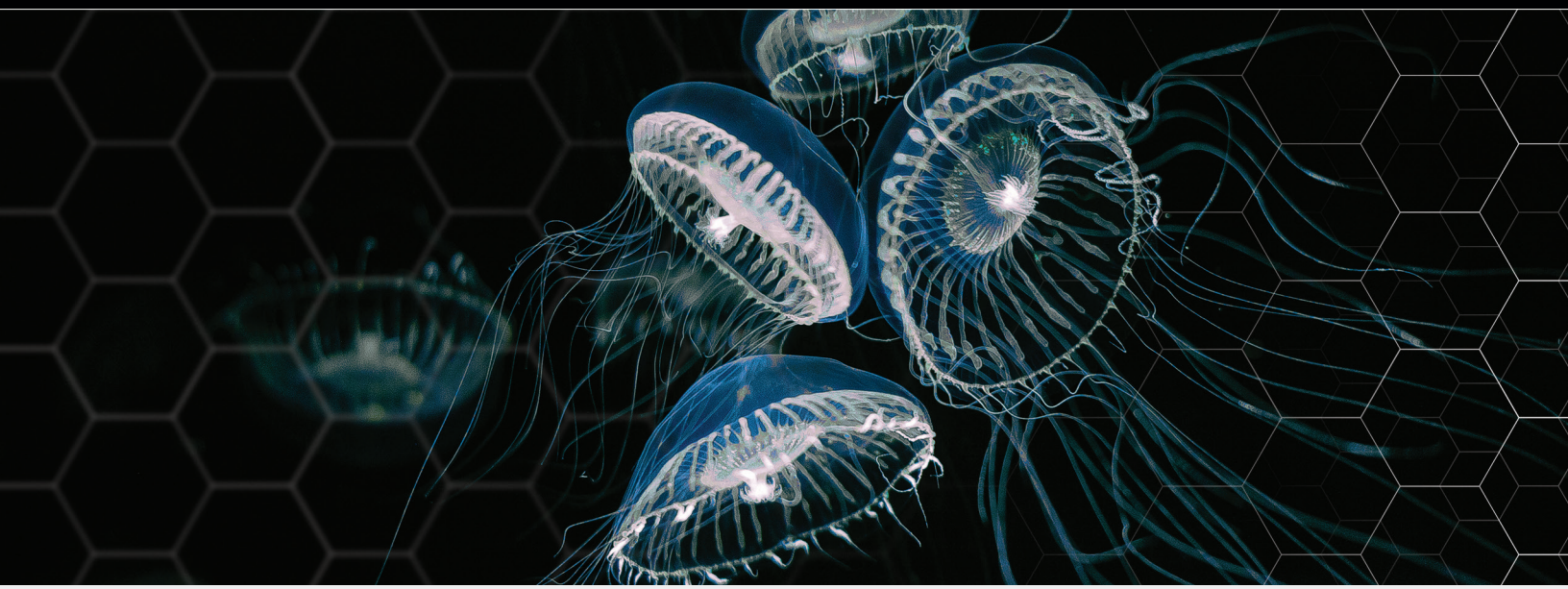
BIO-RAD Explorer



pGLO Bacterial Transformation Kit for General Biology

Catalog #17006991EDU

Instructor Guide

For technical support, call your local Bio-Rad office, or in the U.S. call **1-800-4BIORAD** (1-800-424-6723) option 2.

BIO-RAD

Dear Instructor

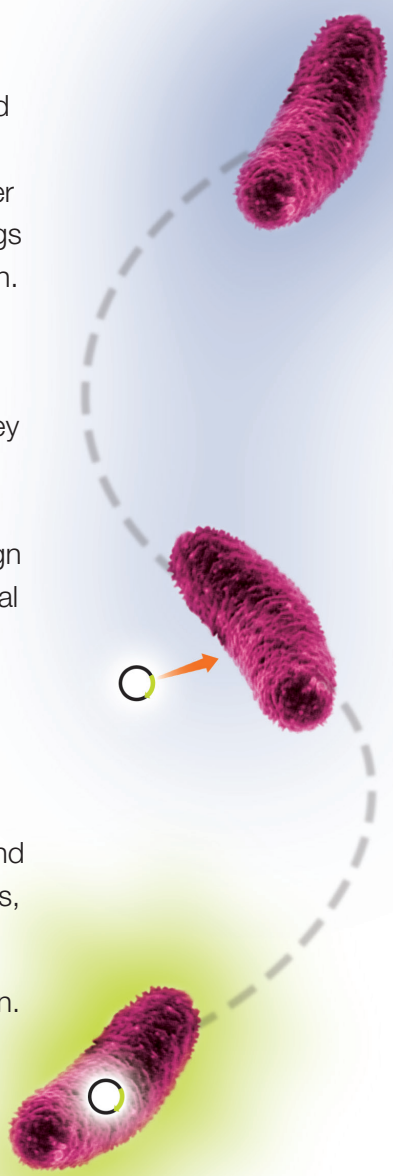
A brilliant flash of glowing green has the power to spark excitement and a sense of wonder. In these activities, your students will genetically engineer their own green glowing bacteria with a gene borrowed from *Aequorea victoria*, a bioluminescent jellyfish. It was such a transference of genetic information that ignited an era of unprecedented advancement in life science and has become a cornerstone of biotechnology. As your students discover how the vast diversity of life relies on a shared four-letter code and make sense of the tension and dramatic inner workings of a cell, they participate in a longstanding scientific conversation.

The lessons in the kit are designed for your students to make sense of gene expression and bacterial transformation through progressive rounds of careful observation and data analysis. They will be asked to describe their thinking and to justify their claims using evidence, each time with more complex and nuanced explanations. The experience culminates in an engineering design challenge in which your students apply their new skills in bacterial transformation to address a real world problem.

