition | Example Sentence | My Sentence |
|---|---|---|---|---|
| **LB**<br>*Luria Broth* |  | A nutrient-rich liquid used to grow bacteria | *LB* is food for the bacteria. | |
| **Ampicillin** |  | A type of antibiotic (kills some bacteria) | *Ampicillin* kills bacteria. | |
| **Arabinose** |  | A type of sugar that binds to the transcription factor AraC | When *arabinose* binds to the AraC protein, the GFP gene will be expressed on the pQuince plasmid. | |
| **Promoter** |  | The region of DNA that initiates transcription of a gene | When RNA polymerase binds to the *promoter* of the GFP gene, it can be transcribed into mRNA. | |
| **Transcription Factor** |  | A type of protein that controls the expression of a gene; it can activate or repress transcription. | AraC is the *transcription factor* that controls expression of GFP on the pQuince plasmid. It acts as an activator in the presence of arabinose and a repressor when arabinose is not present. | |

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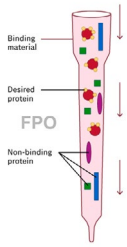
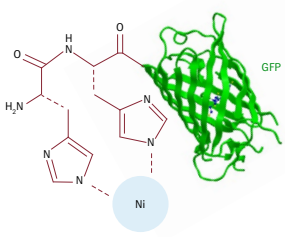
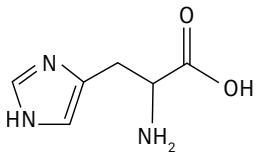
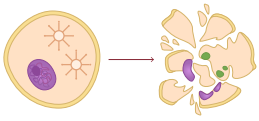
## Vocabulary Tool: Protein Purification

**Directions**
*For each vocabulary word, write a new sentence that helps you practice using it.*

| Word | Image | Definition | Example Sentence | My Sentence |
|---|---|---|---|---|
| **Chromatography** |  | A lab technique for separating the components of a mixture | Nickel Affinity *Chromatography* can be used to separate a protein from a cell. | |
| **Affinity** |  | A linking or similarity between two things (in biology affinity = binding/sticking). | The histidine "tag" on GFP has an *affinity* for nickel. | |
| **Histidine** |  | An amino acid that is used as a tag on GFP; will bind to nickel. | If the GFP did not have a *histidine* tag, we would not be able to separate it from the bacterial cells using nickel. | |
| **Lyse** |  | To break open a cell wall and/or membrane | Before we can isolate GFP from the bacterial cells, we need to *lyse* them to release the proteins inside. | |
| **Elute** |  | To remove a substance by washing it with a solvent | To remove the GFP from the nickel beads, we will *elute* it with imidazole, which will bind to the nickel instead, releasing GFP. | |

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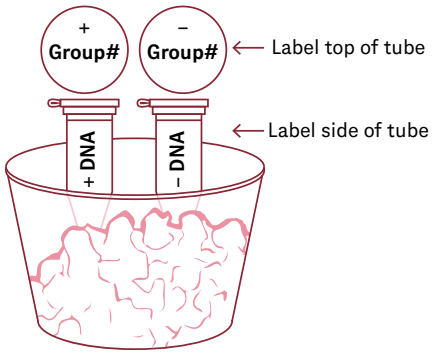
## Student Protocol

*Part 1: Bacterial Transformation*

**Directions**

*Perform this protocol in a group of four. Select one pair of students to be in charge of the "–DNA" tube and one pair of students to be in charge of the "+DNA" tube.*

| | |
|---|---|
| **1** | Label Tubes |
| ☐ | Using a permanent marker, label one tube as *–DNA* and one tube as *+DNA*. Also label tubes with your *Group #*. Label tubes on both the lid and the side. |
| ☐ | Place these tubes in a cup of crushed ice or ice bath. |




| | |
|---|---|
| **2** | Add $CaCl_2$ (calcium chloride) |
| ☐ | Add 300 µL of $CaCl_2$ to each tube using the P1000 micropipette. Keep the same tip. |
| ☐ | Place the tubes back in ice. |

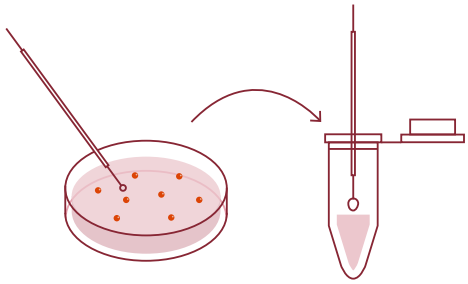
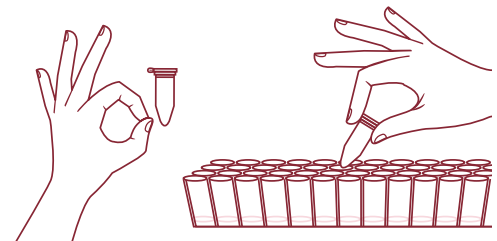
*Continues on next page >*

