Biotechnology Explorer™
CAPTIVATING SCIENCE EDUCATION

pGLO™ Transformation and Inquiry Kit

A ThINQ!™ Investigation

Catalog # 166-0335EDU

AP Biology

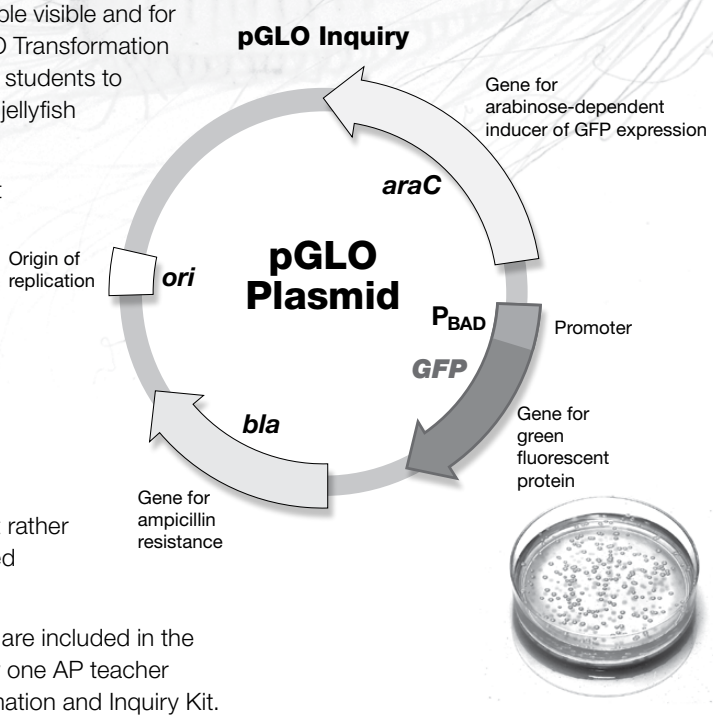
Teacher Model Process

BIO-RAD

Dear Instructor

With the continuing changes in science education, now is the time for addressing the challenges students and teachers face in AP Biology, biotechnology, and molecular biology. One of the biggest challenges is understanding biological concepts that are not visible to the naked eye or sometimes even with a microscope. A more recent challenge for both students and teachers is to engage in student-directed, inquiry-based investigations, just like real scientists. Science education is no longer about memorizing theories or nomenclature and finding the single right answer, it's about discovery, asking relevant questions, and analyzing results to learn from failures as well as successes.

Bio-Rad's Explorer program has a solution for making the invisible visible and for engaging students in the scientific process of inquiry. The pGLO Transformation and Inquiry Kit includes five inquiry investigations that will push students to think like real scientists by using a gene from a bioluminescent jellyfish and its green fluorescent protein (GFP).



GFP fluoresces a brilliant green when viewed with an ultraviolet light source. A modified form of the *GFP* gene has been inserted into Bio-Rad's pGLO plasmid and is available exclusively from Bio-Rad for educational applications. GFP is incredibly bright, so when students use the pGLO plasmid to transform bacteria, they can actually observe gene expression in real time.

The inquiry investigation curriculum guides students through selecting a question and the thought process involved in determining and carrying out a student-directed, inquiry-based experiment. The focus is not solely on the answer or result, but rather on how the result was obtained and how it can be substantiated by careful observation and data analysis.

To facilitate the teacher's role, explanations and interpretations are included in the instructor's guide. The teacher model process also shows how one AP teacher implements inquiry in her classroom using the pGLO Transformation and Inquiry Kit.

Getting students involved with inquiry will help increase their interest in and understanding of the scientific process. Furthermore, we expect that students who engage in this type of process will be better prepared to pass the AP Biology final exam.

Bio-Rad's GFP-based inquiry curriculum is unique and has generated genuine excitement among science educators. We strive to continually improve our curriculum and products. Your input is extremely important to us. We welcome your stories, comments, and suggestions.

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An inquiry-based kit designed to make students think. Developed with master AP Biology teachers, Bio-Rad's ThINQ! Investigation Kits will give you the freedom and tools to successfully guide students through multiple levels of inquiry.