The activities in this kit were developed in partnership with Elizabeth Martinez, an esteemed curriculum developer for the Illinois Mathematics and Science Academy in Aurora, Illinois.

We strive to continually improve our curriculum and products, and your input is extremely important to us. We welcome your stories, comments, and suggestions.

Share your students' success on social media @bioradeducation.



Bio-Rad Explorer Team
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explorer@bio-rad.com

Table of Contents

Kit Storage

When you receive the pGLO Bacterial Transformation Kit for General Biology:

1. Record storage location and batch numbers from the product labels.
2. Store the **Transformation Reagent Refill** in the refrigerator (4°C).

3. Store the remaining materials at room temperature.

4. Visit **bio-rad.com/pgloGenBio** to download the most up-to-date instructor and student guides.

Technical Support is available at support@bio-rad.com or 1-800-4BIORAD, option 2.

Safety Guidelines

Basic laboratory safety guidelines should be followed at all times: wear gloves and safety goggles; eating, drinking, smoking, and applying cosmetics are not permitted in the work area.

The *Escherichia coli* bacteria HB101 K-12 strain contained in this kit is nonpathogenic and has been genetically modified to prevent its growth unless grown on an enriched medium. However, handling of the *E. coli* K-12 strain requires the use of standard Microbiological Practices. These practices include, but are not limited to, the following guidelines. Decontaminate work surfaces once a day and after any spill of viable material. Decontaminate all contaminated liquid or solid wastes before disposal. All persons must wash their hands: (i) after they handle material containing bacteria, and (ii) before exiting the laboratory. Perform all procedures carefully to minimize the creation of aerosols.

After the lab activities, place all bacteria plates and any materials that may have contacted bacteria in a 10% bleach solution for at least 20 minutes. Follow local regulations for further disposal recommendations.

Ampicillin may cause allergic reactions or irritation to the eyes, respiratory system, and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing. Ampicillin is a member of the penicillin family of antibiotics. Those with allergies to penicillin or to any other member of the penicillin family of antibiotics should avoid contact with ampicillin.

Kit Components

Each kit contains materials for 12 student workstations.

Item	Quantity
Transformation solution	15 ml
E. coli HB101 K-12, lyophilized	1 vial
pGLO plasmid, lyophilized	20 µg
Ampicillin, lyophilized	20 mg
L(+) arabinose, lyophilized	600 mg
LB nutrient broth, sterile	10 ml
LB nutrient agar powder, sterile	20 g
Plastic transfer pipet, sterile	50
Inoculation loop, sterile, 10 µl	80
Petri dish, 60 mm, sterile	40
Multicolor microcentrifuge tube, 2.0 ml	60
Foam micro test tube holder	8
UV pen light	1

Required Materials (not included in this kit)	Quantity
Microwave oven	1
Temperature-controlled dry bath or water bath	1
Thermometer (0–60°C)	1
Erlenmeyer flask, 1 L	1*
Graduated cylinder, 500 ml	1
Distilled water	500 ml
Ice bath (e.g., ice bucket or foam cup)	12
Marking pen	12
Timer to count seconds	
Laboratory tape	
Household bleach, 10% solution for cleanup	

Recommended Materials (not included in this kit)	Quantity
2–20 µl adjustable-volume micropipet and tips	1
Incubator oven, 37°C	1
Vortexer	1
Tube rack	1
Parafilm laboratory sealing film	

* If your microwave cannot accommodate a 1 L flask, see Appendix A for modified LB agar plate preparation instructions. You will need two 500 ml flasks instead of a single 1 L flask and a balance with at least 10 g capacity.

pGLO Bacterial Transformation Kit for General Biology

Ordering Information

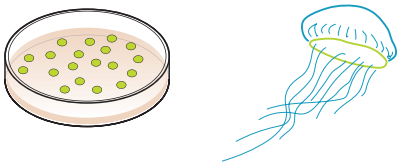
Catalog #	Description
Kits and Refill Packs	
17006991EDU	**pGLO Bacterial Transformation Kit for General Biology**
1660555EDU	**Transformation Reagent Refill**
1660005EDU	**Green Fluorescent Protein Chromatography Kit**
1660013EDU	**pGLO Kit SDS-PAGE Extension**
Consumables	
1660405EDU	**pGLO Plasmid**, lyophilized, 20 µg
1660406EDU	**Arabinose**, lyophilized, 600 mg
1660407EDU	**Ampicillin**, lyophilized, 30 mg
1660408EDU	***E. coli* Strain HB101 K-12**, lyophilized
1660409EDU	**Transformation Solution**, 15 ml
1660421EDU	**LB Broth**, 10 ml
1660600EDU	**LB Nutrient Agar Powder**, 20 g, makes forty 60 mm agar plates
1660472EDU	**LB Nutrient Agar Powder**, 500 g, makes one thousand 60 mm agar plates
1660469EDU	**Petri Dishes**, 60 mm, sterile, pack of 20
1660470EDU	**Petri Dishes**, 60 mm, sterile, pack of 500
1660471EDU	**InoculationLoops**, sterile, pack of 100
1660473EDU	**Colored 1.5 ml Microcentrifuge Tubes**, 6 colors, pack of 600
1660474EDU	**Disposable Plastic Transfer Pipets**, sterile, pack of 500
2239430EDU	**2 ml EZ Micro Test Tubes**, natural, pack of 500
Equipment and Laboratory Supplies	
1660501EDU	**Mini Incubation Oven**, 120 V
1660610EDU	**BR-2000 Vortexer**, 120 V
1660611EDU	**BR-2000 Vortexer**, 220 V for Europe
1660621EDU	**BR-2000 Vortexer**, 220 V for the UK
1660500EDU	**Long-Wave UV lamp**
1660530EDU	**Long-Wave UV Pen Light**
1660481EDU	**Green Racks**, set of 5 racks
1660479EDU	**Jellyfish Foam Floating Racks**, 12-wells, pack of 8

Kit Activity Overview

Activity 1

Transferring Genes between Species

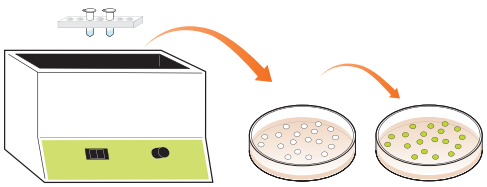
Students observe the growth of green fluorescent bacteria. They assemble descriptive models from their observations and make predictions about how antibiotic resistance might impact growth.



