STO-169

Engineering a Living Medicine

Teacher Information

science take-out
just add students™

Summary

Follow the case of a woman who has phenylketonuria (PKU), an inherited and treatable disorder. She cannot make an enzyme needed to process phenylalanine, an amino acid that is present in almost all foods that contain protein.

- Test the level of phenylalanine in the woman's blood and describe how high phenylalanine levels affect her health.
- Model the production of a "living medicine"—bacteria that could live in the digestive tract and produce enzymes that keep phenylalanine levels low.
- Analyze brief descriptions of an experiment and a clinical trial to test the safety and effectiveness of the bacteria.

Core Concepts

- Models can be designed to illustrate the relationships between components of a system.
- Some genetic diseases cause an inability to produce essential enzymes.
- Genetic engineering can be used to create bacteria that produce enzymes that are essential for human health.
- Clinical trials are scientific studies conducted to test the safety and effectiveness of medicines for humans.

Time Required

Two to three 40-minute class periods

Kit Contains

- **Kaylee's Notes**
- ***E. coli* Diagram**
- Kaylee's blood (simulated)
- Phenylalanine Test Paper (simulated)
- Model pieces:
  - Large clear ring (chromosome)
  - Tubes (genes)
  - Beads (enzymes)
  - White rings (transport proteins)
  - "Phenylalanine" paper strips

Teacher Provides

- Safety goggles
- Paper towels for clean-up

Warning: Choking Hazard

This Science Take-Out kit contains small parts. Do not allow children under the age of seven to have access to any kit components.

Teacher Resources

This activity is loosely based on a real "living medicine" that is under development by synthetic biologists at a company called Synlogic. The following links offer more information on how their work may lead to a treatment for PKU and other metabolic disorders.

- **New Treatments for Phenylketonuria Aim to Loosen Reins on Strict Diet**
  https://www.the-scientist.com/news-opinion/new-treatments-for-phenylketonuria-aim-to-loosen-reins-on-strict-diet-36699
- **Synthetic Bacteria Help Treat PKU in Mice**
  https://www.the-scientist.com/news-opinion/synthetic-bacteria-help-treat-phenylketonuria-in-mice-64656
- **Scientists are Retooling Bacteria to Cure Disease**
  https://www.nytimes.com/2018/09/04/health/synthetic-biology-pku.html
- **Reprogramming Gut Bacteria as Probiotics**
  http://news.mit.edu/2016/startup-synlogic-reprogramming-gut-bacteria-living-therapeutics-0405
- **Development of a synthetic live bacterial therapeutic for the human metabolic disease phenylketonuria**
  https://www.nature.com/articles/nbt.4222
- **Safety and Tolerability of SYNB1618 in Healthy Adult Volunteers and Adult Subjects with Phenylketonuria**
  https://clinicaltrials.gov/ct2/show/NCT03516487

Teacher Suggestions

- Students should have multiple experiences with the NGSS science and engineering practice of developing and using models. ("Develop, revise and/or use a model based on evidence to illustrate and/or predict the relationships between systems or between components of a system.") If your students have limited experience with developing models, they may struggle initially. Direct students to the information **Kaylee's Notes** in their kit and encourage them to use that information to create their models.

- Students do not need detailed information on DNA, RNA, and protein synthesis for the activities in this kit. They simply need to understand that genes carry information for how to make proteins/enzymes.

- This activity models synthetic biology research that engineers a synthetic chromosome that can be inserted into *E. coli* bacteria. This activity it is NOT an example of transformation using plasmids that are recombinant DNA from two different organisms. See the Teacher Resources section above for more information about synthetic biology.

- Consider introducing students to PKU by using the Science Take-Out kit entitled **Testing for Genetic Disorders that Cause Brain Damage** (STO-132). This kit follows the case of twins who have newborn screening for genetic disorders.

- For advanced classes, consider giving students a sense of the complexity of the synthetic biology processes involved in creating and testing a living medicine. Have them read one or both of these articles:
    - **Development of a synthetic live bacterial therapeutic for the human metabolic disease phenylketonuria** https://www.nature.com/articles/nbt.4222
    - **Safety and Tolerability of SYNB1618 in Healthy Adult Volunteers and Adult Subjects with Phenylketonuria** https://clinicaltrials.gov/ct2/show/NCT03516487

Reusing the Kit

Teachers will need to instruct students on how to handle clean-up and return of the reusable kit materials. For example, teachers might provide the following information for students:

Discard	Return to kit bag

• Used Phenylalanine Test Paper	• **Kaylee's Notes**
	• ***E. Coli* Diagram**
	• Kaylee's Blood tube and dropper
	• Vial for phenylalanine test paper
	• Model Pieces:
	  ○ Bacteria Cell Diagram
	  ○ Large clear ring
	  ○ Tubes
	  ○ Beads
	  ○ Small white rings

Refills for **Engineering a Living Medicine** kits are available at www.sciencetakeout.com. The **10 Kit Refill Pack** includes the following materials:

- 10 pieces of Phenylalanine Test Paper (simulated)
- 15 mL of Kaylee's Blood (simulated)
- Transfer pipet for refilling microtubes
- 50 yellow "Phenylalanine" paper strips

NGSS Correlation

Working Towards Performance Expectation

HS-LS1-1. Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins, which carry out the essential functions of life through systems of specialized cells.