One Teacher's Story of Implementing Inquiry

Overview

For many teachers, "inquiry" conjures a nightmare scenario of turning 40 students with minimal skills loose in a lab with unlimited resources and asking them to design experiments. Although trends in science education have shifted from teacher-led coverage of content to inquiry-based, student-directed activities, most lab investigations at the high school and AP levels still require teacher guidance. However, investigations should allow students to ask questions and model the behavior of scientists as they explore answers to those questions. By designing and conducting experiments to test hypotheses, analyze data, and communicate results, students focus on understanding concepts by merging content with the reasoning skills essential to the study of biology. An inquiry-based approach engages students and promotes collaborative learning.

Tips and Tricks

Activities such as these can be performed by students working in small groups, or they can be assigned for homework. They can be conducted either before or after the lab investigation as a review and exercise in connecting concepts. These activities and the lab investigation can, for example, serve as a bridge between the concepts of genetics and evolution.

Bio-Rad's pGLO Transformation and Inquiry Kit accommodates students with varying levels of mastery and proficiency. As students work through each investigation, the teacher asks probing questions to assess their understanding of concepts and address misconceptions and technical challenges. Students are encouraged to use available resources (other than the teacher) for information, such as the Internet using computers and tablets. Obtaining meaningful data is the goal of scientific investigation, but great teachable moments occur when students explore causes of unexpected data and troubleshoot their experimental designs.

The following teacher model process provides examples of how inquiry-based investigation labs can take place using the pGLO Transformation and Inquiry curriculum:

- Pre-lab: Prepare students to investigate pGLO Bacterial Transformation — review concepts surrounding bacterial transformation and extension to other related concepts, such as evolution and genetically modified organisms (GMOs)
- Day 1: Perform pGLO Bacterial Transformation lab
- Day 2: Collect and analyze data (determine transformation efficiency)
- Days 3 and 4: Perform inquiry lab 1, examining variables affecting transformation efficiency

Pre-Lab: Prepare Students to Investigate pGLO Bacterial Transformation

Having been introduced to the concepts of genes, gene expression, and the process of bacterial transformation, the students in Ms. Z's class are anticipating a laboratory exercise on the same topic. In preparation for the pGLO bacterial transformation lab, Ms. Z organizes her classroom for a review of the topic and a relevant inquiry-based discussion. She arranges the desks into groups of four and places two large sheets of paper and several colored markers at the center of each group. The goal of this exercise is to assess their understanding of the topic and challenge them to extend their understanding through several inquiry-based activities.

Activity #1: Inquiry-Based Review of Concepts

As the students filter into the classroom and take their seats, Ms. Z writes a list of terms on the whiteboard:

- Bacterial cellular structure (*Escherichia coli*)
- DNA, RNA, and enzymes
- Transcription and translation
- Operon model of gene regulation in prokaryotes
- GFP
- Bacterial reproduction (conjugation)
- Regulator proteins
- Plasmid
- Ampicillin/antibiotic
- Arabinose

She turns to the class and says, "Over the last week, we learned about DNA, RNA, and protein . . . about genes and gene expression. We discussed how genes from one organism, say a jellyfish, can be transformed, or moved into and expressed in another organism, such as bacteria. So these terms on the board should be familiar to you."

She looks around the room and is encouraged to see the students reading the list and some even nodding in agreement.

"It's one thing to know the meanings of these terms. It's another to understand how they are related to each other. So here is the exercise for this morning. I'd like you to work in groups and use the large sheet of paper and colored markers on your desks to draw a picture or a diagram, whatever you prefer, to show how those terms apply to a very common laboratory experiment: plasmid-based bacterial transformation. Show how the terms are connected."

The students begin to construct their diagrams. Some draw pictures of bacterial cells, others draw diagrams showing relationships between the terms. Ms. Z walks among the groups, periodically asking and answering questions.

“What other terms might we add to our list to describe transformation?” she entices as she stands next to group 2 in the far corner of the classroom.

“Evolution?” blurts Alex from group 1.

“What about evolution? How does transformation relate to evolution?” asks Ms. Z as she turns to face Alex and group 1.

Alex continues his thought, “If the bacteria become resistant to the antibiotic, they’ve adapted to their environment. You know — Darwin, survival of the fittest, natural selection.”

“That’s right,” says Ms. Z.