Activity 2

Bacterial Transformation Laboratory Activity

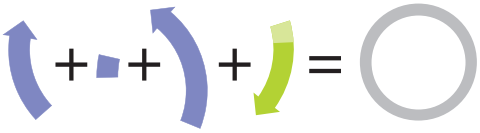
Students transform bacteria with an engineered plasmid without arabinose. Then they design an experiment to use arabinose to turn on the gene for green fluorescent protein.



Activity 3

Bacterial Transformation Design Challenge (optional)

Students use their new understanding of bacterial transformation to design a plasmid-based biosensor that can be used to solve real world problems.



Activity Timelines

The activities in this kit are designed to take five 50 min class periods as shown in Table 1.

Table 1. Suggested timeline for daily 50 min class periods.

Class Period 1	Class Period 2	Class Period 3	Class Period 4	Class Period 5
Activity 1 Part 1. Observe Fluorescent Organisms Part 2. Model the Processes that Occur in Green Fluorescent Bacteria Part 3. Analyze the pGLO Plasmid Finish group work out of class or spend an extra day for Activity 1.	**Activity 2** Part 1. Transform Bacteria with pGLO Plasmid Incubate plates overnight at 37°C.	**Activity 2** Analyze results from Part 1 Part 2. Switch on the GFP gene Students conduct their experiments and incubate plates overnight at 37°C.	**Activity 2** Analyze results from Part 2 **Activity 3** (optional) Bacterial Transformation Design Challenge	**Activity 3** (optional) Continue work and share out

Curriculum Fit

Required prior knowledge

- Basic DNA, RNA, and protein structure and function
- Central dogma (DNA → RNA → Protein → Trait) — the provided lesson sequence requires that students have a basic understanding of the central dogma. If students are not yet familiar with central dogma, you can use the phenomenon in Activity 1, Part 1 to get students engaged in asking questions that may lead to further instruction
- How bacteria grow and divide
- How to culture bacteria on solid media
- How to use a pipet

Concepts, topics, and skills

- **Gene expression and regulation** — students will compare the transcription and gene expression of *Amp*^{r}, the gene whose product confers resistance to ampicillin, and *GFP*, which codes for green fluorescent protein (GFP)
- **Bacterial transformation** — students will perform a bacterial transformation and apply what they learn to design their own plasmid-based biosensor
- **Genetic engineering** — manipulating or engineering the genetic information of an organism to change phenotype is the goal of genetic engineering. Students will introduce a gene naturally found in jellyfish into bacteria using bacterial transformation
- **Artificial selection** — after students complete the transformation, they will be confronted with evidence of artificial selection. Bacteria that take up the pGLO plasmid, which includes an ampicillin resistance gene, will grow in the presence of ampicillin, while those that do not take up the plasmid will not grow
- **Biosensors** — students have the opportunity to apply their new knowledge toward the design of a biosensor, an engineered biological system that responds to an input

Preparation Instructions

Preparation step	Time required	When to begin preparation
Prepare LB agar plates	1–2 hr plus 2 days to dry plates	3–28 days before Activity 2
Rehydrate *E. coli*	5 min plus 8–24 hr incubation at 37°C	2 days before the activity
Streak starter plates	20 min plus 24 hr incubation at 37°C	24 hr before the activity
Dispense solutions	30 min	Up to 3 days before the activity

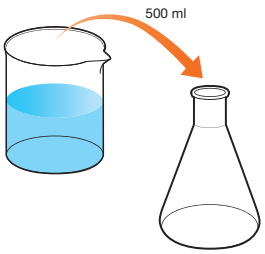
Tips

- If you do not have an incubation oven, incubate rehydrated *E. coli* and starter plates at room temperature (20–25°C) for 72 hr total. Incubating at room temperature for 72 hr may help coordinate preparation over a weekend
- Visit **bio-rad.com/pglo** for tutorial videos that explain the preparation techniques used

Prepare LB Agar Plates 3–28 days before Activity 2

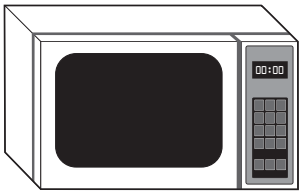
If your microwave cannot accommodate a 1 L flask, see Appendix A for modified plate pouring instructions.

1. Add 500 ml distilled water to an empty 1 L flask.



2. Add 20 g LB agar powder to the flask. Swirl to mix.

3. Heat the flask to boiling in a microwave, about 3 min. Swirl and repeat boiling at least twice more until all the agar has dissolved (no more clear specks).

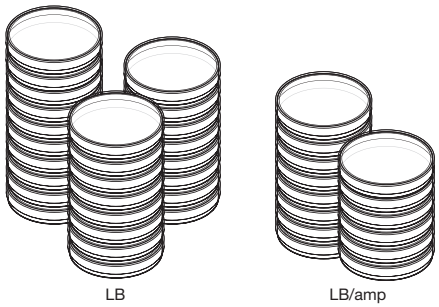


Safety! Use a hot pad or mitt when handling the hot flask. Be careful not to allow the LB agar to boil over. Use a lower power microwave setting and watch it carefully. Always allow the molten agar to cool slightly (about 30 sec) before swirling to prevent sudden boil over.

