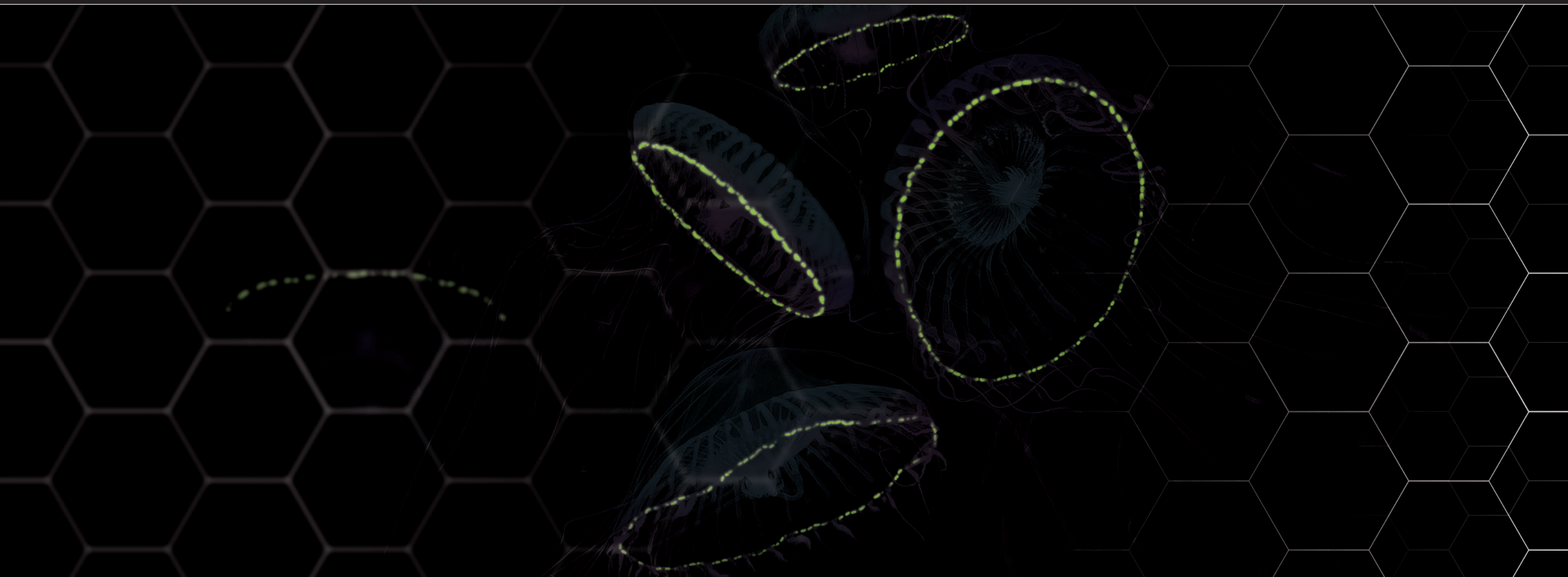

pGLO Bacterial Transformation Kit for General Biology

Catalog #17006991EDU

Student Guide

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Activity 1

Transferring Genes between Species

Part 1. Observe Fluorescent Organisms

Fluorescent Bacteria

A. Watch the video of bacterial growth and record your observations in the table below. Go to bio-rad.com/glow to see the video.

Describe how the bacteria on both plates look at the **beginning** of the video.	
Describe how the bacteria on both plates look in the **middle** of the video.	
Describe how the bacteria on both plates look at the **end** of the video.	

B. Talk with your group about your observations. As a group, develop and write 2–3 questions about what you saw in the video.

Fluorescent Jellyfish

The species of jellyfish shown in Figure 1, *Aequorea victoria*, are naturally fluorescent and glow green under ultraviolet (UV) light. The jellyfish genome has a gene that codes for green fluorescent protein (GFP). Each cell of the jellyfish has a full copy of the genome that includes the gene that codes for GFP. The cells use the gene to synthesize GFP inside themselves.

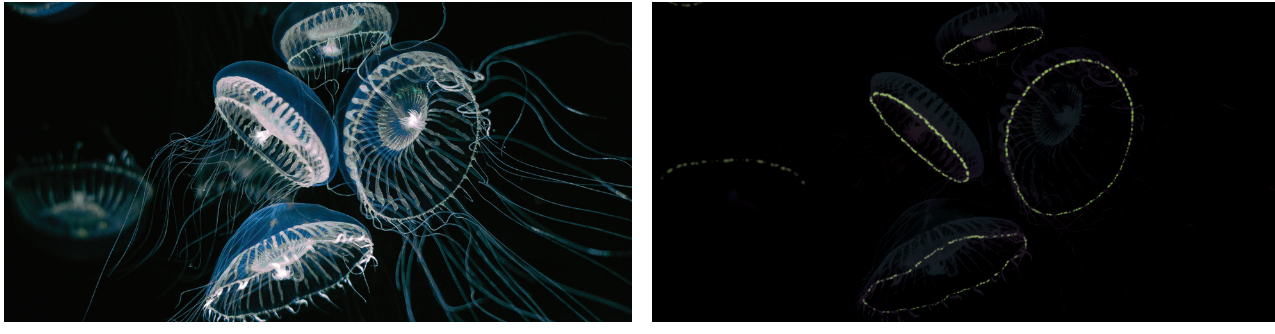


Fig. 1. *Aequorea victoria* jellyfish glow shown under visible light on the left and under UV light on the right.

C. What are some similarities you notice between the bacteria you observed in the video and the jellyfish in Figure 1?

D. What are some differences you notice between the bacteria you observed in the video and the jellyfish in Figure 1?

E. With your group, write down some key ideas about how jellyfish cells use the GFP gene to make green fluorescent protein.