Anna in group 2 adds, “Evolution doesn’t have to take millions of years. It can happen overnight in a high school lab!”

“Excellent point!” says Ms. Z. “Now, who remembers what the antibiotic does during the bacterial transformation process?”

“It kills* the bacteria . . . ,” says Jake as Sanjay adds, “That’s right, just like the antibiotics we take when we have an ear infection.”

Ms. Z nods in agreement and continues this conversation saying, “We know why antibiotics are important when we have an ear infection, but why are they important for bacterial transformation?“

Alex again contributes his thought, “The antibiotics kill off the bacteria that don’t have the plasmid, the ones that aren’t transformed. That way, only the transformed bacteria grow.”

“Correct,” says Ms. Z. “Now, in the experiment we are going to do, how else will we know the bacteria we see on the plate are transformed? How do we know they haven’t simply evolved overnight in a high school lab?”

Silence greets her, so she continues, “What other genes are in the pGLO plasmid?”

Andrew says, “The green one — the one that makes the bacteria glow green.”

The students discuss the use of a fluorescent marker protein and conclude that it’s very cool that they will be making glowing bacteria.

Activity #2: Inquiry-Based Extension and Connection of Concepts — GMOs

Ms. Z now asks the students to take the second sheet of paper and place it in the center of their desks. She surprises them with this question: “If you have ever eaten corn flakes for breakfast, please raise your hand.”

A majority of the students raise their hands.

“Now, how many of you would eat them tomorrow if you knew that the corn used to make them had been genetically modified to produce a toxin to kill caterpillars and earthworms that munch on the corn?”

“Ewwwww,“ says Karlie. “No way!” exclaims Andrew.

“Well,” explains Ms. Z, “You should know, then, that scientists are able to insert genes from the bacterium *Bacillus thuringiensis* (Bt) into the corn genome. When the genes are expressed, they produce a natural insecticide. This means the corn plant with the genes from Bt can kill off some of its pests, so it stays healthier and the farmer doesn’t have to use as much insecticide.”

“But how can that be safe?” asks Karlie.

“You tell me. Who has heard of genetically modified organisms, or GMOs?” Nearly all the students raise their hands. “Please take out your tablets or cell phones and for the next 15 minutes or so, search for and write down other uses of genetically modified products. Karlie, you may want to look up more about why scientists think this corn is safe for humans. All of you ask yourselves: are there any safety issues concerning the use of GMOs? Any ethical or other issues?”

The students investigate the questions and list their findings on the paper given to them by Ms. Z, who again circulates throughout the classroom, asking probing questions.

“Who knows someone who takes insulin to control their blood sugar level?” Three students raise their hands.

Ms. Z nods and then asks, “Who knows someone who’s been told they had an infection that was resistant to antibiotics?”

Olivia raises her hand. “Me. My cousin had pneumonia, and penicillin didn’t work.”

* Ampicillin does not kill bacteria; rather, it inhibits bacterial growth. For the purpose of this lesson, however, it may be advisable not to curb the discussion by pointing this out.

Ms. Z replies, “I hope the doctors were able to find an effective antibiotic for your cousin.”

Olivia nods. “They were! They used another type of antibiotic that worked.”

After 15–20 minutes, Ms. Z goes to the whiteboard and asks group 1 to tell her one of the uses for GMOs they discovered.

“Making medicine, like insulin. Insulin used to come from animals, but it’s cheaper and better to make it in genetically engineered bacteria,” says Cade. Ms. Z writes, “Manufacturing medicine (insulin)” on the board and then asks, “Group 2. What else did you find?” “Cleaning up contamination,” is the response from Sanjay. And so the discussion continues through all the groups until Ms. Z has a list that includes:

- Manufacturing medicine (insulin, human growth hormone, clotting factors, and vaccines)
- Cleaning up contamination (mercury, arsenic)
- Crop resistance to pests (insects, viruses)

“Did you read anything about safety or ethical concerns?” asks Ms. Z.

“Yeah,” says Andrew. “What about bacteria that have become resistant to antibiotics? Isn’t that a bad thing? And what about the GMO corn? What will happen if scientists find out 20 years from now that these ‘Frankenfoods’ aren’t healthy?”