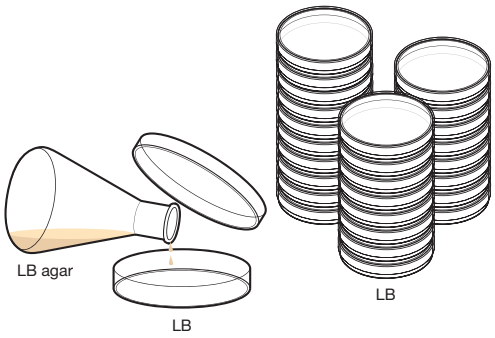
4. Label 26 plates **LB** and 14 plates **LB/amp**.

   Note: Label the plates on the bottom, close to the edge with a permanent marker. Do not label the lids.

   Note: This protocol produces two extra **LB** and two extra **LB/amp** plates which can be used as replacements or for further inquiry.

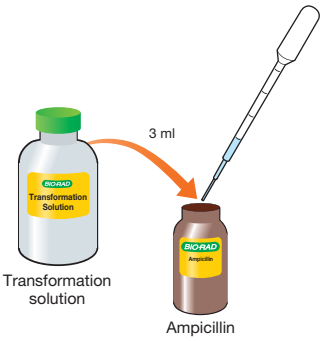


5. Fill 26 **LB** plates one-third to one-half full (~10 ml) with molten **LB** agar. Replace the lids immediately after pouring the agar.



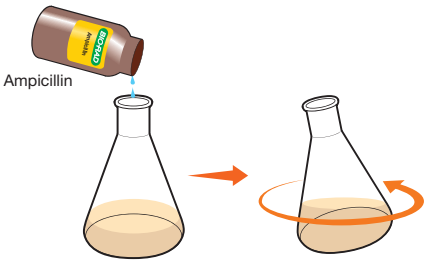
6. Add 3.0 ml transformation solution to the vial of ampicillin. Vortex or mix by pipetting until dissolved.

   Note: Transformation solution is used because it is a convenient sterile solution. If you have sterile water, that may be used instead.

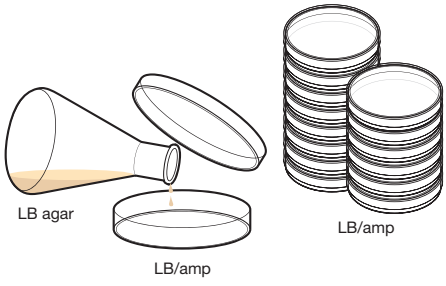


7. Once the flask has cooled enough that it can be comfortably held (~50°C), add the rehydrated ampicillin. Gently swirl to mix.

   Note: Excessive heat (>60°C) will destroy ampicillin. Be sure not to add it until the agar has cooled enough to handle. However, the agar will solidify at 27°C so be sure to pour your plates before it has cooled too much. Placing your flask in a 50°C water bath will help prevent the agar from cooling too quickly.



8. Fill 14 plates one-third to one-half full (~10 ml) with molten **LB/amp** agar.



9. Once solidified (~30 min), allow the plates to dry unwrapped at room temperature (20–25°C) for two days.

   Note: Allowing plates to dry for two days improves the uptake of the liquid transformation samples in the student lesson.

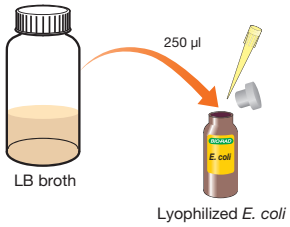
10. Stack and wrap plates in plastic or in the original plastic sleeve packaging taped closed. Store plates upside down and refrigerated at 4°C. Plates can be stored this way for up to one month before use.

Rehydrate bacteria 2 days before Activity 2

If you will be incubating rehydrated *E. coli* and starter plates at room temperature, rehydrate bacteria 4 days before Activity 2.

11. Using a new pipet, add 250 µl LB broth to the vial of *E. coli*. Recap the vial and shake gently to resuspend the bacteria.

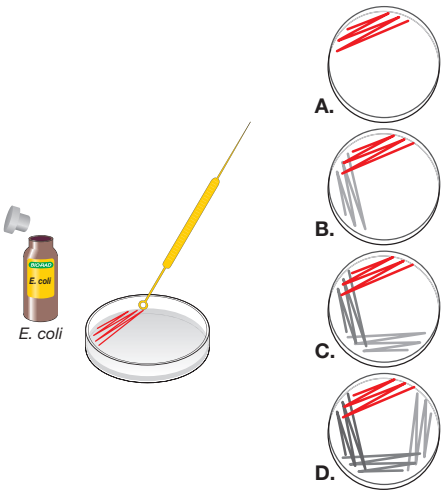
12. Incubate the vial 8–24 hr at 37°C.



Streak starter plates 1 day before Activity 2

If you will be incubating the starter plates at room temperature, streak plates three days before Activity 2.

13. Using a new inoculation loop, streak the rehydrated *E. coli* onto 12 LB plates.



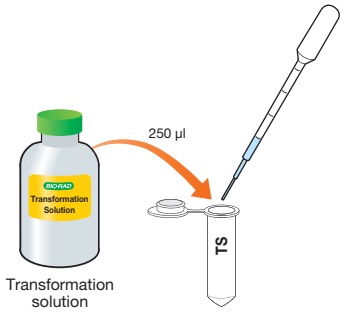
Important! Follow the streaking pattern shown in steps A–D. Dip the loop into the rehydrated *E. coli*, then streak the plate side to side at the edge of one quadrant (A). Rotate the plate about a quarter turn. Pass the loop from side to side through the previous streaks multiple times, extending into the next quadrant (B). Repeat twice more (C, D).