Bacterial Transformation

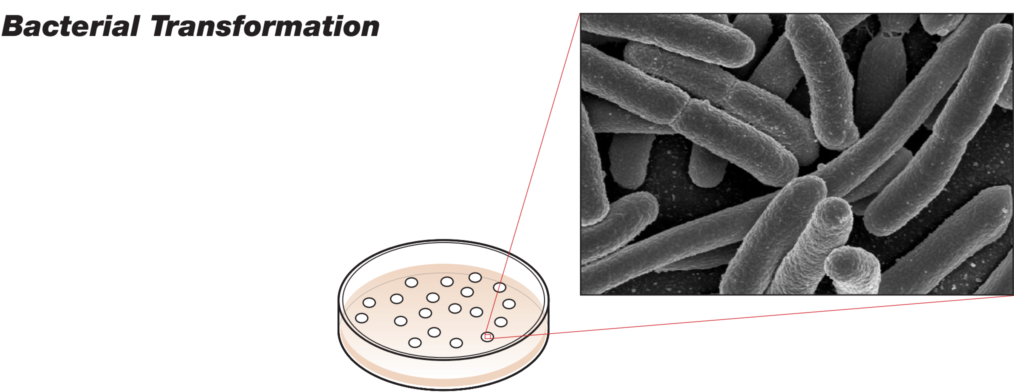


Fig. 2. Electron micrograph of *E. coli* bacteria.

The bacteria you saw growing in the video are called ***Escherichia coli*** or *E. coli* for short. *E. coli* are rod-shaped bacteria that are naturally white or off-white and do not normally glow under UV light. Scientists use *E. coli* as a tool for many reasons but especially because they are very well studied and understood. That makes it much easier, although not simple, to interpret the outcome of an experiment.

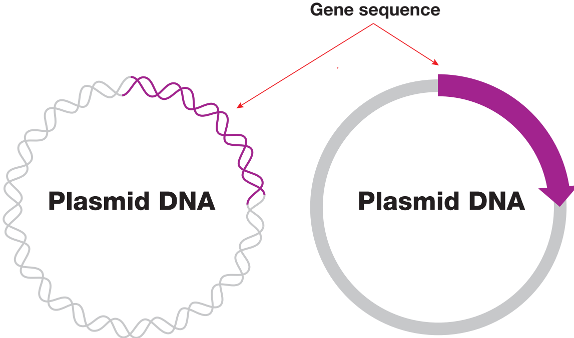


Fig. 3. Two representations of plasmid DNA.

An advantageous feature of *E. coli* is their ability to take in small pieces of circular DNA called plasmids. Plasmids are separate from the chromosome but may include gene sequences. Two representations of the same plasmid DNA are shown in Figure 3. In nature, bacteria exchange genes with each other by swapping plasmids. A gene that allows bacteria to survive in the presence of an antibiotic, for example, might be found on a plasmid. As bacteria pick up and exchange plasmids they can more quickly adapt to a changing environment. Scientists can make use of this feature of bacteria by designing plasmids to include gene sequences of their choice, such as genes from other species. Then they can insert the plasmid into bacterial cells using a process called bacterial transformation. As bacteria divide and multiply, they make copies of the plasmids which are distributed among the offspring.

F. Talk again with your group and describe how bacterial transformation might have produced the results you observed in the video.

Part 2. Model the Processes that Occur in Green Fluorescent Bacteria

The video you watched in Part 1 showed bacteria growing on two plates, one of which glowed green. The glowing color is the result of a protein called green fluorescent protein (GFP) that glows bright green when exposed to ultraviolet (UV) light. The gene sequence that codes for GFP was originally discovered in the jellyfish species *Aequorea victoria*. The *GFP* gene can be included on a plasmid.

G. Draw three diagrams in the spaces provided below that explain your current thinking about what you observed in the video at each stage. Also, write a description of what is happening in each diagram using complete sentences. In every diagram, be sure to include:

- ***bacterial cells, DNA, GFP***
- ***arrows to indicate actions that occur***
- ***labels for all the components***

Before the video	Description
During the video, while the bacteria are multiplying	Description
At the end of the video under UV light	Description

H. Refer to the questions you wrote in step B. If you now have any answers to those questions, write them here.

I. With your group, develop and write 2–3 new questions that will help you better understand what happened in the video.

Part 3. Analyze the pGLO Plasmid

A **plasmid map** highlights features of a plasmid sequence such as genes, promoters, and the origin of replication. There are many other features that can also be shown in a plasmid map. Typically, however, plasmid maps do not show nucleotide sequences (for example, ATTACG). Figure 4 shows a simplified map of one particular plasmid, called **pGLO**.

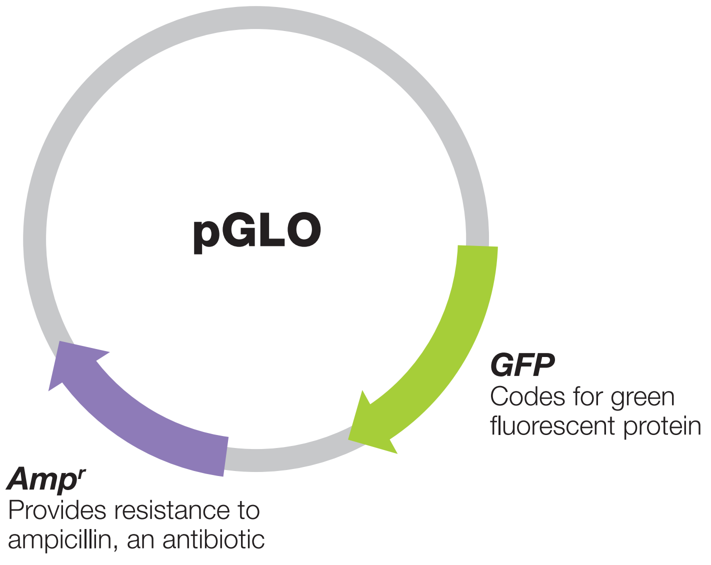


Fig. 4. Simplified pGLO plasmid map. Some components are not shown.

Figure 4 shows two of the genes on the pGLO plasmid. The genes are shown as arrows along a circle, which represents the full circular DNA. The direction of the arrows indicates the direction in which a protein called **RNA polymerase transcribes** the gene.

J. What traits might you expect a bacterial cell to have if it has taken up the pGLO plasmid?

Antibiotic Resistance

Ampicillin is an antibiotic that stops the growth of some bacteria, including *E. coli.* It is used clinically as a treatment for patients with certain bacterial infections. It is also used in laboratories to prevent bacterial growth. Some bacteria have or acquire genes, such as Amp^r, that allow them to grow even in the presence of some antibiotics. Those bacteria are said to be resistant to those antibiotics.

K. With your group, think of a way to use ampicillin to determine whether a sample of* E. coli *has been successfully transformed with pGLO or not.

