

# pGLO™ Transformation and Inquiry Kit

A ThINQ!™ Investigation

**Science Case Study**

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## Science Case Study

### “Hacking the Gut Microbiome: Could Engineered Bacteria Relieve Dysentery Symptoms?”

#### Part 1 – Setting the Scene.

Dr. Karen Cruz felt like she hadn't slept for a week. Running back and forth from one bed to another in the hot and arid climate of Afghanistan had taken its toll on her. In a far-flung military outpost outside Kandahar, she had been tending to the soldiers for several weeks after a dysentery outbreak gripped the troops. Even her nursing staff had succumbed to the illness, and she was short staffed to begin with. Now, sitting at her desk, she began to review her notes:

January 21

*Today 15 more soldiers have presented symptoms of what I suspect is a quickly spreading bacterial infection that causes dysentery. Symptoms include fever, chills, bloody diarrhea, loss of appetite, and dehydration. The total count on bed rest was 56, plus these 15 make 71, with only 30 beds in our medical tents. Keeping everyone quarantined from the remaining 75 personnel on base is more and more difficult.*

*Luckily there have been no casualties, since we are well stocked with IV fluids to keep the most gravely ill hydrated. However, I wonder if there is anything that can be done to relieve their symptoms. I can tell the soldiers are doing their best to keep a positive attitude, but morale is beginning to diminish.*



Because it was nearly impossible for the troops at the outpost to avoid infection from the local food and water, Dr. Cruz hoped maybe she could at least diminish the symptoms and help her patients recover more quickly. Dr. Cruz kept up to date with medical research and noted that there has been a lot of recent interest in microbiomes — the collective genomes of all the microorganisms living in and on an organism, including a human, and how they influence the outcome of acute and chronic illnesses. She also remembered that one of her medical school professors was involved in research funded by Defense Advanced Research Projects Agency (DARPA) to synthesize genetically engineered bacteria to combat gastrointestinal diseases like dysentery. She decided to email her former professor to share her experience and find out more about his research. She was pleasantly surprised by his response:

Dear Dr. Cruz,

Thanks for reaching out to me and thank you for supporting our troops! The initial phases of my research have proven quite successful and may be of use to you. Our research question is: Can genetically engineered bacteria be used to reduce the symptoms of dysentery? We decided to genetically engineer *E. coli* using transformation as a method to program the *E. coli* to sense infection in the gastrointestinal tracts of mice and then stimulate the mouse's own immune system to fight the infection faster than it normally would—so the mice would be sick for half the normal time. Our hypothesis is that it is possible to 1) engineer bacteria to detect infection and 2) engineer the bacteria to induce a host immune response faster than it would naturally occur once the engineered bacteria detected infection.

I've attached our initial data here with further explanation. Let me know what you think.

Sincerely,

Dr. Richardson

Dr. Cruz was excited to hear of the advancements in Dr. Richardson's research. She hoped that this work would allow troops in the future to recover from dysentery sooner or even prevent them from becoming ill. Dr. Richardson's email made sense, but there was still a lot Dr. Cruz did not understand about genetically engineered bacteria: how they are made and how they work in the mice's intestines. Before looking at the data, she decided to do a little research of her own to determine whether the engineered bacteria would be useful to treat dysentery. She would need to understand the process of engineering the bacteria. She decided to consult with her head nurse, Ms. Brady, who had a background in microbiology and genetics.



**Dr. Cruz:** Nurse Brady, I'm really optimistic that Dr. Richardson's research can help in the fight against bacterially transmitted diseases like dysentery. But there are still several things I don't fully understand about his team's research. I'm hoping you can help me. First off, could you give me a refresher on the process of bacterial transformation?

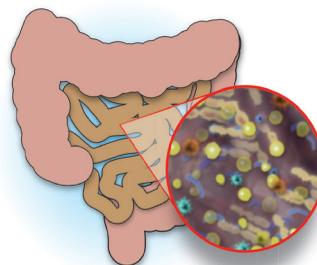
**Nurse Brady:** Sure, I'm glad to help! Genetic transformation in bacteria is a technique that scientists use to insert genes into an organism, in this case the bacterium *E. coli*. In addition to one large chromosome, bacteria naturally contain one or more small circular pieces of DNA called plasmids. These plasmids can be re-created by scientists to include genes they want to express in the bacterial cells. In the case of Dr. Richardson's research, it sounds like they plan to transform *E. coli* with a plasmid expressing a gene that triggers the mouse's immune system when infection is present.