14. Incubate starter plates upside down in a 37°C incubator oven for 24 hr. Do not refrigerate before use.

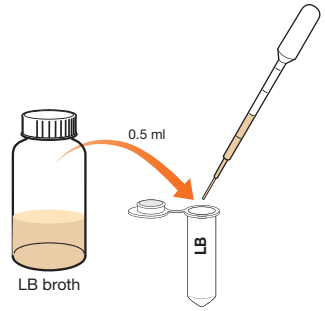
    Note: Alternatively, starter plates may be incubated upside down at room temperature (20–25°C) for 72 hr.

Dispense solutions up to 3 days before Activity 2

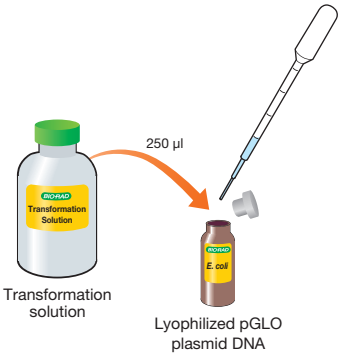
15. Add 250 µl transformation solution to each of 24 tubes labeled **TS**.



16. Add 0.5 ml **LB** broth to each of 12 tubes labeled **LB**.

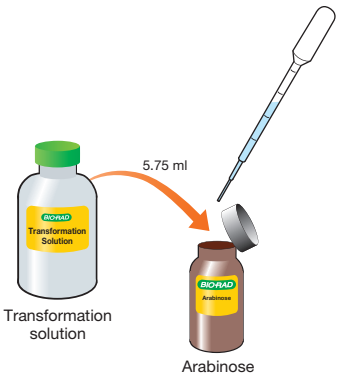


17. Add 250 µl transformation solution to the pGLO plasmid vial.



18. Add the remaining 5.75 ml of transformation solution to the vial of arabinose. Vortex or mix by pipetting for about 30 sec to begin dissolving. Briefly vortex or mix every few minutes until fully dissolved. Arabinose may take more than 10 min to fully dissolve.

    Note: Transformation solution is used here because it is sterile, which is essential. If you have sterile water, that may be used instead.



Prepare these workstations the day of Activity 2 Part 1

The kit includes materials for 12 student workstations.

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

Prepare these workstations the day of Activity 2 Part 2

Student workstation

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

Common workstation

Materials	Quantity
Arabinose solution and shared transfer pipet	~6 ml
UV light	1
Incubator oven set to 37°C (recommended)	1

➜ ***Tip:*** To ensure that each group has enough arabinose solution, limit each group to no more than 250 µl arabinose solution. Use the gradation marks on the transfer pipet to measure. If you have extra tubes and transfer pipets, you can dispense this amount ahead of time and add it to the student workstation along with a transfer pipet.

Instructor Background

Gene Regulation with the pGLO Plasmid System

Bacterial Transformation

With the help of biotechnology techniques, it is possible to transfer genes from one organism to another through a process generally called genetic transformation. In this activity sequence, students learn about inserting genes from one organism into bacteria with the aid of a plasmid. In addition to one large chromosome, bacteria may naturally contain one or more small circular pieces of DNA called plasmids. Plasmid DNA usually contains genes for one or more traits beneficial to bacterial survival. In nature, bacteria can transfer plasmids between themselves, which may permit them to adapt to new environments. For example, the development of bacterial resistance to antibiotics is often due to such transmission of plasmids.

The pGLO Plasmid

In this activity sequence, students will genetically transform bacteria with a plasmid called pGLO that contains a gene encoding green fluorescent protein (GFP). The source of this gene is the bioluminescent jellyfish *Aequorea victoria*. When exposed to ultraviolet (UV) light, GFP fluoresces green.

Bio-Rad's pGLO plasmid contains genetic features essential to its function including:

GFP — a gene from *Aequorea victoria* encoding green fluorescent protein
araC — a gene from the arabinose operon that encodes the arabinose-dependent repressor protein AraC
P_{BAD} — the promoter for *GFP,* which is repressed by AraC in the absence of arabinose and activated by AraC in the presence of arabinose
Amp^r — a gene that encodes beta-lactamase (bla), an enzyme that degrades ampicillin and allows bacteria to survive in the presence of ampicillin
ori — the sequence where plasmid replication is initiated

