DESIGNER BACTERIA

TEAM GUIDE

A Design-Based Immersion Learning Unit

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THIS TEAM GUIDE BELONGS TO . . .

DESIGNER BACTERIA TEAM NAME:

THE TEAM MEMBERS (ID NUMBERS ONLY):

1.

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DESIGNER BACTERIA TEAM GUIDE

INTRODUCTION

WHAT EXACTLY IS ENGINEERING?

Engineering is the application of scientific and technical knowledge to solve human problems. In their work, engineers use imagination, judgment, and reasoning to apply science, technology, mathematics, and practical experience. The result is the design, production, and operation of useful objects or processes to solve a problem or satisfy a need.

The jobs of engineers and scientists are often confused. Scientists explore nature in order to discover general principles; engineers apply established principles drawn from mathematics and science in order to develop economical solutions to technical problems. So,

SCIENTISTS DEVELOP

TO LEARN

AND

ENGINEERS LEARN

TO DEVELOP.

The work of engineers is the link between social needs and commercial applications. Engineers consider many factors when they develop a new product. For example, to develop an industrial robot, engineers must specify precisely the functional requirements, design and test the robot's components, integrate the components to produce the final design, and evaluate the design's overall effectiveness, cost, reliability, and safety. This process applies to the development of many different products, such as chemicals, computers, engines, aircrafts, and toys, just to name a few.

ENGINEERING: The application of scientific and technical knowledge to solve human problems.

Engineers design products to make our lives easier or better through a series of steps:

- Determine exactly what the product should do.
- Decide what features are required to make the product work.
- Test the parts, or subsystems, of the product.
- Once all of the different subsystems have been tested, integrate the results to optimize the design.
- Evaluate how well the product solves the problem.
- Start the process over again to make the product better!

INTRODUCTION

WORKING IN TEAMS

Teamwork is a useful way to share ideas and to work towards solutions for problems. In this unit, you will be part of a team of genetic engineers in charge of creating 'Designer Bacteria' that will be fluorescent green or pigmented blue. Below are a few suggestions for making the most of this teamwork experience.

LISTEN AND RESPECT

The two basic rules for successful teamwork are to listen to each other's ideas and to respect each other's ideas.

RECORD IDEAS

It is important that none of your ideas is lost. One helpful way to keep these ideas is to write them down in ways that engineers and scientists record ideas.

REFLECT AND ASSESS

Learning always includes reflection and assessment. You will think about what you have done (reflect) and evaluate how successful it was (assess) using this team guide.

TEAM ROLES

Each member of your team will have a different role. These roles will change every few days so that everyone has a chance to take on every role. If members of your team are absent, then those members who are present will have to take over additional responsibilities.

LEAD BIOLOGIST

- Supervises team work
- In charge of lab procedures
- Wears gloves
- Conducts experiments
- Assisted by general biologist as needed
- Keeps other members on task

ANALYTICAL BIOLOGIST

- Collect the *Team Guide*
- Records lab procedures
- Completes the team guide based on team work

GENERAL BIOLOGIST

- Obtains all necessary supplies for lab procedures
- Wears gloves
- Assists lead biologist with lab procedures as needed

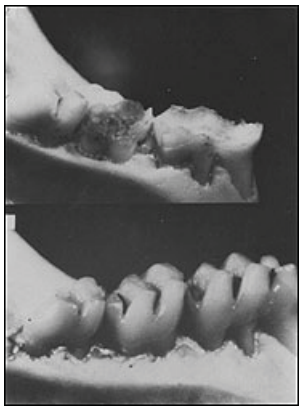
QUALITY ASSURANCE BIOLOGIST

- Makes sure the lab space is clean both before and after lab procedures.
- Rates team members' performance for that lab's procedures using a scale of 1-5.
  - 0 - absent
  - 1 - poor
  - 2 - fair
  - 3 - good
  - 4 - excellent
  - 5 - superior
- A table to record these ratings for each activity is located at the end of this guide.

TEAM GUIDE

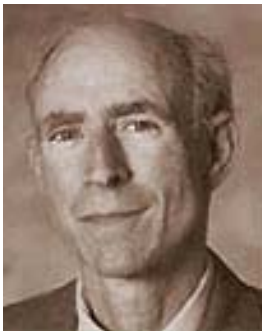
The purpose of this team guide is for you and your team to create a record of the progress that you make while you design and develop your prototype.