FUTURELAB+

## Student Protocol

*Part 1: Bacterial Transformation*

*Continued*

| | |
|---|---|
| **3** | Pick up Bacteria for *–DNA* Tube |
| ☐ | Get a sterile loop without touching the ends of the loop. |
| ☐ | Use the large end of the loop to swipe a small amount of bacteria (just barely enough for you to see) off the starter plate. *Avoid gouging (digging) into the agar.* |
| ☐ | Dip the loop into the *–DNA* tube. Tap and twist the loop several times to *make sure the bacteria goes into the tube*. Nothing should be seen on the loop when finished.  |
| **4** | Pick up Bacteria for *+DNA* Tube |
| ☐ | Repeat Step 3 for the + DNA tube using a new loop. |
| **5** | Mix the Bacteria |
| ☐ | Hold a tube of bacteria in one hand and flick with the other or drag the tube across the rack to thoroughly mix the bacteria. |
| ☐ | Check that there are no clumps of bacteria in the tubes—it should look cloudy or hazy.  "Rack" your tube by applying firm downward pressure. |

*Continues on next page >*

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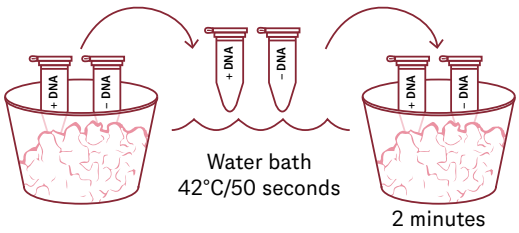
## Student Protocol

*Part 1: Bacterial Transformation*

*Continued*

| | |
|---|---|
| **6** | Add plasmid DNA |
| ☐ | Add 10 µL plasmid DNA to the +DNA tube using the P20 micropipette and flick to mix. |
| ☐ | DO NOT add plasmid to the "–DNA tube" (it is the negative control). |
| **7** | Incubate on Ice |
| ☐ | Place both tubes in ice for *10 minutes*.<br>*Make sure the tubes are in contact with the ice.* |
| ☐ | While waiting, read through the next step as it is very important for success. |
| **8** | Heat Shock |
| ☐ | Keep your tubes on ice and bring them to the *42˚C* water bath. |
| ☐ | Heat shock your bacteria by immediately transferring both tubes from the ice to a tube floater in the water bath for *50 seconds*.<br>*Make sure the tubes have full contact with the warm water.* |
| ☐ | After 50 seconds, *quickly* place both tubes back on *ice for another 2 minutes*.<br>  <br><br>**Note >** *It is VERY important to watch the time and speed of the transfers.* |

*Continues on next page >*

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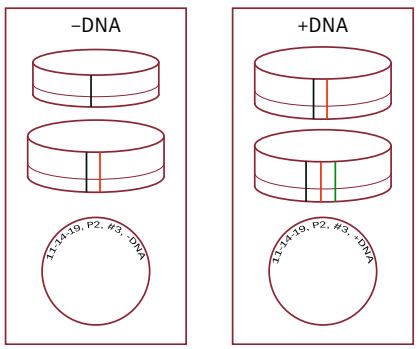
## Student Protocol

*Part 1: Bacterial Transformation*

*Continued*

| | |
|---|---|
| **9** | Feed the Bacteria. |
| ☐ | Take your tubes out of the ice and place them in a tube rack. |
| ☐ | Add *300 µL of LB broth* (food) to each of the tubes using the P1000 micropipette. Do not touch the bacteria with the tip. |
| ☐ | Close the lids and mix each tube turning the tubes upside-down (inverting) several times. |
| **10** | Let the Bacteria Recover. |
| ☐ | Incubate the tubes for at least *5 minutes at 37˚C and up to 24 hours.* |

**Note >** *Potential stopping point until next class.*

| | |
|---|---|
| **11** | Label Plates. |
| ☐ | Get the following four agar plates:<br>— One LB plate (plate with one black stripe)<br>— Two LB/Amp plates (plates with one black and one red stripe)<br>— One LB/Amp/Ara plate (plates with one black, one red, one green stripe) |
| ☐ | On the bottom of the plate (not the lid) – label with:<br>— Date<br>— Period #, Group #<br>— +DNA or –DNA<br>  |