- ***Explain your plan in complete sentences***
- ***Describe the expected outcome for a sample that was successfully transformed with pGLO***
- ***Describe the expected outcome for a sample that was NOT successfully transformed with pGLO***

Activity 2

Bacterial Transformation Laboratory Activity

Background

In this experiment you will transform *E. coli* with the **pGLO** plasmid. Then you will grow, or culture, those bacteria overnight on solid media called **LB agar**, which is a nutrient source for bacteria.

You will have two *E. coli* samples, one with pGLO plasmid added, called +pGLO, and another without pGLO called –pGLO. You will follow the same bacterial transformation protocol for both samples. Then, you will culture each sample on LB agar plates with and without ampicillin. The +pGLO sample will be added to one side of each LB agar plate and the –pGLO sample to the other side. Then the plates will be incubated overnight to let the bacteria grow.

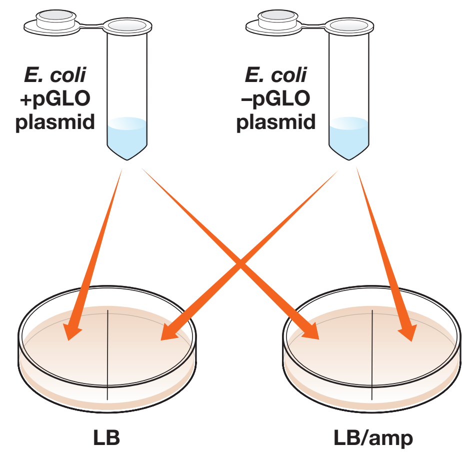
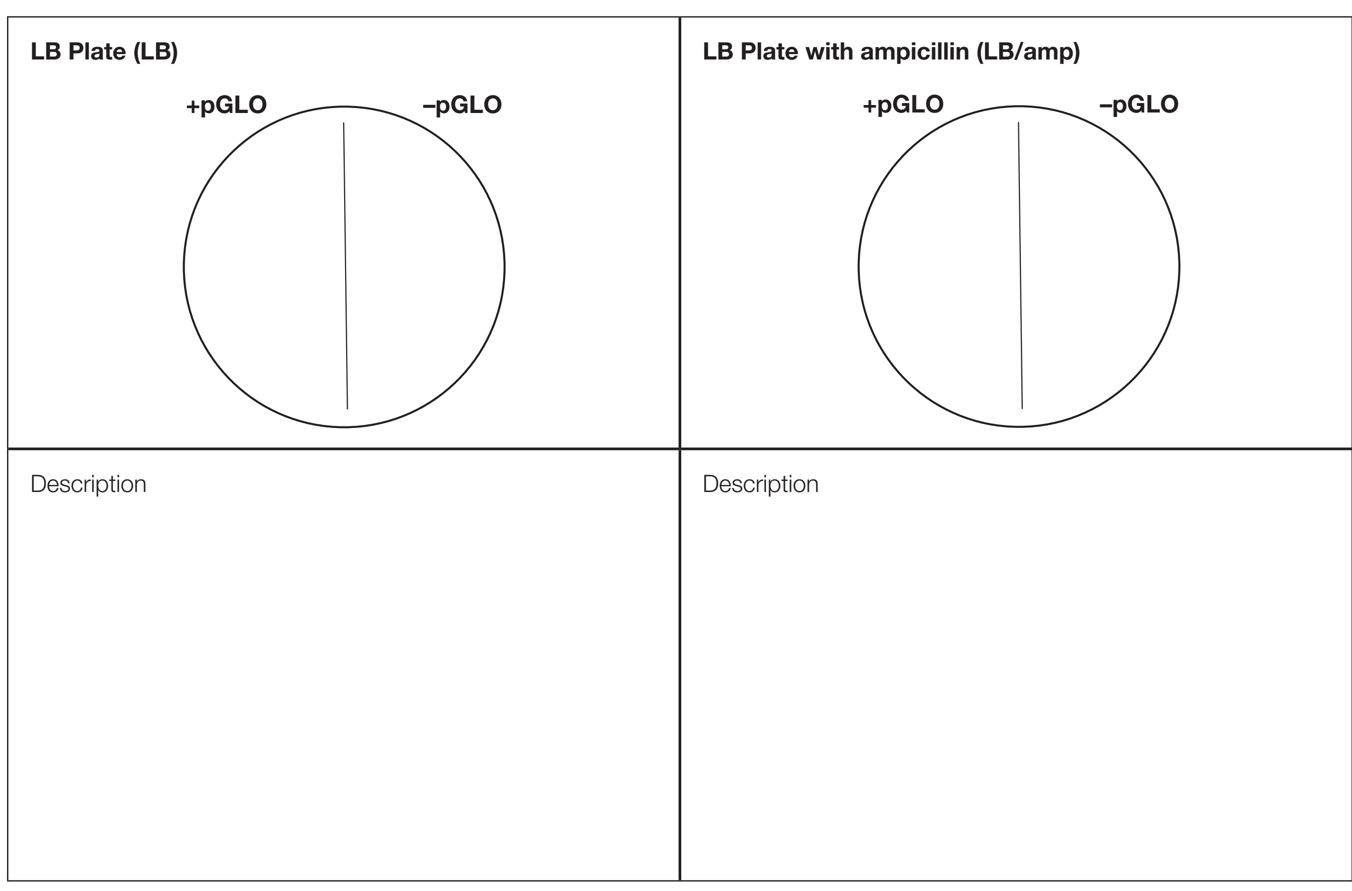


Fig. 5. pGLO bacterial transformation experiment setup.

A. In Table 1 below, sketch and describe the results you expect after you complete the experiment.

Table 1. Predicted results.