**Dr. Cruz:** I see. So Dr. Richardson and his team plan to transform the *E. coli* to express a gene that it normally wouldn't, and that may help mice recover from dysentery more quickly. Another thing that Dr. Richardson mentioned is that the engineered *E. coli* can help combat the "bad" bacteria and viruses in the gut.

**Nurse Brady:** Yes, exactly. It's true that some bacteria can cause illnesses such as dysentery when they are present in high numbers in the gut. They can do this in several ways. Some produce toxins that cause inflammation of the intestinal lining, resulting in diarrhea. Others may cause ulcers to form in the intestines, resulting in blood in solid waste. Some *E. coli* strains are harmful, but other strains are not, particularly those used in research labs like *E. coli* HB101, which are engineered so that they are not harmful. Dr. Richardson must be making use of these harmless *E. coli* strains.

**Dr. Cruz:** That makes sense. Can you tell me more about microbiomes? I have read a little, but I gather that you have been reading much more on the topic.

**Nurse Brady:** Well, the first thing to consider is that mammals, including humans, have more bacterial cells in their gastrointestinal, or GI, tracts than the number of cells making up their entire bodies. There are up to 100 trillion bacteria in your GI tract and approximately 37 trillion cells that make up your body. As you know, the GI tract includes the series of organs through which food passes, nutrients are absorbed, and solid waste is eliminated. Bacteria live on the surfaces of the GI tract and these communities, made of many different types of bacteria, are referred to as your GI tract's microbiota.



Several biotechnology companies are investigating how those bacteria can influence our overall health, how where we live can determine what types of bacteria make up our microbiota, and how what we eat (or don't eat) can influence the composition of our microbiome. When bad bacteria or viruses grow in number in your GI tract and you get sick, you can think of the gut microbiota as being out of balance. The purpose of adding these genetically engineered *E. coli* is to allow the immune system to more quickly respond to the infection and rid the body of the harmful bacteria. This would then allow the normal balance of microbiota in the gut to reestablish itself.

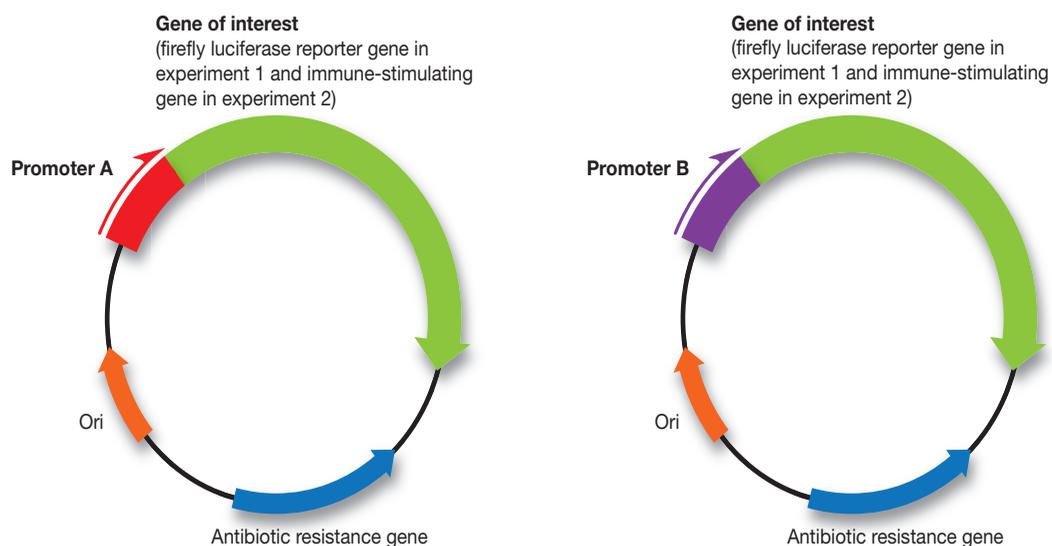
**Dr. Cruz:** Thanks so much. That helps clear things up for me. Let's take a look at the data Dr. Richardson shared and determine whether we think his work could help to reduce the number of days that patients experience dysentery symptoms. Maybe this research will eventually be useful in reducing the severity as well as the duration of dysentery symptoms in humans. I see his email attachment starts with a note.