**Note >** ***Do not write*** *in the middle of the plate, write in a curve along the edge.*

*Continues on next page >*

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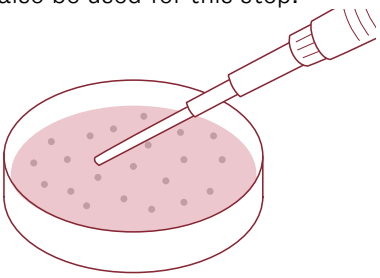
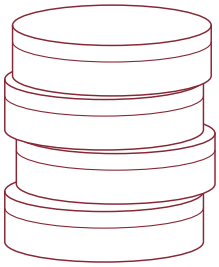
## Student Protocol

*Part 1: Bacterial Transformation*

*Continued*

| | |
|---|---|
| **12** | Plate Bacteria. |
| ☐ | Remove the lids from the agar plates. |
| ☐ | Transfer 150 µL of the *–DNA* bacteria to each plate labeled with *–DNA*.<br>*Dribble directly onto the agar plate (not on the lid).* |
| ☐ | Repeat for *+DNA*, using a new tip. |
| ☐ | Swirl to spread the bacteria evenly. Allow bacteria to soak into the agar plates until you do not see a pool of liquid on the agar. A sterile polystyrene cell spreader may also be used for this step.<br>  |
| **13** | Prepare Plates for Incubation. |
| ☐ | Stack the plates together with the lids on top. |
| ☐ | Your teacher will place plates (upside down) into a 37˚C incubator until the next day or when bacteria colonies are visible. Alternatively, stack the plates into a warm spot in the classroom. It may take 2–3 days for bacterial colonies to appear.<br>  |
| **14** | Clean and wash up! |
| ☐ | Clean up your trash and instruments. Wipe down all lab surfaces with disinfectant and wash your hands. |
| ☐ | Per teacher instruction, prepare your lab station so it is ready for the next class. |

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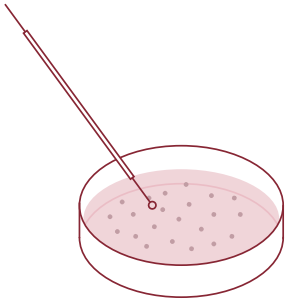
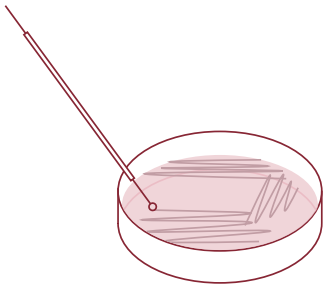
## Student Protocol

*Part 2: Protein Purification*

**Directions**

*Perform this protocol in a group of two.*

| | |
|---|---|
| **1** | **Retrieve transformed bacteria** |
| ☐ | Ensure *E. coli* is successfully transformed by confirming that they glow under UV light. |
| **2** | **Label a fresh LB/Amp/Ara plate** |
| ☐ | On the edge of the plate, write your name or group number, the date, period and plasmid (pQuince). |
| **3** | **Pick up a glowing colony** |
| ☐ | Use the large side of a sterile loop to *gently* lift up a bacterial colony. |
| ☐ | Gently touch or scrape the colony off the plate without gouging the agar.  |
| **4** | **Streak the colony** |
| ☐ | Remove the lid from the LB/Amp/Ara plate you labeled. |
| ☐ | Start with your loop at the top left of the plate and gently move it over the surface of the agar to the right in a *zigzag motion*.  |

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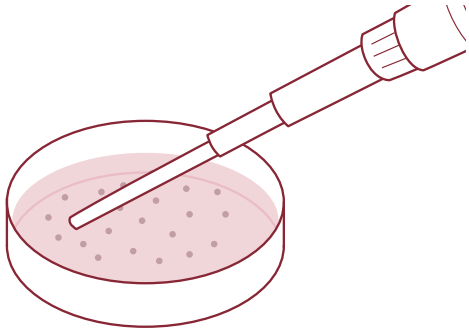
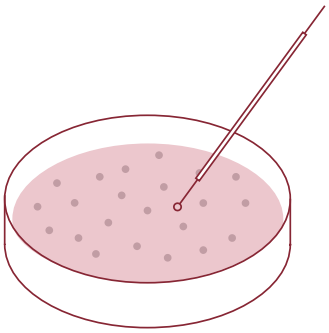
**Student Protocol**
*Part 2: Protein Purification*