Science and Engineering Practices

Develop, revise, and/or use a model based on evidence to illustrate and/or predict the relationships between systems or between components of a system.

Plan an investigation individually and collaboratively to produce data to serve as the basis for evidence, and in the design: decide on types, how much, and accuracy of data needed to produce reliable measurements and consider limitations on the precision of the data (e.g., number of trials, cost, risk, time), and refine the design accordingly

Apply concepts of statistics and probability (including determining function fits to data, slope, intercept, and correlation coefficient for linear fits) to scientific and engineering questions and problems, using digital tools when feasible

Disciplinary Core Ideas

LS1.A: Structure and Function

- Systems of specialized cells within organisms help them perform the essential functions of life.
- All cells contain genetic information in the form of DNA molecules. Genes are regions in the DNA that contain the instructions that code for the formation of proteins, which carry out most of the work of cells.
- Multicellular organisms have a hierarchical structural organization, in which any one system is made up of numerous parts and is itself a component of the next level.

Cross Cutting Concepts

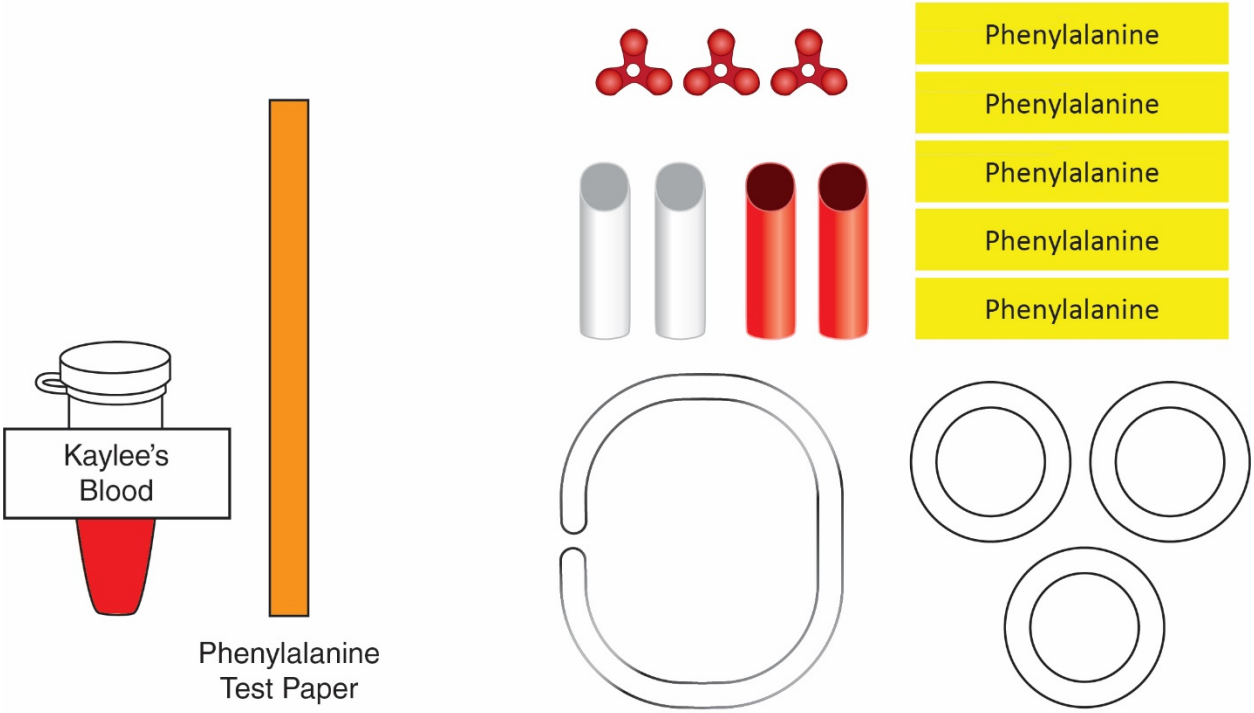
Structure and Function

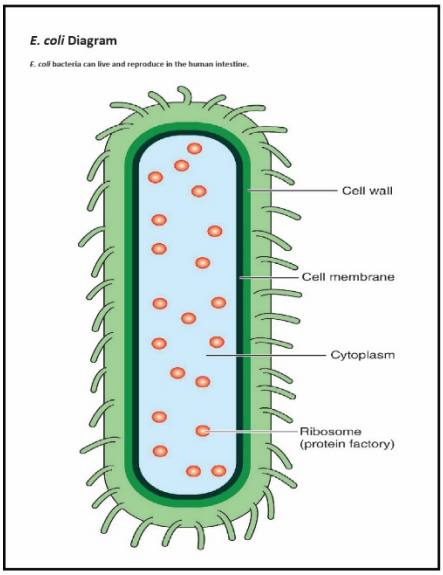
- The functions and properties of natural and designed objects and systems can be inferred from their overall structure, the way their components are shaped and used, and the molecular substructures of its various materials.

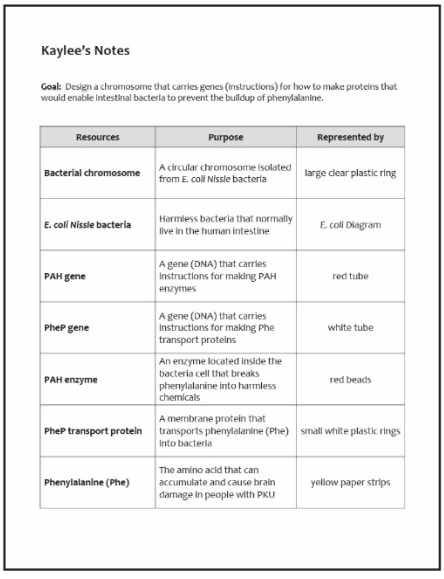
Cause and Effect

- Cause and effect relationships can be suggested and predicted for complex natural and human designed systems by examining what is known about smaller scale mechanisms within the system.

Kit Contents Quick Guide







Kaylee's Notes

Goal: Design a chromosome that carries genes (instructions) for how to make proteins that would enable intestinal bacteria to prevent the buildup of phenylalanine.