LB Plate (LB) **+pGLO** **–pGLO**	**LB Plate with ampicillin (LB/amp)** **+pGLO** **–pGLO**
Description	Description

Part 1. Transform Bacteria with the pGLO Plasmid

Student Workstation

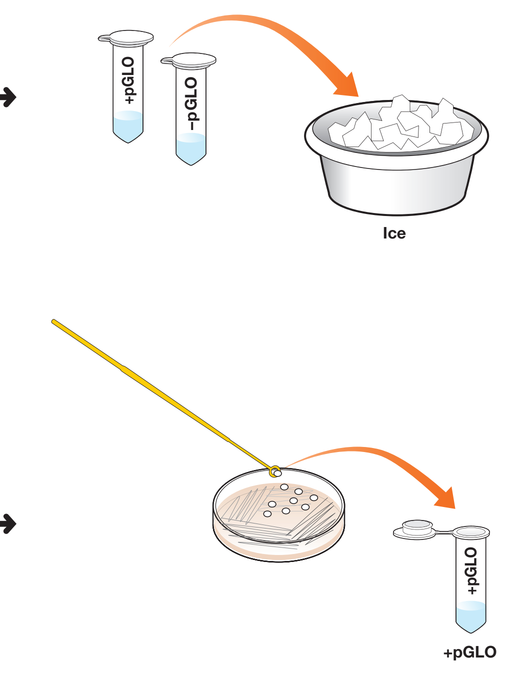
Materials	Quantity
E. coli starter plate (shared between two groups)	1
LB plate (**LB**)	1
LB/amp plate (**LB/amp**)	1
Transformation solution (**TS**), 250 µl	2
LB nutrient broth (**LB**), 0.5 ml	1
Inoculation loop (1 pack of 10 shared between two groups)	5
Disposable plastic transfer pipet	3
Foam tube holder/float (shared between two groups)	1
Ice bath with crushed ice	1
Marking pen	1
Tube rack (recommended)	1

Common Workstation

Materials	Quantity
pGLO plasmid DNA	1
Water bath or dry bath set to 60°C	1
Thermometer (if using water bath)	1
UV light	1
Incubator oven set to 37°C (recommended)	1
2–20 µl adjustable volume micropipet and tips (recommended)	1

Protocol

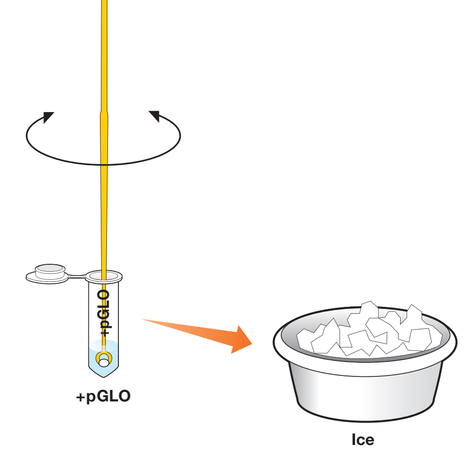
1. *Label one TS tube **+pGLO** and another **–pGLO**. Place both on ice.*



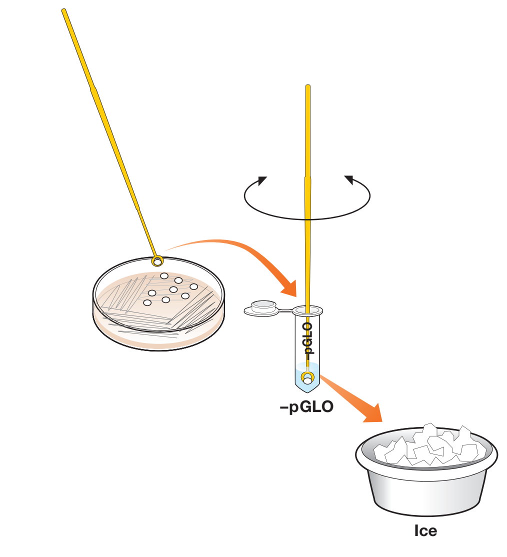
2. *Use a new, sterile inoculation loop to pick 2–4 round bacterial colonies from the starter plate.*

   ***Note:** It is important to take individual colonies and not a swab from the dense area of bacteria.*

3. *Dip the loop into the transformation solution in the **+pGLO** tube. Spin the loop between your fingers until the colony is completely distributed in the transformation solution (no chunks remaining). Close the tube and put it back on ice.*

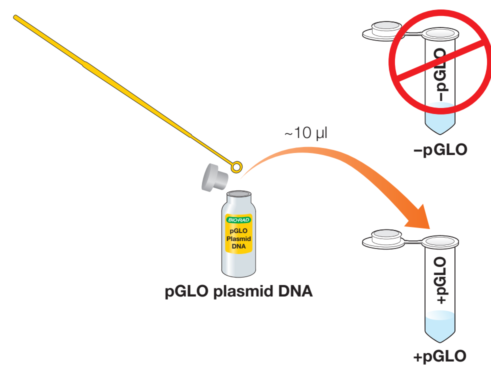


4. Using a new, sterile inoculation loop, repeat steps 2 and 3 with the* –pGLO *tube.

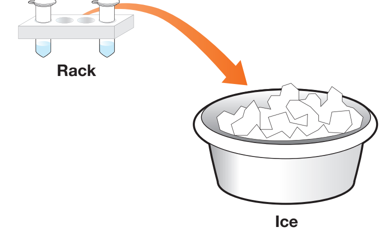


5. Use a new, sterile inoculation loop to transfer a loopful (~10 µl) pGLO plasmid into your* +pGLO *tube. There should be a film of plasmid solution across the ring just like when blowing soap bubbles. Swirl the loop in the* +pGLO *tube to mix. Close the tube and put it back on ice.

***Important!** Do not add pGLO plasmid to the* **–pGLO** *tube.*



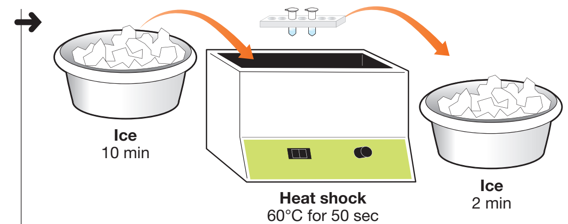
6. Transfer your tubes to the foam rack and then set them on ice for 10 min. Make sure the bottoms of the tubes are pushed all the way through the racks and are touching the ice.