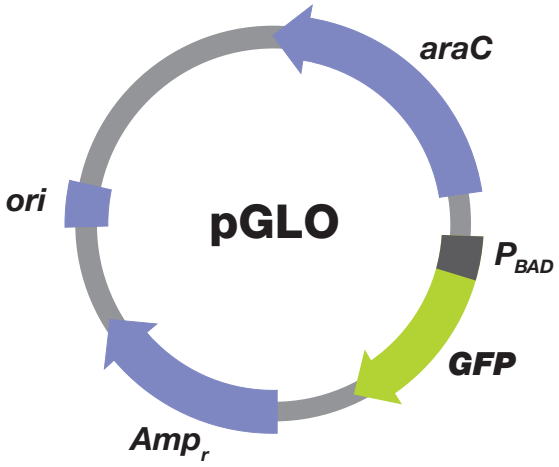
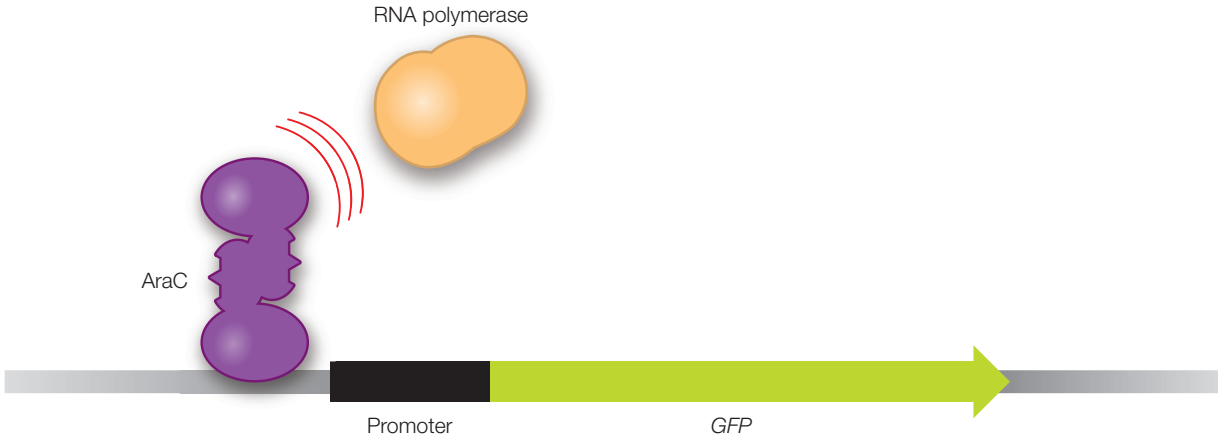


Fig. 1. pGLO plasmid map

Gene regulation

Gene expression in all organisms is carefully regulated to allow adaptation to differing conditions and to prevent wasteful overproduction of unneeded proteins. The genes involved in the breakdown of different food sources are good examples of highly regulated genes. For example, the simple plant sugar arabinose is a source of both energy and carbon for bacteria. The bacterial genes that make digestive enzymes to break down arabinose for food are not expressed when arabinose is not in the environment. But when arabinose is present, these genes are turned on. When the arabinose runs out, the genes are turned off again. Arabinose initiates transcription of these genes by promoting the binding of RNA polymerase. In the genetically engineered pGLO plasmid, some of the genes involved in the breakdown of arabinose have been replaced by the jellyfish gene that codes for GFP, but under the same arabinose control. Once transformed with pGLO, bacteria will begin to express the regulatory protein AraC. In the absence of arabinose, AraC blocks the P_{BAD} promoter and prevents RNA polymerase from transcribing GFP. In the presence of arabinose, AraC changes shape, no longer blocks P_{BAD}, and instead recruits RNA polymerase to transcribe *GFP,* leading to green glowing bacteria.

Without arabinose



With arabinose

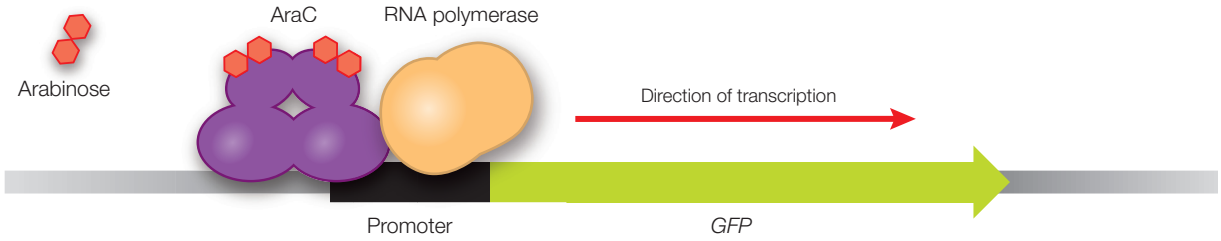


Fig 2. Regulation of the GFP gene by AraC

Antibiotic (Artificial) Selection

In bacterial transformation, only a very small fraction of bacteria successfully take up a plasmid. Without any intervention, these few transformed bacteria would be overwhelmed by the growth of neighboring bacteria. Since only those bacteria with a plasmid are of interest, an ampicillin resistance marker is included on the pGLO plasmid. Ampicillin will arrest the growth of *E. coli.* Those bacteria with the pGLO plasmid and therefore the ampicillin resistance gene will survive exposure to ampicillin. Those without the pGLO plasmid will not grow. The result is the selected growth of only those bacteria with the plasmid.

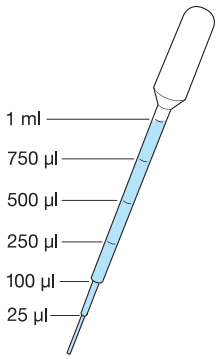
Laboratory Skills and Materials

Sterile Technique

This activity sequence is a great opportunity for students to practice their sterile technique. As with any microbiology procedure, contamination can ruin the pGLO bacterial transformation experiments. Contaminating bacteria are found on fingertips, benchtops, other surfaces, and even in the air. When students are working with the inoculation loops, pipets, and agar plates, stress to them not to touch the end of inoculation loops, the tip of pipets, nor the surface of agar plates. While some contamination will likely not ruin the experiment, students would benefit from an introduction to the idea of sterile technique.

Plastic Transfer Pipets

Before beginning the laboratory sessions, point out the graduations on the pipette the students. The 100 and 250 µl and 1 ml marks will be used as units of measurement throughout the labs.



Media

LB (lysogeny broth or Luria-Bertani broth) and LB agar provide a mixture of carbohydrates, amino acids, nucleotides, salts, and vitamins as nutrients for bacterial growth. Agar is derived from seaweed. It melts when heated, forms a solid gel when cooled (analogous to Jell-O), and functions to provide a solid support on which to culture bacteria.

Transformation Solution

This solution of calcium chloride ($CaCl_2$) greatly increases transformation efficiency. It is thought that the Ca^{2+} cations neutralize the repulsive negative charges of the phosphate backbone of the DNA and the phospholipids of the cell membrane to allow the DNA to enter the cells.