Anna adds, “I read about butterfly populations dying off and concerns that were linked to the GMO corn.”

Ms. Z says, “Those are common and often-debated concerns. Much scientific evidence supports the idea that GMOs are safe for human consumption, but the debate continues. And we don’t fully understand how GMOs affect the environment, either. That is why there is such a big debate raging about whether GMOs are ‘good’ or ‘bad.’ It’s important for you to understand what GMOs really are, how they are made and used, so that you can form your own educated opinions. But here is a question for you: Haven’t humans been creating ‘Frankenfoods’ all along?”

The students look puzzled.

“Corn, tomatoes, wheat . . . dogs, cats, cows, horses . . .,” she continues. “All these organisms have been bred over centuries to have characteristics (a phenotype) we humans prefer. What do you think? Is that different from, say, putting a fluorescent protein into bacteria in a single step? Why or why not?”

She leaves them with these questions, adding, “Remember all these questions as you conduct your lab investigations tomorrow. Please read the pGLO lab background and protocol and then answer the questions for homework, so we can jump right in to the investigation tomorrow. Also, you’ll have an opportunity later in the week to design your own experiments based on the pGLO lab. So as you’re reading the protocol and answering the questions, start thinking about and writing down questions and observations you may have while performing the experiment.”

DAY 1: Investigation #1: pGLO Bacterial Transformation (structured inquiry)

Ms. Z’s students are arranged in groups again and are working through the pGLO transformation protocol. The room is buzzing with conversation as students help each other with techniques and tools they have not used before. Ms. Z circulates among the groups and stops at group 1, asking Antonio, “Why did you add calcium chloride to the reaction mixture?”

Antonio looks up, a blank expression on his face. “Calcium? I don’t know . . . Because they need strong bones,” he replies with a grin.

Ms. Z smiles back and continues, “Think about the chemistry of calcium chloride. What kind of compound is it?”

“It’s . . . a salt?” he asks.

“Yes, it is a salt,” says Ms. Z. “What are the components of this salt?”

“Calcium and chloride?”

“Great! Which of those is positively charged and which is negatively charged?”

“Calcium is positive, and chloride is negative,” replies Antonio, gaining confidence.

Tips and Tricks

In small groups, students follow the step-by-step procedure outlined for the pGLO Bacterial Transformation lab. Consider the protocol a learning module; many students will be unfamiliar with the tools and techniques used to transform bacteria using a plasmid system, such as reading the metric graduations on a plastic DPTP or thermometer, or following sterile technique. Impatient students often skip steps in the procedure, while others may be daunted by the mathematical formulas to determine transformation efficiency. As you circulate among the teams, ask provocative questions that will allow you to assess students’ understanding of concepts while promoting independence, reasoning skills, and the application of good science practices.

Ms. Z decides to keep Antonio on this train of thought and asks, “Now how about DNA. What kind of charge does it carry?”

“A negative charge . . . because of the phosphate groups.”

“Correct again!” assures Ms. Z. “For transformation to occur, what does the DNA molecule need to do?”

“It needs to get into the bacterial cell,” answers Antonio.

“What does it need to cross to get into the cell?”

“The membrane,” says Antonio, wondering where all this is going.

“Now, think about the membrane. It has phosphate groups sticking out into the growth medium, right? What happens when the negatively charged DNA comes close to it?”

“They repel each other,” says Antonio. “Oh! I get it! The calcium ions are positive, so they neutralize the negative charges so the DNA can be taken up by the bacteria.”

Ms. Z is impressed. “Well said! You understand chemistry! Actually, the calcium binds the DNA phosphate group and the membrane phosphate group, bringing them closer together.”

Stopping in front of group 3, she notices the series of tubes on ice and asks, “Who can explain why you need to incubate your microcentrifuge tubes on ice and then heat shock them?”

Matt answers, “The ice makes the membrane of the bacteria less liquidy, kind of like butter in a refrigerator is thicker and more rigid than butter at room temperature. So the heat shock probably increases membrane permeability. I read that it’s tricky for cells to take up plasmids. They’re not very competent.” He laughs as other students groan at his pun.

Alex asks, “What happens if we forget to heat shock the cells?”

Ms. Z responds, “What do you think might happen?”