7. While the tubes incubate on ice, label the bottom of your two agar plates as shown in the diagram. Add your initials.

8. ***Bring your ice container and tubes to the water bath or dry bath, if using. Place the foam rack and tubes into the 60°C water bath for exactly 50 sec. Use a timer. Make sure the bottoms of the tubes are touching the water.***

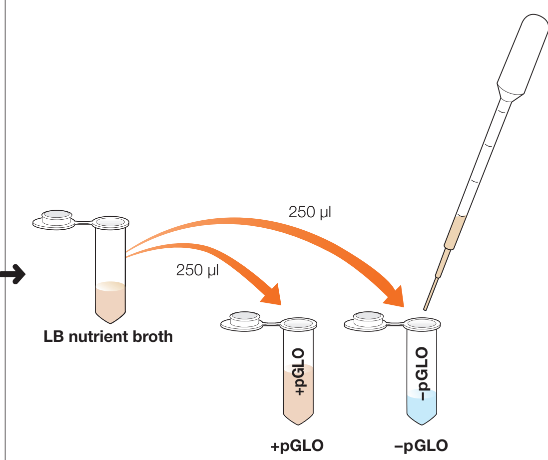
   ***Note:** If using a dry bath, transfer the tubes from the rack directly into holes in the aluminum block.*



9. ***Return the tubes back to ice. Incubate for 2 min.***

10. ***Transfer the rack with the tubes onto the benchtop.***

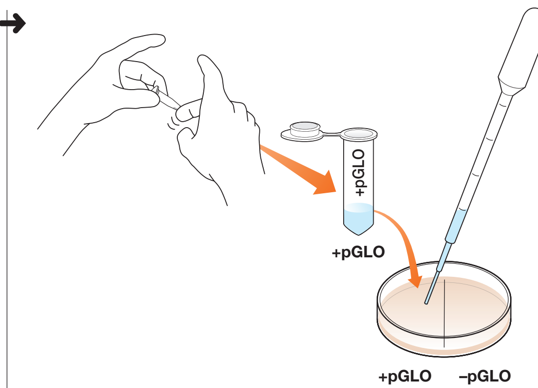
11. ***Use a new sterile transfer pipet to add 250 µl LB nutrient broth to each sample tube. Be careful not to touch the tip of the transfer pipet to the sample tube or liquid. Close both tubes.***



12. ***Incubate both tubes at room temperature (20–25°C) for 10 min.***

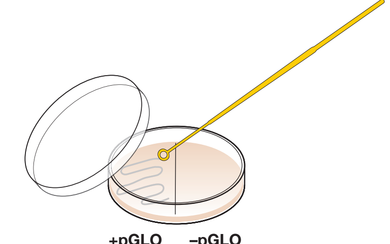
***Stop.** Ask your instructor whether to proceed now or tomorrow.*

***13.* Gently flick the closed tubes to mix. Use a new sterile transfer pipet to add one drop of bacterial suspension from the +pGLO tube to the +pGLO side of each plate.**



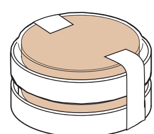
***14.* Use a new sterile inoculation loop to spread the bacteria suspension evenly on the +pGLO side of each plate.**

***Note:** To spread the bacteria, skate the loop gently across the surface of the agar several times.*



***15.* Use a new sterile transfer pipet and a new sterile inoculation loop to repeat steps 13 and 14 with –pGLO on the –pGLO side of the plate.**

***16.* Stack your plates and tape them together. Add your initials to the tape.**



***17.* Place your stack upside down in the 37°C incubation oven overnight.**

Results Analysis

Gather your plates from the previous activity. Keep the lids on while you inspect them. Use a UV light to check for fluorescence.

B. In Table 2 below, sketch and describe your results from the transformation activity. Draw any growth and include labels.

Table 2. Results from the bacterial transformation activity.

LB plate (LB)	**LB plate with ampicillin (LB/amp)**
+pGLO **–pGLO**	**+pGLO** **–pGLO**
Description	Description
Differences from prediction in Table 1	Differences from prediction in Table 1

C. In Table 2, describe any differences between your predictions in step A and your actual results.

D. Before you started the transformation activity, what did both plates have in common?

E. Before you started the transformation activity, what was different between the two plates?

Look at the bacterial growth on each plate. You may see two types of growth: individual circles of bacteria called colonies and areas where there are so many colonies that they merge together. That is called a bacterial lawn.

F. On your plates, where was there no bacterial growth?

G. On your plates, where did bacteria grow as colonies?

H. On your plates, where did bacteria grow as a lawn?

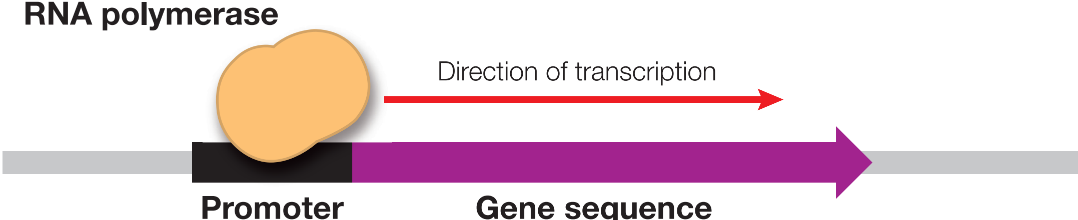
I. Write a claim about how the presence or absence of ampicillin impacted bacterial growth. Use your observations from both plates as evidence to support your claim. Use complete sentences.

J. Do you think the gene for green fluorescent protein was successfully transformed into any of the bacteria on either of your plates? Justify your answer using your observations, the pGLO plasmid map in Activity 1, Part 3, and any other resources available. Use complete sentences.

Part 2. Switch ON the GFP Gene

Background

In all organisms, the expression of genes is tightly controlled. Some genes might be turned ON, or expressed, while other genes might be turned OFF. When a gene is turned ON, RNA polymerase binds to the **promoter** sequence ahead of the gene and then continues to transcribe the gene into messenger RNA (**mRNA**).



Some genes are always ON. For example, on the pGLO plasmid, *Amp*^{r} is designed to always be ON. Other genes are turned OFF and require an input to switch ON. The pGLO plasmid also includes a gene called ***araC*** (Figure 6) that codes for the protein of the similar name, AraC. AraC acts as an ON/OFF switch. When **arabinose** is present, AraC switches the *GFP* gene ON. Without arabinose, AraC switches the *GFP* gene OFF.