Heat Shock

Heat shock increases the permeability of the cell membrane to DNA. While the mechanism is not known, the duration of the heat shock is critical and has been optimized for the type of bacteria used and the transformation conditions employed. Be sure that students carefully time the heat shock and keep their samples on ice both before and after the heat shock. The rapid change of temperature is also very important for successful transformation.

Recovery

The 10-min incubation period following the addition of LB nutrient broth allows the cells to recover and to express the ampicillin resistance protein beta-lactamase so that the transformed cells survive on the ampicillin selection plates. The recovery culture can be incubated at room temperature or at 37°C for 1 hr to overnight.

Activity 1

Transferring Genes between Species

Goal: Students make observations and generate questions that lead to a working model of gene expression in transformed bacteria.

Begin this lesson by showing students a time-lapse video of transformed *E. coli* growth and exposure to UV light. Visit **bio-rad.com/glow** to see the video. All three parts of this activity are designed for students to observe and predict. At this stage there are no correct or incorrect answers.

	Teaching tips and notes
Part 1. Observe Fluorescent Organisms	• It is important that students generate ideas about how bacterial transformation might be involved in producing fluorescent bacteria. At this stage, refrain from correcting them or providing explanations • Ensure students understand that bacterial growth on agar media, both as individual bacterial colonies and lawns, comprises millions of individual bacteria cells. Probe this understanding in Part 2 as students draw models • **Formative assessment opportunity:** Review student answers to question E to determine whether they need additional support with how a gene leads to a trait
Part 2. Model the Processes that Occur in Green Fluorescent Bacteria	• If your students are new to modeling or if you are teaching remotely, visit **bio-rad.com/pglogenbio** to download a digital PowerPoint model template for students to use • **Formative assessment opportunity:** Discuss models with your students and press them to identify information that would help them to refine their models
Part 3. Analyze the pGLO Plasmid	• The plasmid map is presented with increasing complexity to scaffold student interpretation. Adjust as appropriate for your students • As students think of ways to use ampicillin to determine if bacteria have taken up the pGLO plasmid, it is very likely that they will predict the bacteria to glow green since they are not yet aware of *araC* • **Option:** if your students have experience with gene regulation, show them the full pGLO plasmid map at this stage and request more thorough explanations

Activity 2

Bacterial Transformation Laboratory Activity

Goal: Students collect experimental evidence to develop new ideas about gene regulation and to refine their models of gene expression in transformed bacteria.

In this activity, students will collect experimental evidence to compare and explain the expression of the genes *Amp*^{r} and *GFP*. Following each stage of the protocol, students will be invited to update their thinking and finally revise their models to include all the information they have gathered.

	Teaching tips and notes
Part 1. Transform Bacteria with the pGLO Plasmid	• See the Answer Guide in the kit box for expected results • There is an optional stopping point after step 14. Leave samples at room temperature overnight • Although tempting, do not explain to students why none of their bacteria glow. It is important for students to design an experiment in the next part that will help them explain this observation • **Formative assessment opportunity:** Ask students to predict how the bacteria would grow if they were to inoculate a new LB/amp plate with a colony from their current LB/amp plate
Part 2. Switch ON the *GFP* Gene	• See the Answer Guide in the kit box for expected results • Fluorescence should develop in about 24 hours after students apply the arabinose solution. Check for a green glow using a UV light • Each student group is instructed to use only 250 µl of arabinose solution, which is about 5 drops. There are actually closer to 450 µl available for each group. The discrepancy ensures that there is enough for all groups. Have students apply arabinose solution a drop at a time. A forceful squirt will both use too much solution and spread a bacterial colony creating a small lawn. • Students may struggle to explain why no bacteria glowed green on the +pGLO side of the LB plate. This result is a great opportunity to discuss environmental pressure and selection. A very small proportion of bacterial cells take up the pGLO plasmid during the transformation process. Without pressure, the antibiotic (ampicillin) resistance does not increase reproductive fitness for those bacteria with the pGLO plasmid. Since bacteria without the pGLO plasmid are much more numerous, they overwhelm those with the pGLO plasmid

Activity 3

Bacterial Transformation Design Challenge

Goal: Students apply the knowledge they gained using science practices to solve a real world problem using engineering practices.

Introduction

Bacterial transformation has many applications in a wide variety of fields, including pharmaceuticals, life science research, food science, and even cosmetics. In some applications gene regulation is utilized in a way that allows bacteria to "detect" an input that stimulates the expression of a gene such as *GFP*. Engineers may use genetically modified bacteria like these as part of biosensors, which are biological devices that respond to an input. In this activity, students will design a basic biosensor system that combines inputs and outputs of their choice to address a real world problem.

Teaching tips and notes:

- Have a discussion with your class about why it might be useful to use bacterial transformation to solve problems
- Have your students research examples of how bacterial transformation has already been used to solve important problems. See the Resources section for ideas
- Discuss the rubric with your students to answer any questions and even make modifications that everyone agrees on
- Keep students focused on the overall design of their biosensor; details of the mechanism of each input option can be avoided or included in later design iterations
- Consider having students revisit and modify their solutions multiple times throughout the year as they learn new concepts and gather information

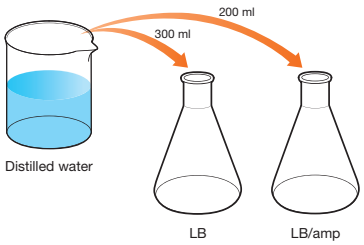
Bacterial Transformation Design Challenge Rubric

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 detail 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.