Resources	Purpose	Represented by
Bacterial chromosome	A circular chromosome isolated from *E. coli* Nissle bacteria	large clear plastic ring
***E. coli* Nissle bacteria**	Harmless bacteria that normally live in the human intestine	*E. coli* Diagram
PAH gene	A gene (DNA) that carries instructions for making PAH enzymes	red tube
PheP gene	A gene (DNA) that carries instructions for making Phe transport proteins	white tube
PAH enzyme	An enzyme located inside the bacteria cell that breaks phenylalanine into harmless chemicals	red beads
PheP transport protein	A membrane protein that transports phenylalanine (Phe) into bacteria	small white plastic rings
Phenylalanine (Phe)	The amino acid that can accumulate and cause brain damage in people with PKU	yellow paper strips

Read these instructions before using Science Take-Out kits

Parental or Adult Supervision Required

This kit should be used only under the supervision of an adult who is committed to ensuring that the safety precautions below, and in the specific laboratory activity, are followed.

Chemicals Used in Science Take-Out Kits

Every effort has been made to reduce the use of hazardous chemicals in Science Take-Out kits. Most kits contain common household chemicals or chemicals that pose little or no risk. Safety Data Sheets (SDS) provide specific safety information regarding the chemical contents of the kits. SDS information for each kit is provided in the accompanying teacher instructions. We encourage students to adopt safe lab practices when performing laboratory activities involving chemicals.

Warning: Choking and Chemical Hazard

Science Take-Out kits contain small parts that could pose a choking hazard and chemicals that could be hazardous if ingested. Do not allow children under the age of seven to have access to any kit components.

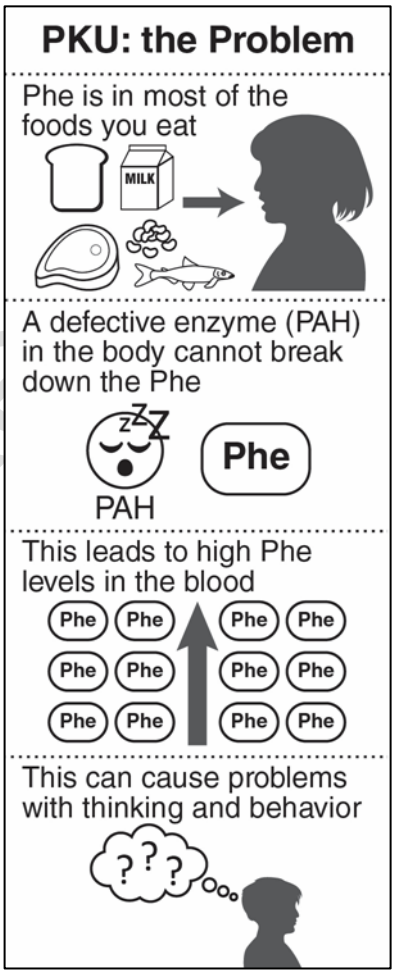
No blood or body fluids from humans or animals are used in Science Take-Out kits. Chemical mixtures are substituted as simulations of these substances.