“I’m guessing that our experiment won’t work very well. That’s a question we can investigate. Maybe we can skip the ice, too.”

Ms. Z nods. Her students are on the right track to inquiry. She then moves on to group 2. “I can’t help but notice that your microcentrifuge tube looks like it has more than 250 microliters of transformation solution in it.”

Sanjay says, “I wasn’t sure about the markings on the pipet.”

“How can you find out if you’re reading them correctly?”

“Compare the real pipet to the one in the diagram? I should’ve thought of that.” He checks the diagram and then draws the solution from the microcentrifuge tube back into the pipet. “Yikes! Almost 500 micro-whatevers.”

Anna corrects him. “Microliters. And you’re touching the tip of the pipet with your finger. Everything’s supposed to be sterile, remember?”

Sanjay nods and says, “And I should probably wear safety glasses.”

Ms. Z is monitoring the discussion but allowing students to self-correct and collaborate, as both skills are essential to inquiry. “How will you be able to tell if your cells have been transformed?” she asks, since making predictions before analyzing results — hypothesizing — is another important practice in science. But she is greeted with silence.

Walking toward Jake, she says, “What types of genes did we have on the plasmid? Does anyone remember?”

“The jellyfish one, the one that makes the jellyfish glow green,” says Jake. “If the cells took up the plasmid, they’ll glow green under UV light,” he continues.

“Will we have to wait to have a UV light then, to know if the cells are transformed?”

Again, she is met with silence, and so continues with, “What other gene did we have in the plasmid? Will all the bacterial colonies growing on all of our plates be transformed? How might we know without using a UV light?”

“The plasmid had the gene for antibiotic resistance. The transformed bacteria will be able to grow on the plates with ampicillin,” says Jake.

Antonio adds, “And the cells that aren’t transformed will die. The ones that took up the plasmid will be resistant, just like the ones that infected Olivia’s cousin!”

Ms. Z says, “Looks like you’re ready to finish up. You can incubate your plates either overnight at 37°C or for several days at room temperature. Which do you think will be more effective?”

Antonio replies, “Can we try both?”

DAY 2: Collecting and Analyzing Data (calculating transformation efficiency)
The students arrive in Ms. Z’s classroom eager to check their plates for transformed cells. They crowd around the incubator and Anna immediately exclaims, “Wow! We’ve got about 200 colonies growing on the plate with LB, ampicillin, and arabinose.”

Alex says, “Check this out! We have a ton of colonies glowing bright green under the UV light! Cool!”

Tips and Tricks

In small groups, students examine the results of the transformation experiment performed the day before. Now their analytical and critical thinking skills are put to the test as they calculate transformation efficiency and start to think of the variables that may have influenced their results.

“We have some bacteria growing on our control plates, but not on the plates with ampicillin and arabinose,” comments Jake rather sadly.

Ms. Z comes over to look at the plate Jake is holding. “Hmm. That’s surprising. Do you have any idea why you don’t have transformed cells?”

Jake says, “Maybe we forgot to add plasmid. That’s the most logical answer.”

The students in Jake’s group begin talking about the factors that could have interfered with the transformation process. Did they add enough bacteria or plasmid to the reaction mixture? Was the temperature of the water bath for the heat-shock treatment too high or too low? Others chime in and ask if they used the right amount of $CaCl_2$ or waited too long to return the cells to ice following the heat shock.

Karlie asks, “How about the plates themselves? Did you spread enough transformed culture on them?”

Alex, looking at the plate Jake is holding, asks, “Why are your plates so wet inside?”

Group 3 realizes that they had placed their plates in the incubator right side up instead of upside down; condensation had dripped onto the agar.

“We know how we messed up. We accidentally diluted everything!” says Matt.

“Mystery solved! I have extra supplies, so you can repeat the experiment. You might have to come in after school, though,” says Ms. Z. “Okay, it’s time to analyze results and determine transformation efficiency. You’ll need the equations from the protocol and calculators. When you have your results, add them to the class data table on the whiteboard. Cade, can you help us out by drawing a table?”

Ms. Z circulates among the lab groups, asking questions to determine whether they have sufficient mathematical skills to calculate transformation efficiency and understand which factors influence efficiency. Visiting group 1, she examines their calculations and asks, “You calculated a transformation efficiency of 1,187. What does that mean?”