BACTERIA CAN SAVE YOUR TEETH!



The top picture shows rat teeth with severe decay. The bottom picture shows rat teeth treated with genetically engineered bacteria. © Oragenics

Tooth decay affects over 5 billion people today! One scientist, Dr. Jeffrey Hillman from the University of Florida founded a biotech company, Oragenics, and made bacteria, through genetic modification, that can actually stop cavities from forming!



Jeffrey Hillman, D.M.D, Ph.D, © Oragenics

Dr. Hillman dedicated himself to find a solution to the very common problem of tooth decay. He studied dentistry at Harvard and received his D.M.D. Afterwards, he went on to earn a Ph.D. in microbiology. Dr. Hillman felt that one way to combat tooth decay was to understand the genes of the bacteria that were responsible for it. By understanding the bacteria that cause tooth decay, he would find a way to modify those bacteria so they could be introduced into your mouth to prevent tooth decay.

GENE: Unit of heredity; a section of DNA that codes for RNA and / or protein that causes an organism to look a certain way.

OKAY, SO THIS GUY MADE BACTERIA THAT I AM GOING TO PUT IN MY MOUTH. GROSS!

Dr. Hillman broke down his prototype of genetically modifying bacteria into pieces. The first piece he needed to explore was the virulence of the bacteria. Virulence is the ability of bacteria to cause infection. Dr. Hillman wanted to find a bacteria that could not hurt you. The second piece that needed to be explored was the bacteria's ability to grow or colonize. In the human mouth, there are many different types of bacteria. Dr. Hillman was searching for one particular type of bacteria that would be able to colonize and wipe out the 'bad' bacteria in your mouth.

COLONIZE: When bacteria become established in a habitat; this habitat could include an agar plate, a host, or a wound.

Dr. Hillman conducted over 1,000 genetic manipulations before he had developed a strain of bacteria that met his two requirements! This process took him over 25 years to complete and he is not finished yet! His product, or therapy in this case, still is being tested to make sure that it is safe.

Could you say that Dr. Hillman's work was engineering? Why or why not.

What was the problem Dr. Hillman was trying to solve? How did Dr. Hillman go about solving the problem?

If you could engineer a bacteria to do anything, what problem would you solve? How would you go about solving that problem with your engineered bacteria?

GENETICALLY MODIFIED PRODUCTS

WHAT IS GENETIC ENGINEERING?

Genetic engineering is the process of manipulating genes outside of an organism's normal reproductive process. Genetic engineers make existing animals, bacteria, and plants do things better or do things they would not normally do to solve a problem. The aim is to introduce new characteristics or attributes physiologically or physically, such as making a crop resistant to a herbicide, introducing a novel trait, or producing a new protein or enzyme. Examples can include the production of new types of experimental mice such as the OncoMouse (cancer mouse) for research, the genetic redesign of human insulin through the use of modified bacteria, or the blending of two fruits to make a novel fruit such as the mango nectarine.

GENETIC ENGINEERING: Scientific alteration of the structure of genetic material in a living organism. It involves the production and use of recombinant DNA and has been employed to create bacteria that synthesize insulin and other human proteins.

WHAT CAN GM DO?

Genetic modifications (GM) happen in many living systems, ranging from bacteria to plants. GM has the potential to solve many problems in our world today. For instance, GM potatoes in the United Kingdom are resistant to the late potato blight fungus. This fungus wiped out almost the entire crop of potatoes in Ireland between 1845 and 1851. This famine is estimated to have caused over one million deaths. Although farming techniques have improved significantly since this terrible famine, still there is a potential for this fungus to strike again. This deadly plant disease now is preventable; thousand of lives and billions of dollars may be saved because of GM.

The Sketch of a Woman and Children represents Bridget O'Donnel. Her story is briefly this:--

'. . .we were put out last November; we owed some rent. I was at this time lying in fever. . . they commenced knocking down the house, and had half of it knocked down when two neighbours, women, Nell Spellesley and Kate How, carried me out. . . I was carried into a cabin, and lay there for eight days, when I had the creature (the child) born dead. I lay for three weeks after that. The whole of my family got the fever, and one boy thirteen years old died with want and with hunger while we were lying sick.'