General Safety Precautions

1. Never taste, smell, or ingest any chemicals provided in the kit - they may be hazardous.
2. Chemicals used in Science Take-Out experiments may stain or damage skin, clothing or work surfaces. If spills occur, wash the area immediately and thoroughly.
3. Report any chemical spills or contact with chemicals to your teacher.
4. Work in a clean, uncluttered area. Cover the work area to protect the work surface.
5. Read and follow all instructions carefully.
6. Pay particular attention to following the specific safety precautions provided by your teacher or included in the kit activity instructions.
7. Do not use the contents of this kit for any other purpose beyond those described in the kit instructions.
8. Do not leave experiment parts or kits where they could be used inappropriately by others.
9. Do not eat, drink, or apply make-up or contact lenses while performing experiments.
10. Wash your hands before and after performing experiments.

Engineering a Living Medicine

Part 1: A Blood Test for Kaylee



Kaylee is a 32-year-old woman who was diagnosed at birth with phenylketonuria (PKU), a rare inherited disorder. PKU is caused by a defect in the gene that carries instructions for how to make an enzyme called phenylalanine hydroxylase (PAH). The PAH enzyme is needed for normal breakdown of excess phenylalanine (Phe), an amino acid found in almost all proteins.

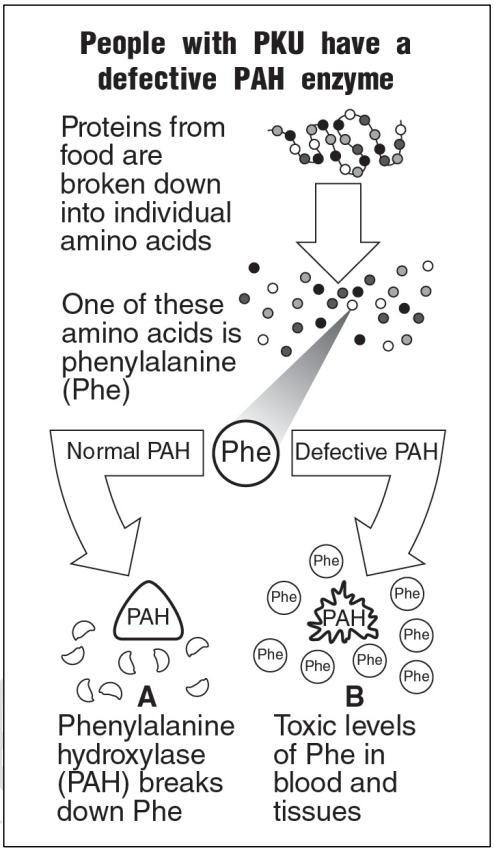
Because Kaylee cannot make the PAH enzyme, a dangerous buildup of phenylalanine (Phe) can develop in her blood when she eats foods that contain protein. High phenylalanine levels in the blood can poison brain cells, leading to severe intellectual disability in children or problems with memory and attention in adults.

1. Write the three letter abbreviation (**PKU, PAH, or Phe**) most closely associated with each of the following words or phrases. *Hint: Refer to the text above and the diagram on the right.*

   - _____ Phenylalanine, an amino acid
   - _____ Phenylketonuria, a genetic disorder
   - _____ Phenylalanine hydroxylase, an enzyme
   - _____ An inherited inability to produce an enzyme
   - _____ An enzyme that breaks down phenylalanine
   - _____ An amino acid found in proteins
   - _____ A chemical that can accumulate and poison brain cells

2. The diagram on the right shows phenylalanine (Phe) metabolism in a person with PKU and a person who does not have PKU. Which side of the lower part of the diagram (**A** or **B**):

   - Shows a person with PKU? _____
   - Should be labeled "People with normal PAH enzyme can break down phenylalanine"? _____
   - Shows what happens if a person with PKU eats a diet high in protein? _____



3. Kaylee needs a monthly blood test. Explain the purpose of this blood test.

4. Test Kaylee's blood using the contents of the **Phenylalanine Blood Test Kit:**

   a) The tube labeled **Kaylee's Blood** contains a sample of Kaylee's blood that has been mixed with a special reaction mixture to test for phenylalanine.

   b) Dip one end of the phenylalanine test paper into the tube of Kaylee's Blood then remove the test paper from the tube.

   c) If the phenylalanine test paper changes to a green or blue color it indicates that the blood phenylalanine level is above 10 mg/dL.

   d) People with PKU should keep their blood phenylalanine levels in the safe range, which is between 1 mg/dL and 10 mg/dL. Complete the following table to record the results of Kaylee's blood test:

Color of Test result	Amount of phenylalanine in blood	Safe or unsafe range?