*Continued*

| | |
|---|---|
| **5** | Incubate the plate |
| ☐ | Place the plate upside down in a 37°C incubator for 24 hours. |
| ☐ | **Stop >** *You cannot continue until the bacteria has grown.* |
| **6** | Label tube |
| ☐ | Write your name or group name and number on an empty 1.5 mL microtube. |
| **7** | Add PBS |
| ☐ | Take the lid off your bacterial plate and use a P1000 micropipette to transfer 1 mL of PBS buffer onto the plate. |
| ☐ | Gently rock the plate back and forth to ensure that the entire plate is covered with PBS buffer.  |
| **8** | Detach bacteria |
| ☐ | Gently spread a sterile loop along the surface of the plate without gouging the agar.  |

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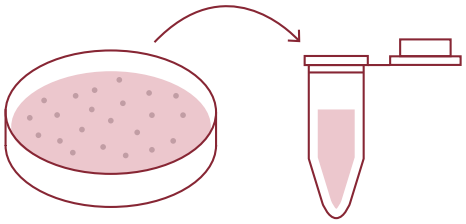
## Student Protocol

*Part 2: Protein Purification*

*Continued*

| | |
|---|---|
| **9** | Remove bacteria/PBS mixture |
| ☐ | Tilt the plate so the liquid with cells pool at the edge of the plate and use a P1000 micropipette to *carefully remove the bacteria/PBS buffer mixture* from the surface of the agar plate. |
| ☐ | Transfer the cell suspension into the 1.5 mL microtube you labeled in Step 6. |
| ☐ | Pipette the cell suspension up and down until the *cells are thoroughly mixed* into solution (i.e., no cell clumps are visible).<br>  |
| **10** | Add lysozyme |
| ☐ | Use a P200 micropipette to add 100 µL of lysozyme to your bacteria/PBS mixture. |
| ☐ | Mix the lysozyme thoroughly into the cell suspension by flicking the closed tube thoroughly. |
| **11** | Incubate cell lysate |
| ☐ | Incubate the tube at room temperature for 5 minutes. |
| ☐ | Invert the tube occasionally during this time (turn upside-down and back). |
| **12** | Store lysate in freezer |
| ☐ | Freeze overnight or longer. |
| ☐ | The ice crystals formed will help physically lyse the cells.<br>**Stop >** *You cannot continue until the lysate has been stored in the freezer overnight.* |
| **13** | Defrost lysate |
| ☐ | Remove the cell lysate from the freezer and defrost at room temperature. |

*Continues on next page >*

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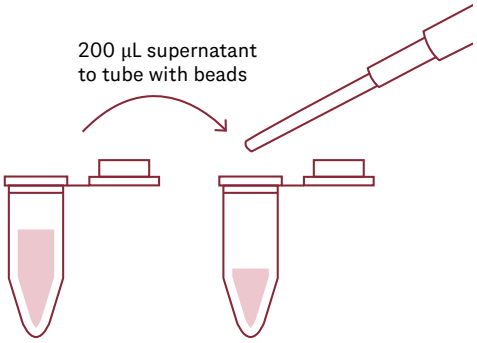
## Student Protocol

*Part 2: Protein Purification*

*Continued*

| | |
|---|---|
| **14** | Centrifuge lysate |
| ☐ | Place lysate tubes in a balanced centrifuge and spin at the highest rpm for five minutes. |
| ☐ | A pellet should be located at the bottom of the tube after centrifugation. |
| **15** | Label tube |
| ☐ | Get a tube with 100 µL of nickel bead slurry and label it with your name or group name and "purified GFP." |
| **16** | Transfer 200 µL of the supernatant |
| ☐ | Use a P200 micropipette to transfer 200 µL of the supernatant (the liquid above the cell pellet) to the tube with the nickel bead slurry. *Do not disturb the pellet.*   |
| **17** | Incubate supernatant |
| ☐ | Incubate the supernatant with the nickel beads at room temperature for five minutes. |
| ☐ | Invert the tube several times each minute. If the beads become lodged in the bottom of the tube, flick the tubes a little more vigorously to dislodge them. |
| **18** | Remove supernatant |
| ☐ | Put the tube into a rack and allow the beads to settle to the bottom. |
| ☐ | Use a P200 micropipette to remove the supernatant to a waste bucket. |
| ☐ | *Do not disrupt the beads*—the fluorescent protein is attached to them! |