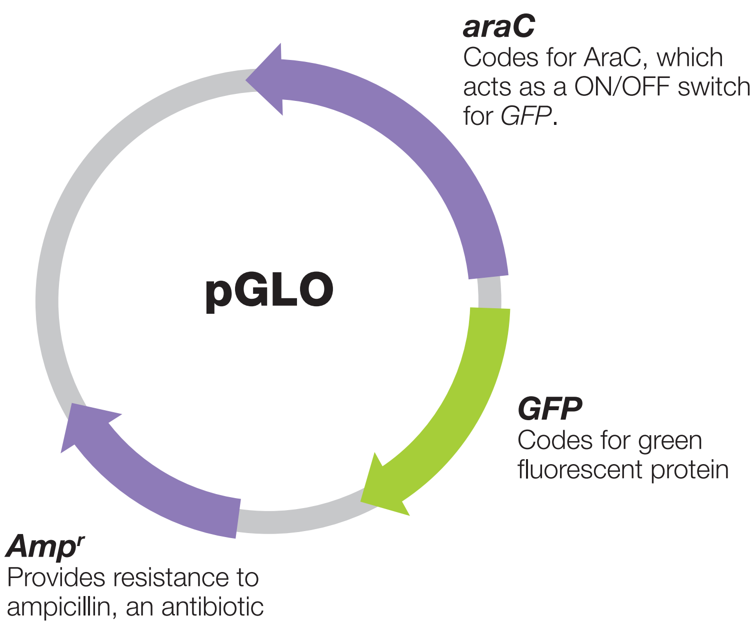


Fig. 6. Simplified map of the pGLO plasmid showing the *araC* gene.

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K. Refer back to your results from Activity 2. Talk with your group about whether the GFP gene was switched ON or OFF in **E. coli** ***on your plates and how you know. Describe your thinking in complete sentences.***

Experimental Design

With your group, design an experiment using the available materials to determine whether the bacteria on your plates from Part 1 have the *GFP* gene.

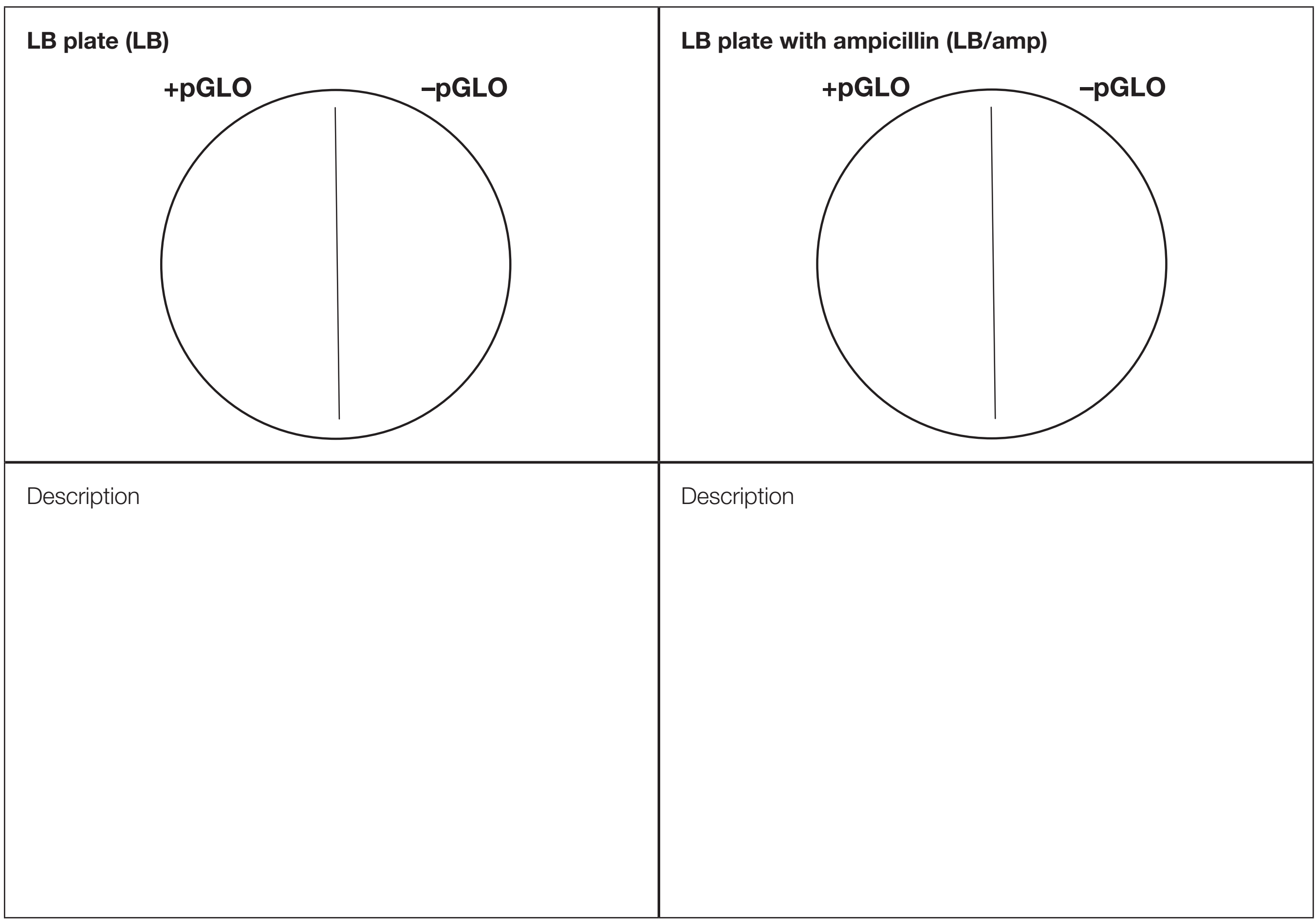
Available Materials	Quantity
Results plates from Part 1 (LB plate and LB/amp plate)	1
Marking pen	1

Common workstation	Quantity
Arabinose solution and shared transfer pipet (per group)	250 µl
UV light	1
Incubator oven set to 37°C (recommended)	1

L. Describe the steps of your experiment below. A drawing may be helpful. Remember to include labels.

M. Sketch and describe your expected results in Table 3.

Table 3. Predicted results for the GFP switch activity.