5. What substance can people who do not have PKU make to prevent the buildup of phenylalanine?

6. What physical symptoms might Kaylee show if she continues to have the phenylalanine levels determined by the blood test?

To keep her blood phenylalanine levels low, Kaylee drinks a specially formulated dietary supplement (a shake) that contains protein (with no phenylalanine), vitamins, minerals and calories. She eats measured amounts of low protein fruits, vegetables, bread, pasta and cereals. Kaylee should not eat high protein foods such as milk and dairy products, meat, fish, chicken, eggs, beans and nuts which contain large amounts of phenylalanine.

7. Why do you think it might be difficult for Kaylee to follow the diet that keeps her phenylalanine levels in the safe range?

Part 2: Kaylee's Unusual Idea

It is difficult for Kaylee to remain on a highly restrictive, low phenylalanine diet. She knows that if she doesn't stay on a low phenylalanine diet it can result in damage to her neurological (brain) function. Kaylee would like to be able to eat all kinds of foods without worrying about the level of phenylalanine in her blood. She wishes scientists could solve the problem that people with PKU face so that they would not need to be on a very low protein diet.

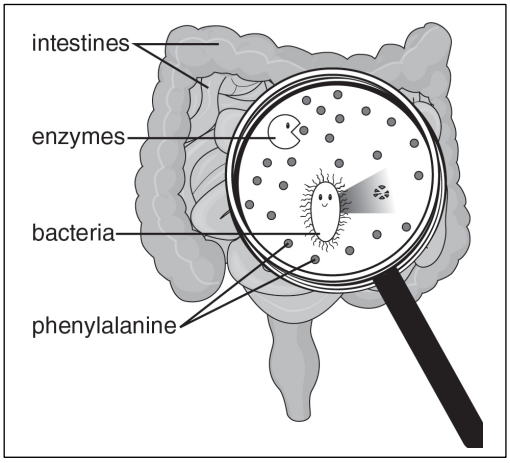
Identify the Problem:

1. What problem does Kaylee wish scientists could solve?

2. How would you know if this problem has been solved?

Kaylee is the head of a new synthetic biology laboratory at a local university. Synthetic biology combines science and engineering to create new biological systems and organisms that don't already exist in the natural world. Her lab specializes in using genetic engineering to synthesize artificial bacterial chromosomes that can be inserted into bacteria cells.

Kaylee has an unusual idea. She knows that the small and large intestines of humans are populated by a huge variety of beneficial bacteria. She wonders whether it might be possible to engineer a "living medicine" of beneficial bacteria for people who have PKU. These bacteria could live in the human intestine and produce PAH enzymes that break down phenylalanine.



3. Why are people with PKU not able to prevent the buildup of phenylalanine?

4. What gene could be added to bacteria to make them useful as a "living medicine" that keeps phenylalanine levels in the blood low?

Kaylee would like you to make a model that shows a design for a genetically engineered chromosome. This engineered chromosome, when inserted into bacteria, should turn the bacteria into a "living medicine" for people with PKU. The "living medicine" bacteria could live in human intestines and produce enzymes that break down phenylalanine.

To help you get started, Kaylee has already done these two things:

- She wrote some ideas (see **Kaylee's Notes** in your kit) about what the engineered chromosome should be able to do and what resources might be useful in your design.
- She put together a bag of **Model Pieces** that you can use to make a model chromosome.

Identify Goals and Resources:

5. What specific goal does Kaylee want you to achieve?

6. What information and resources are available to help you get started with building the model chromosome?

Brainstorm:

7. Brainstorm ideas for how you could change normal bacteria into a living medicine. *Hint: What genes could be added to a normal chromosome to make an engineered chromosome that would turn bacteria into a "living medicine"?*

Build a model of an engineered chromosome:

8. Use **Kaylee's Notes** and the bag of **Model Pieces** to make a model of an engineered chromosome. This chromosome should include genes that would enable bacteria to convert phenylalanine into harmless substances. ***Hint: You should only use three of the model pieces to make the engineered chromosome.***

9. Draw and label your model of an engineered chromosome in the space below.

The engineered chromosome contains gene instructions for how to make the PAH enzymes and PheP transport proteins. However, the engineered chromosome is not a "living medicine" because it has no way to produce these enzymes and proteins. To make a "living medicine," the engineered chromosome must be inserted into a living bacterial cell. The bacterial cell has ribosomes (protein factories) that can follow the gene instructions to produce the PAH enzymes and PheP transport proteins. The bacteria will also be able to reproduce and produce millions of identical bacteria that could be used to treat many people.