*Continues on next page >*

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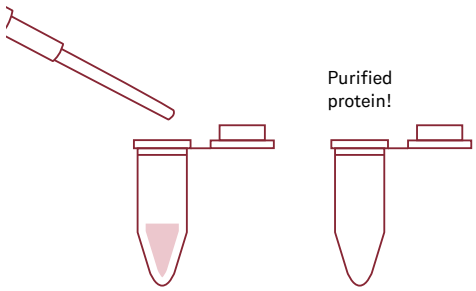
## Student Protocol

*Part 2: Protein Purification*

*Continued*

| | |
|---|---|
| **19** | Wash the beads |
| ☐ | Use a P1000 micropipette to addd 1000 µL of PBS buffer to the beads and invert the tube several times. |
| ☐ | Put the tube into a rack to allow the beads to settle to the bottom of the tube. |
| **20** | Remove supernatant |
| ☐ | Use a P1000 micropipette to remove the PBS buffer (supernatant) to a waste bucket. |
| ☐ | *Do not disrupt the beads*—the fluorescent protein is attached to them! |
| **21** | Elute |
| ☐ | Use a P200 micropipette to add 200 uL of elution buffer to the tube. |
| ☐ | Incubate the tube at ***room temperature for five minutes*** to elute (release) the bound protein from the beads. |
| ☐ | Invert the tube several times each minute. If the beads become lodged in the bottom of the tube, gently shake the tube a little more vigorously to dislodge them. |
| **22** | Congratulations, you just isolated a fluorescent protein! |
| ☐ | Optional: Remove the supernatant (purified protein) to a newly labeled tube. |
| ☐ | Use a *UV transilluminator* to observe the color. Make sure to wear UV-rated goggles if your light source does not include a UV shield. |



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## Student Guide, Part 1: Pre-Lab

**Directions**
*In this lab, you will play the role of a molecular biologist exploring how to modify the DNA of an organism to create and isolate a useful protein product. To begin, carefully examine each station provided by your teacher and record two observations and two questions about what you see.*

1. Phenomenon Stations

| | Station 1 | Station 2 | Station 3 |
|---|---|---|---|
| **Observations**<br>*I notice...*<br>*... reminds me of...* | | | |
| **Questions**<br>*I wonder...*<br>*Could... be...* | | | |

**What do you think these three stations have in common?**

*Continues next page >*

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## Student Guide, Part 1: Pre-Lab

*Continued*

2. Choose one of the three stations and draw a scientific model that explains how the images in the stations are related to each other. Consider the following questions to help you draw your model: What is going on inside the organisms? How was the product made?

*Continues next page >*

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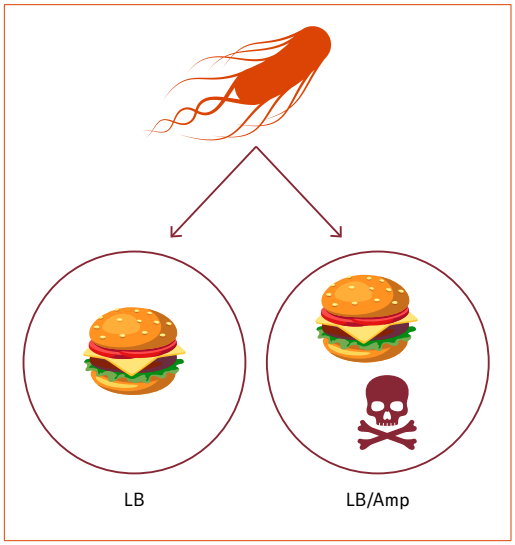
## Student Guide, Part 1: Pre-Lab

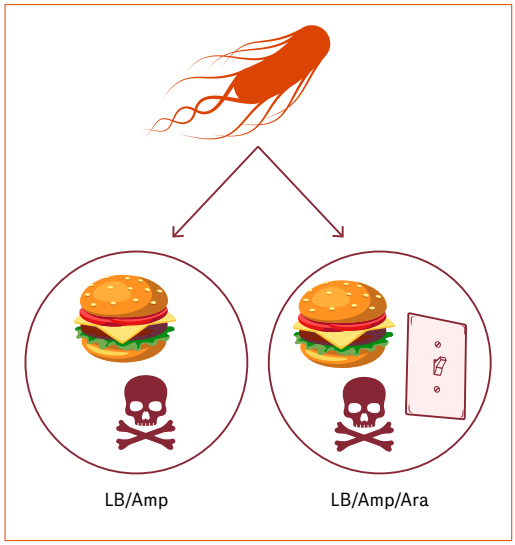
*Continued*

3. Predictions: Below is a diagram of how your experiment will be set up in *Student Protocol, Part 1: Bacterial Transformation*. Use the information in the diagram and your understanding of bacterial transformation from the background reading to make predictions in the tables on the next page.