Jake says, “It means we have about 1.2×10^3 transformed colonies per microgram of DNA that we spread on the plate.”

“How does this compare to your prediction?”

Antonio confidently says, “We think it’s good because scientists from Bio-Rad claim we can expect between 8.0×10^2 and 7.0×10^3 transformants per microgram using this protocol.”

“Good work. How can you improve transformation efficiency? Can you design another experiment?”

Visiting group 2, Ms. Z asks Anna, “What’s your calculated transformation efficiency?”

“About 94.”

“Is that what you were expecting?”

Sanjay answers, “No. We were expecting more than that. It’s not even close to what we saw when we looked it up online.”

“How many colonies are growing on the plate with plasmid, ampicillin, and arabinose?”

“About 150,” says Anna, a hint of confusion in her voice.

Cade overhears this conversation and adds, "We had about 200 colonies and got a transformation efficiency about 10 times more than that. Are you sure you did the math right?" He takes her calculator and starts plugging in numbers. "For 150 colonies, and assuming you spread 0.16 micrograms of pGLO DNA on the plate, your transformation efficiency should be 938, not 94. Your transformation efficiency is actually great."

Anna replies, "Seriously?" She and the rest of the group check over their work. "I put the decimal point in the wrong place! No wonder we're way off."

The groups finish calculating their results, and as they enter them in the class data table on the whiteboard, Ms. Z starts to summarize the factors they discussed that could have affected transformation efficiency. "Plasmid concentration, amount of bacteria, ice treatment, temperature and duration of heat shock . . . What other factors can affect transformation efficiency?"

With the help of the class, she ends up with a list that includes the following factors:

- Plasmid concentration
- Amount of bacteria
- $CaCl_2$ concentration
- Is $CaCl_2$ the only salt we could use?
- Ice incubation prior to heat shock (10 min)
- Temperature of heat shock
- Amount of LB broth
- Bacterial growth: 37°C?

She reads the list to the class and then adds, "What about the plasmid itself? Do you think expressing a green fluorescent protein from jellyfish affects the growth of transformed *E. coli*? Is that something you could test?"

She continues, "For homework tonight, I'd like you to go over this list and design an experiment for a factor or variable and hypothesize its effect on transformation efficiency. There are steps in your lab manual to help you. Bring your experimental design to class tomorrow so you can work with your group to decide which one you will perform."

DAY 3: Inquiry Investigation #2: Transformation Efficiency (guided inquiry)

Ms. Z greets her class and points to the list of variables from the day before. "Yesterday, we discussed these variables, or factors we could manipulate, in an effort to improve transformation efficiency. For homework, you looked through the list and thought about ways in which you could test each of them. Today, I'd like each group to agree on a variable to test. Please list your top three choices, so we can be sure the whole class does not investigate the same one."

After several minutes, Ms. Z notices the level of discussion is winding down, so she redirects the class's attention by saying, "Okay, then. Group 1, which variable do you plan to investigate?"

Antonio speaks for the group. "We're going to play with the heat-shock treatment a bit."

Looking for a little more detail, Ms. Z asks "How so?"

Alex says, "Yesterday we determined that heat shocking the cells for 50 seconds at 42°C resulted in a transformation efficiency of about 1,200 transformants per microgram of DNA. Today we're going to try higher and lower temperatures: 60°C and 32°C."

Dias adds, "We could try the different temperatures for different times, because maybe a shorter, hotter heat shock would be better!"

Ms. Z asks, "Dias, if you heat shock at 60°C for 10 seconds and you get fewer colonies than you do at 32°C for 50 seconds, will you know anything about the experiment?"

Dias replies, "Well, we won't know if it was the temperature or the time or both that made the difference. Okay, then we'll do both treatments for 50 seconds so we have only one variable."

Ms. Z says, "That is a better experiment. Time itself is also a variable, so you should keep that constant if you are looking into the effect of temperature. Any predictions?"

Tips and *Tricks*

Students with weak math skills often have difficulty working through the series of calculations necessary to determine transformation efficiency. Collaboration strengthens these skills when students teach each other.