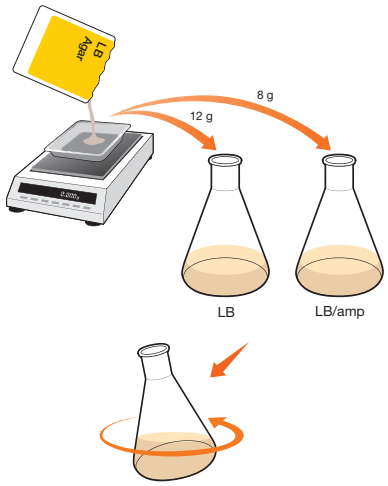
Small Microwave Modified LB Agar Plate Preparation Instructions

If you are using a microwave that cannot accommodate a 1 L flask, use these modified instructions, which utilize 500 ml flasks, to prepare LB agar plates. Then continue with normal preparation.

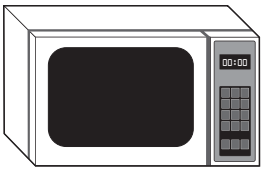
1. Add 300 ml distilled water to a flask labeled **LB** and 200 ml distilled water to a flask labeled **LB/amp**.



2. Add 12 g LB agar powder to **LB** and the remaining 8 g LB agar powder to **LB/amp**. Swirl both to mix.

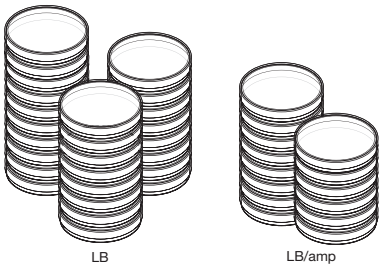


3. Heat each flask to boiling in the microwave, about 1–2 min. Swirl and repeat boiling at least twice more until all the agar has dissolved (no more clear specks).



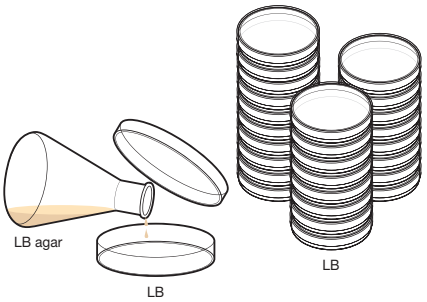
Safety! Use a hot pad or mitt when handling the hot flask. Be careful not to allow the LB agar to boil over. Use a lower power microwave setting and watch it carefully. Always allow the molten agar to cool slightly (about 30 sec) before swirling to prevent sudden boil over.

4. Label 26 plates **LB** and 14 plates **LB/amp**.



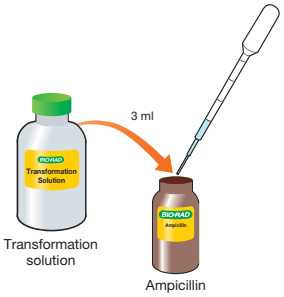
Note: Label the plates on the bottom, close to the edge with a permanent marker. Do not label the lids.

5. Fill 26 **LB** plates one-third to one-half full (~10 ml) with molten **LB** agar.



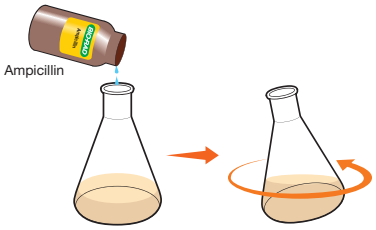
6. Add 3.0 ml transformation solution to the vial of ampicillin. Vortex or mix by pipetting until dissolved.

   Note: Transformation solution is used because it is a convenient sterile solution.



7. Once the **LB/amp** flask has cooled enough that it can be comfortably held (~50°C), add the rehydrated ampicillin. Gently swirl to mix.

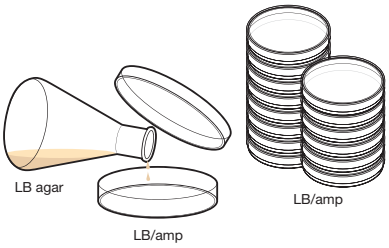
   Note: Excessive heat (>60°C) will destroy ampicillin. Be sure not to add it until the agar has cooled enough to handle. However, the agar will solidify at 27°C so be sure to pour your plates before it has cooled too much. Placing your flask in a 50°C water bath will help prevent the agar from cooling too quickly.



8. Fill 14 plates one-third to one-half full (~10 ml) with molten **LB/amp** agar.

9. Once solidified (~30 min), allow the plates to dry upside down and unwrapped at room temperature (20–25°C) for two days.

   Note: Allowing plates to dry for two days improves the uptake of the liquid transformation samples in the student lesson.



10. Stack and wrap plates in plastic or in the original plastic sleeve packaging taped closed. Store plates upside down and refrigerated at 4°C. Plates can be stored this way for up to one month before use.

Resources

Visit **bio-rad.com/pGLOGenBio** for additional classroom resources.

French C E et al. (2011). A5 Synthetic Biology and the Art of Biosensor Design. In: Institute of Medicine (US) Forum on Microbial Threats. The Science and Application of Synthetic and Systems Biology: Workshop Summary. Washington (DC): National Academies Press. Available from: ncbi.nlm.nih.gov/books/NBK84465/. Accessed August 18, 2020.
A collection of information about biosensors and some of their important applications.

Hanahan D (1983). Studies on transformation of Escherichia coli with plasmids. J Mol Biol 166, 557–580.
Detailed information about the factors that affect bacterial transformation in *E. coli*.

Schlief R (2000). Regulation of the L-arabinose operon in Escherichia coli. Trends Genet 16, 559–565.
A summary of information about the function of the arabinose operon in *E. coli.*

International Genetically Engineered Machine. igem.org
iGEM hosts an annual competition where high school and undergraduate teams compete for the best synthetic biology project. Students projects use synthetic biology to solve important human problems.

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