***E. coli* without pQuince (–DNA)**




***E. coli* with pQuince (+DNA)**




Agar plates:
LB = nutrients (bacteria food)
Amp = ampicillin (antibiotic)
Ara = arabinose (sugar that binds to AraC protein)

*Continues next page >*

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## Student Guide, Part 1: Pre-Lab

*Continued*

### *E. coli* without pQuince (–DNA)

| Type of LB Agar Plate | Bacteria growth? | Glowing green? | Explain Your Prediction<br>*The bacteria will/will not… because…* |
|---|---|---|---|
| LB | ☐ Yes<br>☐ No | ☐ Yes<br>☐ No | |
| LB + Amp | ☐ Yes<br>☐ No | ☐ Yes<br>☐ No | |

### *E. coli* with pQuince (+DNA)

| Type of LB Agar Plate | Bacteria growth? | Glowing green? | Explain Your Prediction<br>*The bacteria will/will not… because…* |
|---|---|---|---|
| LB + Amp | ☐ Yes<br>☐ No | ☐ Yes<br>☐ No | |
| LB +<br>Amp + Ara | ☐ Yes<br>☐ No | ☐ Yes<br>☐ No | |

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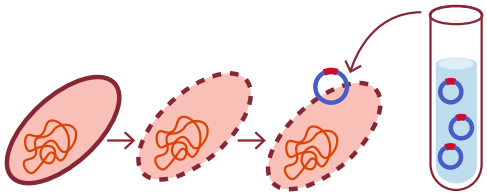
## Student Guide, Part 2: Lab

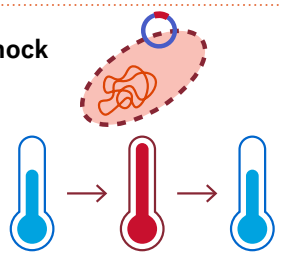
**Directions**
*Answer the questions below to prepare for the lab and record your data after completing the lab.*

1. The image below provides an overview of the steps you will perform to transform your bacteria. Record the purpose of each step as your teacher explains:

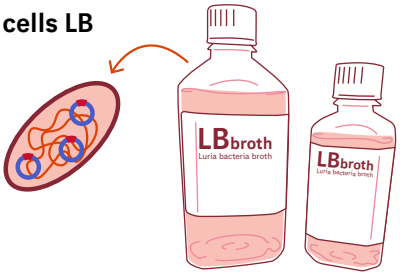
**1. Add bacteria and plasmid to $CaCl_2$**



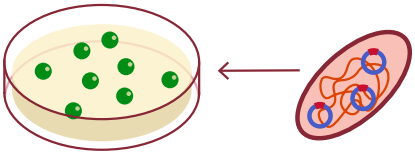
**2. Ice bath/heat shock**



**3. Feed cells LB**




**4. Plate bacteria**



1.

2.

3.

4.

*Continues next page >*

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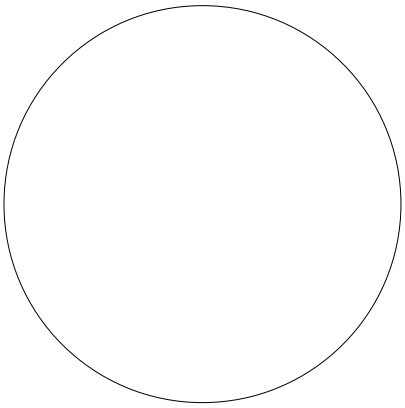
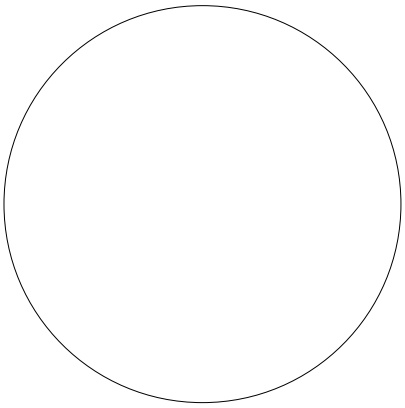
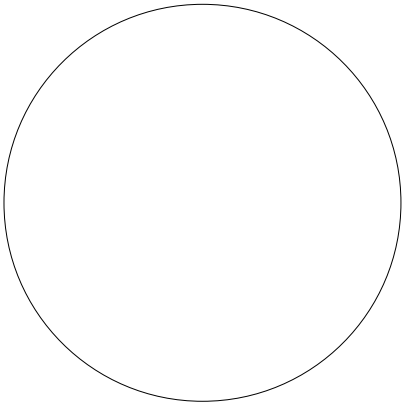
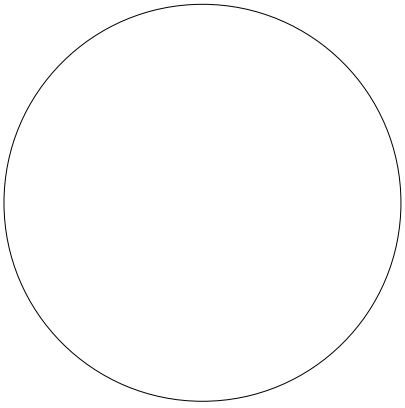
## Student Guide, Part 2: Lab