Tips and *Tricks*

In this activity, students plan and execute experiments to test the variables that affect transformation efficiency. Continuing to work in groups, they base their predictions on prior knowledge by connecting concepts they've explored previously, from the relationship between temperature and behavior of molecules and the structure and function of cell membranes to plasmid-based bacterial transformation. All members of the group participate in designing the independent investigation.

Tips and *Tricks*

Students should be challenged to think through to the end goal of their experimental design. What are they actually trying to determine? Will their setup really show them that? Once again, collaboration strengthens these skills when students teach and challenge each other.

Jake finally gets a word in. “The point of heat shocking the cells is to make the bacterial cell membranes more permeable to take up plasmid. Since the membranes contain lipids, it seems that if the temperature increases, the lipids will move around more and loosen up the membrane. If this is true, then heat shocking the cells at 60°C should improve transformation. Colder temperatures should slow everything down.”

Alex adds, “Unless the higher temperature destroys the membrane or plasmid DNA, like how enzymes are denatured.”

Dias concludes, “We’ll do the math and compare the transformation efficiencies from the three different temperatures.”

Impressed with how much thought they have put into this experiment, Ms. Z says, “Great idea. I’ll look forward to seeing if your data validate your predictions. Group 2, which variable are you going to study?”

Sanjay speaks for the group. “We’re going to study the 10 minute ice treatment before heat shock. That part doesn’t make a lot of sense to me. If heat shock is needed to open the membranes, then having the cells cold first should make things worse, not better.”

Ms. Z asks, “How will you study this variable?”

Anna contributes, “We are going to try the transformation with no cold shock and with a longer cold shock, and then we’ll compare the transformation efficiency of those two sets of cells with the transformation efficiency we got yesterday to see if it makes a difference.”

“Hmmm. Do you think you should compare a new experiment to one you did a day or so ago? How can you be sure everything else will stay the same?”

Hunter adds, “Ms. Z is right. We should also do a 10 minute ice treatment as a control. So we should have at least three treatments: no ice, 10 minutes on ice, and a 20 minute ice treatment.”

Ms. Z seizes this opportunity to explore the concept of controls and their importance to an experiment. “Great, Hunter. Why is the 10 minute treatment a control?”

Hunter says, “Well . . . it’s the time we used yesterday.”

“That’s right. What is a control?”

“A control is the experiment you do that you compare the other ones to. We used 10 minutes yesterday and today we are trying to see if time in the ice makes a difference, so 10 minutes is the control.”

Ms. Z is encouraged that her students are thinking through their experiments. “Okay, everyone, your ideas are really great. Go ahead and get started!”

DAY 4: Examining Variables Affecting Transformation Efficiency

Ms. Z opens the discussion with group 3, asking them, “Okay, group 3. What did you discover about the effect of the length of the heat-shock treatment on transformation efficiency?”

Karlie speaks for the group. “Fifty seconds at 42°C is optimal. When we heat shocked the cells for 90 seconds, we got half the number of transformed colonies. With no heat shock, we got even less — about a tenfold decrease in transformants.”

Andrew adds, “Heat shocking for too long probably disrupts the membrane too much or denatures enzymes involved in the process, like RNA polymerase. With no heat shocking, the membrane stays too rigid and it’s hard for the plasmids to get through it. No plasmid, no transformation. Simple.”

At which Alex groans, “Nothing in biology is simple.”

Ms. Z congratulates the students. “Excellent work, teams. Tomorrow we’ll solve a crime using restriction enzyme analysis.”

Tips and *Tricks*

Students study the results of their experiments, choosing also the means for reporting their results and conclusions. For example, group 1 decides to create a mini-poster, group 2 collaborates on a formal lab report, and members of group 3 submit individual reports. Students examine the data within the context of their question, consider how the experiment was conducted, and think about other experiments that could be performed to extend their inquiry.

Tips and *Tricks*

Students might be inspired to take their investigations in other directions, time and resources permitting. The pGLO Transformation and Inquiry Kit provides supplies and guides for several open inquiry investigations. When the series of investigations wraps up, use the student case study at the end of the manual as a real-world example of an application for bacterial transformation in fighting disease. Finally, circle back to the original questions about the applications of genetic transformation, including GMOs, and possible ethical issues raised by the manipulation of DNA.

Visit **bio-rad.com/web/pGLOinquiryMore** for more information.

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