10. Could the engineered chromosome alone be used to treat someone with PKU? Explain why or why not.

11. Next, you will model what would happen if your engineered chromosome was inserted into an *E. coli* bacterial cell that can live and reproduce in the human intestine. Use the ***E. coli* Diagram** in your lab kit. Follow the steps in the chart below. For each step, describe what you did to model this step.

Steps	Describe what you did to model this process
A. The engineered chromosome is inserted into an *E. coli* bacterial cell.	
B. The bacterial cell uses the instructions on the genes to produce PAH enzymes in the cytoplasm and PheP transport proteins in the cell membrane.	
C. When phenylalanine is present outside the bacterial cell, it enters through the transport proteins in the cell membrane.	
D. When phenylalanine is present in the bacterial cell, the PAH enzyme breaks it down into harmless substances.	

12. Explain how the addition of the engineered chromosome affected the bacteria cell.

    a) What new enzymes/proteins does it make?

    b) How do these new proteins prevent phenylalanine buildup?

Evaluate:

13. Was the engineered chromosome that you designed effective in turning *E. coli* bacteria into a "living medicine" that could be used to fix the problem that people with PKU have when phenylalanine is present? Support your answer with information from steps A through D in the chart on the previous page.

14. Assume that scientists used your design for an engineered chromosome to create millions of *E. coli* bacteria that could be used as a "living medicine." What would the next steps be to find out if this "living medicine" is a reasonable cure for PKU?

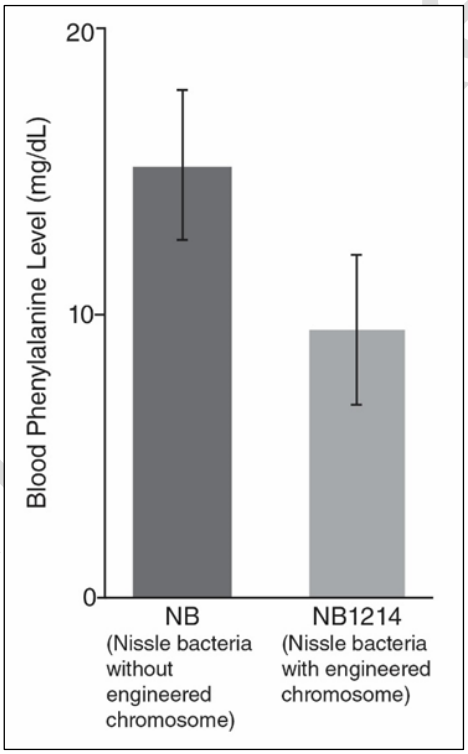
Testing:

Genetic engineers used your design to synthesize a real chromosome that was then inserted into a type of *E. coli* bacteria called *E. coli Nissle* bacteria. The new bacteria with the engineered chromosome were named "NB1214." The scientists then did an experiment to test the ability of the NB1214 bacteria to break down phenylalanine in mice that have PKU.

- 25 mice with PKU were the control group. They were treated with a placebo – **NB** (*E. coli Nissle* bacteria without the engineered chromosome). Blood samples from these mice were then tested for phenylalanine.

- 25 mice with PKU were the experimental group. There were treated with **NB1214** (*E. coli Nissle* bacteria with the engineered chromosome). Blood samples from these mice were then tested for phenylalanine.

The graph below shows the results of the testing.



15. Medibac, a pharmaceutical company, has purchased the rights to produce and sell NB1214. Medibac claims that NB1214 could be used as a "living medicine" for treating adult human PKU patients. Do the results of the testing in mice support this claim? Explain why or why not.

Part 3: Clinical Trials for NB1214

Before NB1214 can be sold as a "living medicine" for PKU patients, Medibac must conduct clinical trials (scientific experiments) to determine whether NB1214 is safe and effective for reducing phenylalanine levels in humans.

Medibac is recruiting people to participate in the clinical trial described below.

Brief description of the NB1214 clinical trial

NB1214 is a possible treatment of PKU. This clinical trial will evaluate the safety and effectiveness of NB1214 on 20 volunteers diagnosed with PKU. The research study will be **randomized, placebo-controlled, and double-blinded**.

All research subjects will undergo initial laboratory tests after they eat a single meal containing a measured amount of protein.

Research subjects will then drink liquid containing NB1214 bacteria or they will drink a placebo liquid (a similar liquid that does not contain NB1214 bacteria).

The research subjects will then undergo final laboratory tests after they eat another single meal containing a measured amount of protein.

A **clinical trial** involves human participants (also called research subjects) and seeks to evaluate the effects of a medicine on human health.

Placebo-controlled refers to a control group receiving a placebo. A **placebo** is an inactive substance given to a research subject in place of medicine. A **control** group is given a placebo while an experimental group is given the medicine being studied. That way, researchers can compare the medicine's effectiveness against the placebo's effectiveness.