*Continued*

2. Record the number of colony forming units (CFU) that grew and sketch your observations for each plate in the chart below. (If there are too many bacteria colonies to count, write "lawn".)

| Plate | ***E. coli* without pQuince** (–DNA) | Plate | ***E. coli* with pQuince** (+DNA) |
|---|---|---|---|
| LB |  <br># colonies<br>glowing green? | LB + Amp |  <br># colonies<br>glowing green? |
| LB + Amp |  <br># colonies<br>glowing green? | LB + Amp + Ara |  <br># colonies<br>glowing green? |

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## Student Guide, Part 2: Lab

*Continued*

3. *Consider your results for Student Protocol, Part 2: Protein Purification:* Did you successfully isolate GFP from the transformed *E. coli* bacteria? What evidence supports your answer?

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## Student Guide, Part 3: Data Analysis

**Directions**

*Analyze your results from the lab by answering the questions below.*

1. Which results were the same as your predictions? What does this mean? *A result that supported my prediction was ….This means that…*

2. Which results were different from your predictions? What could have caused this? *A result that refuted my prediction was…This could have happened because…*

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## Student Guide, Part 3: Data Analysis

*Continued*

3. Describe two potential sources of error in *Student Protocol, Part 1: Bacterial Transformation* (mistakes that lead to unexpected results) and how they may affect the results. Errors include: systematic/procedural, random, and human.

| Source of error | How it may affect results |
|---|---|
| | |
| | |
| | |

4. Consider your results for *Student Protocol Part 2: Protein Purification:* Why is it useful for scientists to be able to isolate a protein product from a genetically modified organism? Describe one example.

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## Student Guide, Part 4: Making a Model

**Directions**

*Review the scientific model you drew in Question #2 of the Student Guide, Part 1: Pre-Lab and consider what changes you could make based on what you have learned. Then, follow the steps below to create a new model.*

1. A scientific model represents the observable (visible) and unobservable (invisible) features of a phenomenon. Draw a model that explains what occurs inside transformed bacterial cells to produce a new protein.