## Part 2 – In Vitro Experimental Results.

Dear Dr. Cruz,

Please see the latest data we gathered during trials with mice that have dysentery. We conducted two experiments to determine 1) how the presence or absence of inducer molecules similar to those associated with gastrointestinal infections would affect expression of the reporter gene firefly luciferase and 2) if engineered *E. coli* expressing an immune-stimulating gene could reduce the number of days mice exhibit symptoms of dysentery.

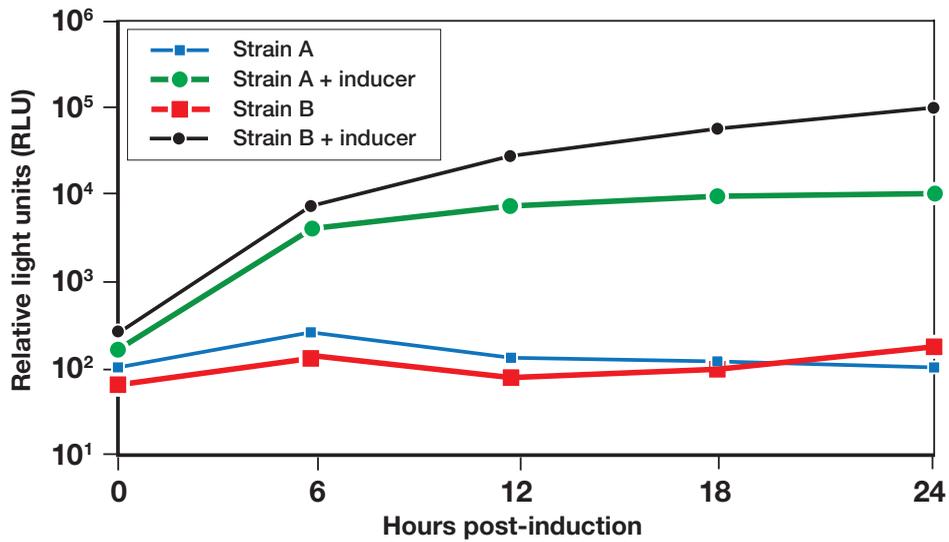
We began by designing promoters that would activate expression of a reporter gene in response to gut inflammatory signals. GI infection produces inflammatory signals that induce these promoters. The reporter gene we used is the luciferase gene, which encodes an enzyme found in fireflies that generates light when it cleaves its natural substrate, luciferin. We cloned multiple versions of the promoter into a plasmid that also had an antibiotic resistance gene, and an origin of replication. We decided to test two different versions of the promoter to see which version would be most effective, A or B (Figure 1).



**Fig 1. Schematic of plasmids used in transformation studies.** For experiment 1, the gene of interest is firefly luciferase gene. For experiment 2, the gene of interest is an immune-stimulating gene.

### Experiment 1:

The purpose of this experiment was to test whether the newly designed promoters are induced by inflammatory signaling molecules and to compare the level of induced gene expression. In order to do this we transformed gut-derived *E. coli* from mice with the plasmid containing Promoter A and the firefly luciferase reporter gene (Strain A) or the plasmid containing Promoter B and the firefly luciferase reporter gene (Strain B). We measured the amount of luciferase expressed downstream of the promoter by performing an assay to measure the amount of light produced (measured as Relative Light Units) for each strain in the presence or absence of the inducer. You can see our results below (Figure 2).



**Fig 2. Amount of gene expression in the presence or absence of inducer as measured in Relative Light Units (RLU) over time.**

Experiment 1 Questions

1. How are the plasmids Dr. Richardson's team used in their experiments similar or different from those you used in your bacterial transformation investigation(s)?
2. In your own words, describe the purpose of Dr. Richardson's experiment.
3. What is the advantage of doing experiment 1 before doing an experiment in mice?
4. Which strain (A or B) in the presence or absence of an inducer generated the greatest amount of firefly luciferase as measured in relative light units?





9. Consider what the next steps in this work may be in order to better understand how the engineered bacteria work in the mouse gut to further reduce dysentery symptoms. Provide your ideas for the following:

a. Formulate a follow-up extension question that builds on the research findings presented in this case:

b. Why is your question useful in thinking about the process taking place in the mouse gut?

c. What is your hypothesis? Be sure to provide a reason for your thinking.

d. What data would need to be collected in order to test your hypothesis and answer your question?

e. What protocol steps would you need to take in order to test your hypothesis?

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