Double-blinded means that neither the research subjects nor the researchers know who is getting a placebo and who is getting the medicine.

Randomized means that research subjects are randomly assigned to either the control group or the experimental group.

1. What data should be collected for the initial and the final laboratory tests?

2. Why is it important to give a placebo to some of the research subjects?

3. If you were to participate in this clinical trial, should you get to choose whether you received NB1214 or a placebo? Explain why or why not.

4. If you were to participate in this clinical trial, should you and the doctors conducting the follow-up know whether you got NB1214 or a placebo? Explain why or why not.

5. If NB1214 is effective, how would the final laboratory tests results be different from the initial laboratory tests? Circle the correct choice (a, b, or c) for the Control Group and for the Experimental Group.

	Effect of a Protein Meal on Phenylalanine Levels of PKU Patients
Control Group Research subjects who received the placebo	a. Phenylalanine levels are **higher** in the final laboratory tests than in the initial laboratory tests. b. Phenylalanine levels are **the same** in the final laboratory tests and the initial laboratory tests. c. Phenylalanine levels are **lower** in the final laboratory tests than in the initial laboratory tests.
Experimental Group Research subjects who received NB1214	a. Phenylalanine levels are **higher** in the final laboratory tests than in the initial laboratory tests. b. Phenylalanine levels are **the same** in the final laboratory tests and the initial laboratory tests. c. Phenylalanine levels are **lower** in the final laboratory tests than in the initial laboratory tests.

6. If you had PKU, would you be willing to be a research subject in this clinical trial? Explain why or why not.

Section 1 Chemical Product and Company Information

Science Take-Out
80 Office Park Way
Pittsford, NY 14534
(585)764-5400

CHEMTREC 24 Hour Emergency
Phone Number (800) 424-9300
For laboratory use only. Not for drug, food or household use

Product	Buffer Solution pH10
Synonyms	"Kaylee's Blood" (simulated)

Section 2 Hazards Identification

This substance or mixture has not been classified at this time according to the Globally Harmonized System (GHS) of Classification and Labeling of Chemicals.

Signal word: WARNING
Pictograms: None required
Target organs: None known

GHS Classification:
Skin Irritation (Category 3)
Eye irritation (Category 2B)

GHS Label information: **Hazard statement(s):**
H316: Causes minor skin irritation.
H320: Causes eye irritation.

Precautionary statement(s):

P264: Wash hands thoroughly after handling.

P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P332+P313: If skin irritation occurs: Get medical attention.

P337+P313: If eye irritation persists: Get medical attention.

Ca Prop 65 - This product does not contain any chemicals known to the State of California to cause cancer, birth defects, or any other reproductive harm.

Section 3 Composition / Information on Ingredients

Chemical Name	CAS #	%	EINECS
Water	7732-18-5	98.98%	231-791-2
Potassium chloride	7447-40-7	0.40%	231-211-8
Boric acid	10043-35-3	0.33%	233-139-2
Sodium hydroxide	1310-73-2	0.19%	215-185-5
Red Food coloring		0.1%	

Section 4 First Aid Measures

INGESTION: Call physician or Poison Control Center immediately. Induce vomiting only if advised by appropriate medical personnel. Never give anything by mouth to an unconscious person.

INHALATION: Remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention.

EYE CONTACT: Check for and remove contact lenses. Flush thoroughly with water for at least 15 minutes, lifting upper and lower eyelids occasionally. Get immediate medical attention.

SKIN ABSORPTION: Remove contaminated clothing. Flush thoroughly with mild soap and water. If irritation occurs, get medical attention.

Section 5 Fire Fighting Measures

Suitable Extinguishing Media: Use any media suitable for extinguishing supporting fire.

Protective Actions for Fire-fighters: In fire conditions, wear a NIOSH/MSHA-approved self-contained breathing apparatus and full protective gear. Use water spray to keep fire-exposed containers cool.

Specific Hazards: During a fire, irritating and highly toxic gases may be generated by thermal decomposition or combustion.

Section 6 Accidental Release Measures

Personal Precautions: Evacuate personnel to safe area. Use proper personal protective equipment as indicated in Section 8. Provide adequate ventilation.

Environmental Precautions: Avoid runoff into storm sewers and ditches which lead to waterways.

Containment and Cleanup: Absorb with inert dry material, sweep or vacuum up and place in a suitable container for proper disposal. Wash spill area with soap and water.

Section 7 Handling and Storage

Precautions for Safe Handling: Read label on container before using. Do not wear contact lenses when working with chemicals. Keep out of reach of children. Avoid contact with eyes, skin and clothing. Do not inhale vapors, spray or mist. Use with adequate ventilation. Avoid ingestion. Wash thoroughly after handling. Remove and wash clothing before reuse.