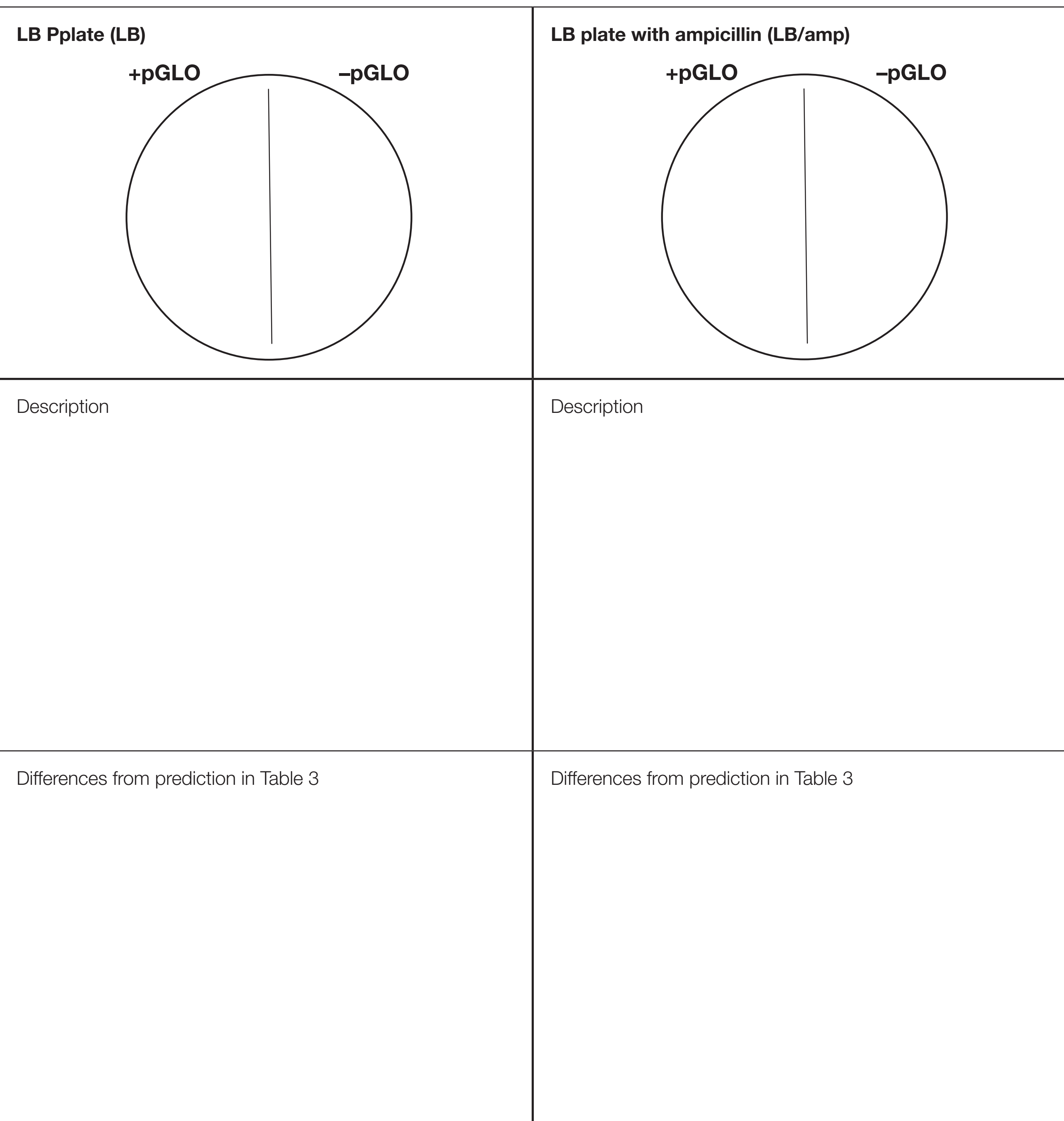
N. Explain how your expected results will allow you to determine whether the bacteria on your plates from Part 1 have the GFP gene.

O. Once you have reviewed your plan with your instructor, carry out your experiment.

Results Analysis

P. Examine your plates in regular light and then in UV light. Sketch and describe your observations below.

Table 4. Results from the GFP switch activity.



LB Pplate (LB)	**LB plate with ampicillin (LB/amp)**
+pGLO **–pGLO**	**+pGLO** **–pGLO**
Description	Description
Differences from prediction in Table 3	Differences from prediction in Table 3

Q. Why did some bacteria glow green while others did not?

R. Write a claim about how arabinose influenced the expression of the GFP gene. Use your observations from both plates as evidence to support your claim. Use complete sentences.

S. Look back to your model in Activity 1, Part 1. Revise the model to include all of the new information you have gathered. The model should now include:

- ***the bacterial cells, pGLO plasmid, GFP, arabinose, and ampicillin***
- ***arrows to indicate actions that occur***
- ***labels for all the components***

Activity 3

Bacterial Transformation Design Challenge

Background

In the pGLO plasmid system, you can think of GFP as an output in response to arabinose as an input. When you add arabinose (input) to bacteria that have been transformed with the pGLO plasmid, they begin to produce GFP (output). By creating a plasmid with different genes than those on pGLO, *E. coli* can be engineered to be responsive to different inputs and produce different outputs. Bacteria that are engineered to be responsive to an input are called **biosensors**.

Table 5. Example inputs and outputs.

Inputs	Outputs
Light responsive Cold responsive Drought responsive Sucrose responsive Low oxygen responsive Heavy metal responsive (like lead or arsenic)	Changes color Produces a smell (bananas, cherries, mint) Makes a protein (insulin, cancer drug) Captures toxins (lead, arsenic, mercury)

Table 5 provides only a small list of example inputs and outputs that engineers might use when designing a biosensor. There are many others that you can investigate on your own. Now it is your turn to develop an idea for a biosensor that could solve a real world problem.

Challenge Instructions

Design and propose a bacterial system that could be used as a biosensor to solve a real-world problem. Use the design proposal template to structure your idea.