*Fill in the blanks below to help plan your model.*

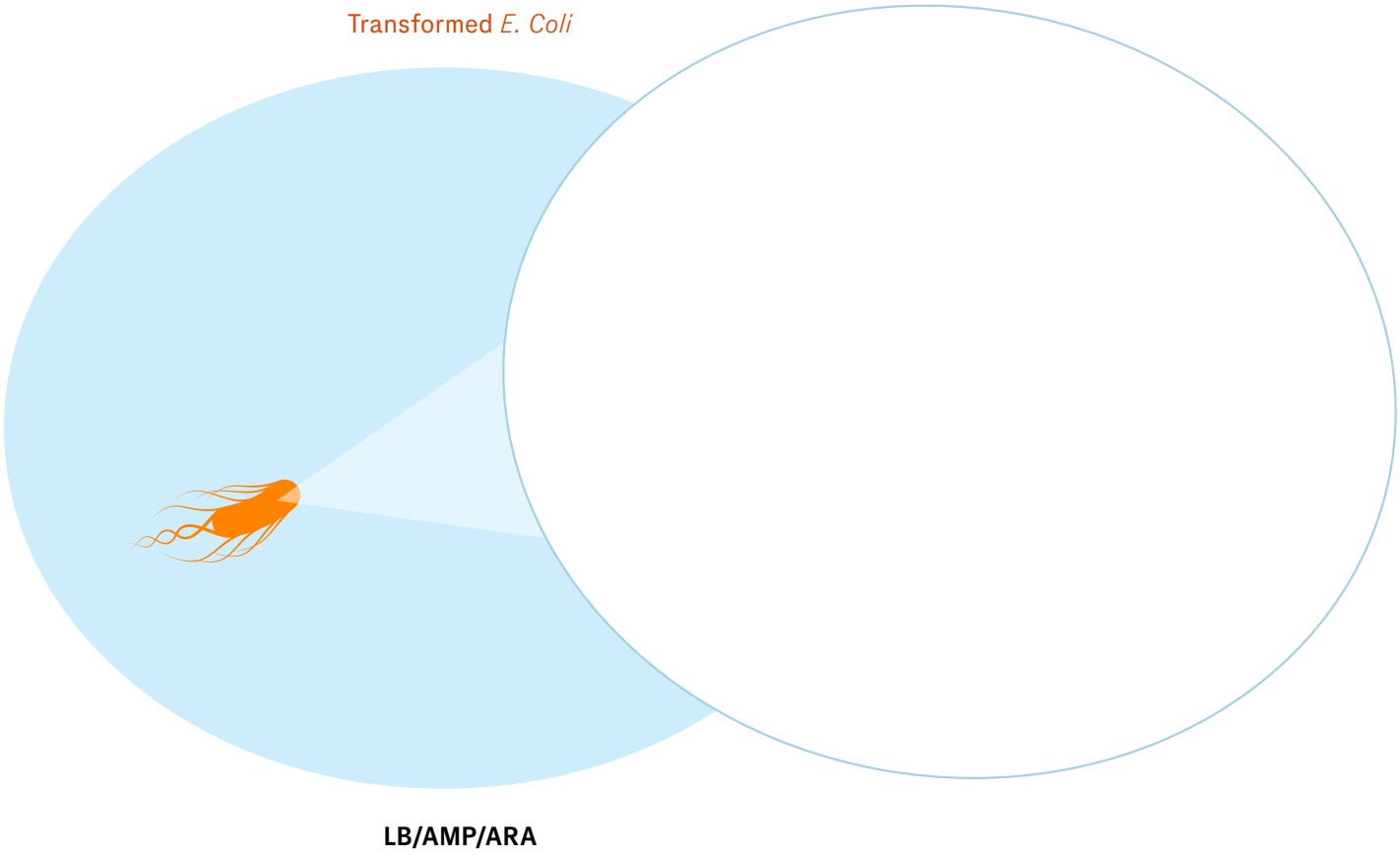
Organism being modified:

Names of new genes:

Proteins for which new genes code:

Traits to which the new genes lead:

Organism from which GFP came:

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## Student Guide, Part 4: Making a Model

*Continued*

2. Work with your group to add on to your model to explain how the GFP produced by the transformed bacteria can be isolated by nickel affinity chromatography. Use the following keywords in your diagram:

— Histidine
— Nickel
— Elute
— Lyse

3. After viewing other models and receiving feedback on your model, describe three limitations of your model. Consider the following questions:

   a. What parts of the bacterial transformation and protein purification system are not shown in the model?

   b. What is an assumption in your model and how does it impact the reliability of the model?

   c. What is estimated, rather than observed directly, in your model?

4. Consider how you could use your model in further investigations. Could you use your model to reliably *predict* how to modify the DNA of any organism to create and isolate a useful protein product? Explain why or why not and provide an example.

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## Scientific Model Rubric

| Score | 4 | 3 | 2 | 1 |
|---|---|---|---|---|
| **Content** | Develops a model that accurately shows all key features of a phenomenon or process and their relationships. | Develops a model that accurately shows most key features (vocabulary, concepts, etc.) of a phenomenon or process and their relationships. | Develops a model that shows some key features of a phenomenon or process and their relationships. | Develops a model that shows few features of a phenomenon or process and attempts to show relationships. |
| **Presentation** | Model is clear, organized, and concise. | Model is clear and organized. | Model is somewhat organized and somewhat clear. | Model is unclear and disorganized. |
| **Limitations** | Clearly and thoroughly describes at least three limitations of the model and to what extent it can be used to make predictions. | Describes three limitations of the model and to what extent it can be used to make predictions. | Describes more than one limitation of the model. | Describes one limitation or irrelevant limitations of the model. |
| **Final Score** | | | | |

*Key features include:*

Plasmid DNA containing three genes

Protein each gene codes for

Relationship between the DNA and each protein product

Relationship between each protein product and its corresponding trait/function

Process of isolating a protein product