Conditions for Safe Storage: Store in a cool, well-ventilated area away from incompatible substances.

Section 8 Exposure controls / Personal Protection

Exposure Limits:

Chemical Name	ACGIH (TLV)	OSHA (PEL)	NIOSH (REL)
Potassium chloride	None established	None established	None established

Engineering controls: Facilities storing or utilizing this material should be equipped with an eyewash facility and a safety shower and fire extinguishing material. Personnel should wear safety glasses, goggles, or faceshield, lab coat or apron, appropriate protective gloves. Use adequate ventilation to keep airborne concentrations low.

Respiratory protection: None should be needed in normal laboratory handling at room temperatures. If misty conditions prevail, work in fume hood or wear a NIOSH/MSHA approved respirator.

Section 9 Physical and Chemical Properties

Appearance: Clear, red liquid.
Odor: No odor.
Odor threshold: Data not available.
pH: 9.0
Melting/Freezing point: Approx. 0°C (32°F) (water)
Boiling point: Approx. 100°C (212°F) (water)
Flash point: Data not available

Evaporation rate (Water = 1): <1
Flammability (solid/gas): Data not available.
Explosion limits: Lower/Upper: Data not available
Vapor pressure (mm Hg): 14 (water)
Vapor density (Air = 1): 0.7 (water)
Relative density (Specific gravity): Approx. 1.0 (water)
Solubility(ies): Complete in water.

Partition coefficient: Data not available
Auto-ignition temp.: Data not available
Decomposition temp.: Data not available
Viscosity: Data not available.
Molecular formula: Mixture
Molecular weight: Mixture

Section 10 Stability and Reactivity

Chemical stability: Stable
Hazardous polymerization: Will not occur.

Conditions to avoid: Excessive temperatures which cause evaporation.
Incompatibilities with other materials: Acids, alkalies, and air will change the buffer's ability.
Hazardous decomposition products: Boron oxide and chlorine gas.

Section 11 Toxicological Information

Acute toxicity: Data not available
Skin corrosion/irritation: Data not available
Serious eye damage/irritation: Data not available
Respiratory or skin sensitization: Data not available
Germ cell mutagenicity: Data not available
Carcinogenity: Data not available

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.
IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.
OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

Reproductive toxicity: Data not available
STOT-single exposure: Data not available
Aspiration hazard: Data not available
STOT-repeated exposure: Data not available

Potential health effects:
Inhalation: May be harmful if inhaled.
Ingestion: May be harmful if swallowed.
Skin: May cause mild irritation.
Eyes: May cause mild irritation.

Signs and symptoms of exposure: To the best of our knowledge the chemical, physical and toxicological properties have not been thoroughly investigated. Specific data is not available. Exercise appropriate procedures to minimize potential hazards.
Additional information: RTECS #: Data not available

Section 12 Ecological Information

Toxicity to fish: No data available
Toxicity to daphnia and other aquatic invertebrates: No data available
Toxicity to algae: No data available
Persistence and degradability: No data available
Bioaccumulative potential: No data available
Mobility in soil: No data available
PBT and vPvB assessment: No data available
Other adverse effects: An environmental hazard cannot be excluded in the event of unprofessional handling or disposal.

Section 13 Disposal Considerations

These disposal guidelines are intended for the disposal of catalog-size quantities only. Federal regulations may apply to empty container. State and/or local regulations may be different. Dispose of in accordance with all local, state and federal regulations or contract with a licensed chemical disposal agency.

Section 14 Transport Information

UN/NA number: Not applicable
Shipping name: Not Regulated
Hazard class: Not applicable
Packing group: Not applicable
Reportable Quantity: No
Marine pollutant: No
Exceptions: Not applicable
2012 ERG Guide # Not applicable

Section 15 Regulatory Information

A chemical is considered to be listed if the CAS number for the anhydrous form is on the Inventory list.

Component	TSCA	CERLCA (RQ)	RCRA code	DSL	NDSL	WHMIS Classification
Potassium Chloride	Listed	Not Listed	Not Listed	Listed	Not Listed	Uncontrolled Product
Sodium hydroxide	Listed	1,000 lbs (454 kg)	D002	Listed	Not Listed	E

Section 16 Additional Information

The information contained herein is furnished without warranty of any kind. Employers should use this information only as a supplement to other information gathered by them and must make independent determinations of suitability and completeness of information from all sources to assure proper use of these materials and the safety and health of employees.
NTP: National Toxicology Program, IARC: International Agency for Research on Cancer, OSHA: Occupational Safety and Health Administration, STOT: Specific Target Organ Toxicity, SE: Single Exposure, RE: Repeated Exposure, ERG: Emergency Response Guidebook.

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