In your proposal you must:

- *Identify a problem that could be solved using a biosensor. Remember that biosensors are great for detecting something. What would be useful to detect?*

- *Explain why it is important to solve this problem*

- *Explain the solution by:*
  - *drawing a model of the system, including the necessary elements of the plasmid*
  - *describing how the system would be implemented*
  - *describing the inputs and outputs of the system*

- *Identify two strengths and two limitations of this solution. Consider some of the following:*
  - *public opinion — what would the public think about this?*
  - *scaling up — what would have to be done to make enough of the product?*
  - *concerns — what are some possible benefits of the solution? What are some of the potential risks?*

Design Proposal Template

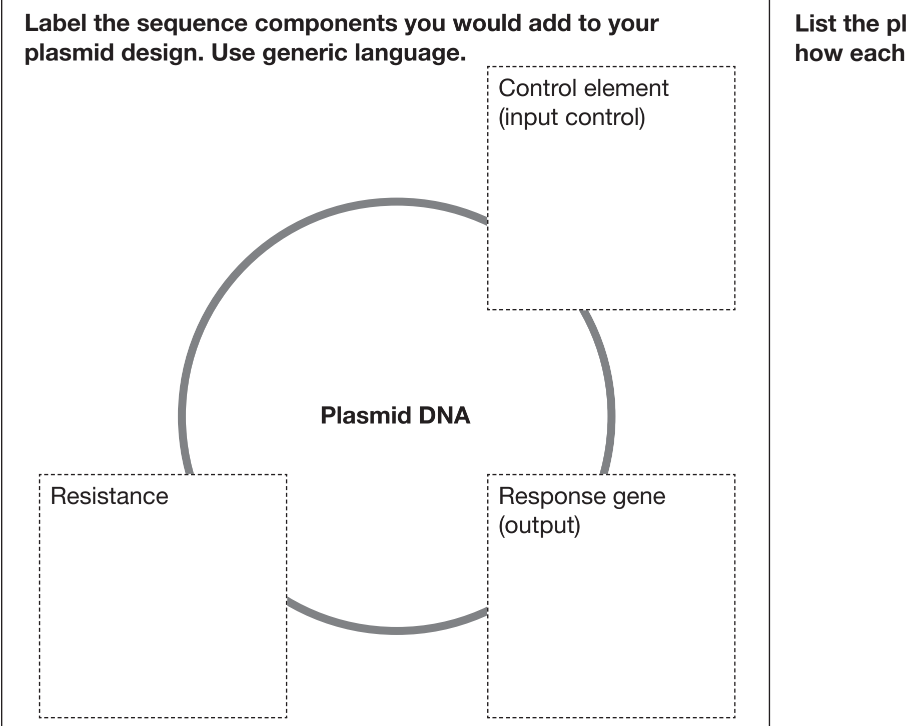
Define the Problem

What is the problem?
Who does the problem affect?
Why is this problem worth solving?

Design the Solution

Describe your solution.
Explain how your solution will solve the problem.
Identify two strengths and two limitations of this solution.

Label the sequence components you would add to your plasmid design. Use generic language.



List the plasmid components here and explain how each of them works in your solution.

Draw a diagram that describes how your system will be implemented to solve the problem. Include labels.

Bacterial Transformation Design Challenge Rubric

Question	Novice	Developing	Proficient
What is the problem?	Details of the problem are missing; demonstrates little understanding of the problem.	Explains some details of the problem but includes irrelevant details or is missing important details; demonstrates some understanding of the problem.	Clearly explains relevant details of the problem; demonstrates a strong understanding of the problem.
Who does the problem affect?	Does not describe the affected individuals with any relevant details.	Describes the affected individuals in some detail but is missing key details or includes irrelevant details.	Clearly describes the individuals affected by the problem using information that is relevant to the problem.
Why is this problem worth solving?	Does not explain the potential impact of solving the problem.	Explains the potential impact of solving the problem in some details but does not provide evidence or justification.	Clearly explains the potential impact of solving the problem and provides quantitative and/or qualitative evidence to justify the importance.
Describe your solution.	Does not explain all the components of the system and is missing important details.	Explains the system but is missing some components or important details.	Clearly describes the inputs, outputs, and context of the system; describes how the components work together.
Explain how your solution will solve the problem.	The solution does not connect to the problem or the connection is not explained.	Explains some connections between the problem and the solution but does not explain how the solution will be successful for the intended audience.	Clearly explains the connection between the solution and the problem; provides details about how the solution will be successful for the intended audience.
Identify two strengths and two weaknesses of your solution.	Does not identify real strengths and weaknesses	Identifies strengths and weaknesses but some are trivial or not well explained.	Identifies and explains two real strengths and two real weaknesses of the proposed solution.
Solution diagram	Some plasmid components are missing, and the diagram is not complete.	Includes all the necessary plasmid components but some are not well described or are not included in the diagram	All the necessary plasmid components are included, described in detail, and included in a detailed diagram.

Glossary

Aequorea victoria — a jellyfish species whose chromosome naturally has the gene for green fluorescent protein and glows green under UV light.

Agar — a gelatinous substance derived from seaweed that can be used to make solid media for bacterial growth.

Ampicillin — an antibiotic in the penicillin family that is commonly used in laboratories. Bacteria with the *Amp*^{r} gene are resistant to ampicillin and continue to grow in its presence.

Amp^r — a gene that provides resistance to ampicillin.

Antibiotic resistance — a trait that allows bacteria to grow in the presence of an antibiotic that normally prevents bacterial growth.

Arabinose — a carbohydrate found in plants that can be a food source for bacteria. It can switch on gene expression that is controlled by *araC*.

AraC — an activator protein that acts as an ON/OFF switch for the P_{BAD} promoter.

araC — a gene that codes for the protein AraC.

Bacterial colony — a circular mass of genetically identical bacterial cells growing on solid media.

Bacterial lawn — a growth pattern in which individual bacterial colonies have merged together.

Bacterial transformation — a process for inserting plasmid DNA into bacteria.

Biosensor — a device that use biological organisms or materials to detect a certain input.

Escherichia coli (E. coli) — a rrod-shaped bacterium commonly used in laboratories and genetic engineering.

Fluorescence — the appearance of glowing or giving off visible light often caused by exposure to UV light.

Gene expression — the general process in which the instructions in a gene are used to make a protein or other molecule.

Green fluorescent protein (GFP) — a protein that fluoresces green when exposed to UV light.

LB (Lysogeny Broth, also known as Luria-Bertani broth) — a mixture of nutrients that is commonly used to grow bacteria.

mRNA (messenger RNA) — a type of RNA that is transcribed from DNA and is involved in protein synthesis.

P_{BAD} — a promoter region that can be regulated by AraC.

pGLO — a specific plasmid that includes the *GFP* gene.

Plasmid — a circular piece of DNA that carries genes and can be inserted into a bacterium in a process called bacterial transformation.

Plasmid map — a diagram that includes annotations about the genes and genetic features of a plasmid.

Promoter — a DNA sequence where RNA polymerase attaches to begin transcribing a gene.

RNA polymerase — a protein that transcribes DNA sequences into mRNA.

Transcription — the process in which RNA polymerase synthesizes mRNA using a DNA sequence as a template.

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