Credit: Illustrated London News, December 22, 1849

Irish Potato Blight References:

The American Heritage® Dictionary of the English Language, Fourth Edition. Retrieved August 28, 2007, from Dictionary.com website: http://dictionary.reference.com/search?q=DNA&x=0&y=0

http:\\en.wickpedia.org/wiki/Irish_Potato_Famine_%281845-1849%29

GENETICALLY MODIFIED PRODUCTS

Donut Peaches

GM, GM EVERYWHERE!!

Genetically modified products are not necessarily a bad thing and much more common in our everyday lives than we think! In fact, there are GM foods in your refrigerator at home right now! Many fruits and vegetables, such as corn, tomatoes, and wheat, have been genetically modified to be healthier for you as well as being resistant to various plant diseases and insect pestilences. By engineering these various foods to be insect and disease resistant, farmers are saving valuable time and money. With GM, farmers are able to create more profit for themselves and spare the environment from harmful, lasting pesticides like DDT. DDT was responsible for endangering our national bird, the American bald eagle.

Some novelty fruits are available at your local grocery store. Fruits such as mango nectarines, donut peaches, and dinosaur egg pluots are all GM fruits. Various genes from each parent fruit were modified in some manner and combined, resulting in a completely new type of fruit that has qualities of each parent fruit. For example, the honeydew nectarine has the fuzzy skin of a nectarine and the light green meaty flesh of a honeydew melon!

Honeydew Nectarines

Mango Nectarines

NEEDS AND NEW IDEAS

NEED: A problem that requires a solution.

Thinking about needs is an important part of the design process. Engineers think about needs when they design new products or improve old products. Once you identify some needs that interest you, then you can begin to think about some new ideas that might meet those needs.

Below record your needs and new ideas for bacteria that are fluorescent green or have blue pigment.

NEEDS	PROTOTYPE IDEAS
Our team needs a fluorescent or pigmented bacteria because . . .	Some types of fluorescent or pigmented bacteria that might meet our needs are . . .

SYSTEM DECOMPOSITION

SUBSYSTEMS

Instead of focusing on the entire design, engineers break it down into its subsystems. This strategy helps them tackle their task by focusing on one part of the prototype at a time. Each subsystem is designed to accomplish one function. Dividing a prototype into its subsystems and functions clarifies the goals for the design. The figure below describes the functions of each subsystem in your prototype.

FUNCTIONS: How a prototype, or the subsystems of a prototype, work.

SUBSYSTEM: The parts of a prototype.

FLUORESCENT – PIGMENTATION SYSTEM

ENVIRONMENT SUBSYSTEM

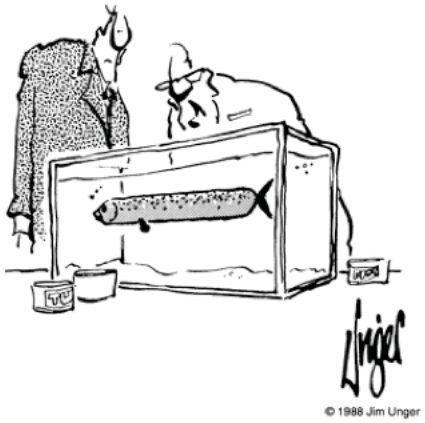
Influences the bacterial growth. Changes in the environment can enhance or hinder the rate of cell growth or death.

GENES SUBSYSTEM

Defines the genetic material available to the bacteria. Changes in the genes determine the characteristics of the cell.

EXPRESSION SUBSYSTEM

Determines the intensity of a trait or characteristic exhibited by the bacteria. Genes expressed by the cell can be turned on or off as the cell interacts with the environment.



"We've genetically engineered a tuna exactly the same diameter as our cans."

Remember, the goal of genetic engineering is to introduce new traits or characteristics by changing the genes in an organism. These new traits or characteristics can be used to solve a problem in that the environment of an organism.

REQUIREMENTS

REQUIREMENTS

Requirements are the features that make up a prototype. There are two types of requirements. Must-have requirements are necessary for your prototype to work. Nice-to-have requirements are not necessary for your prototype to work, but they make it more attractive.

MUST-HAVE REQUIREMENT
A feature that is necessary for a prototype to work.

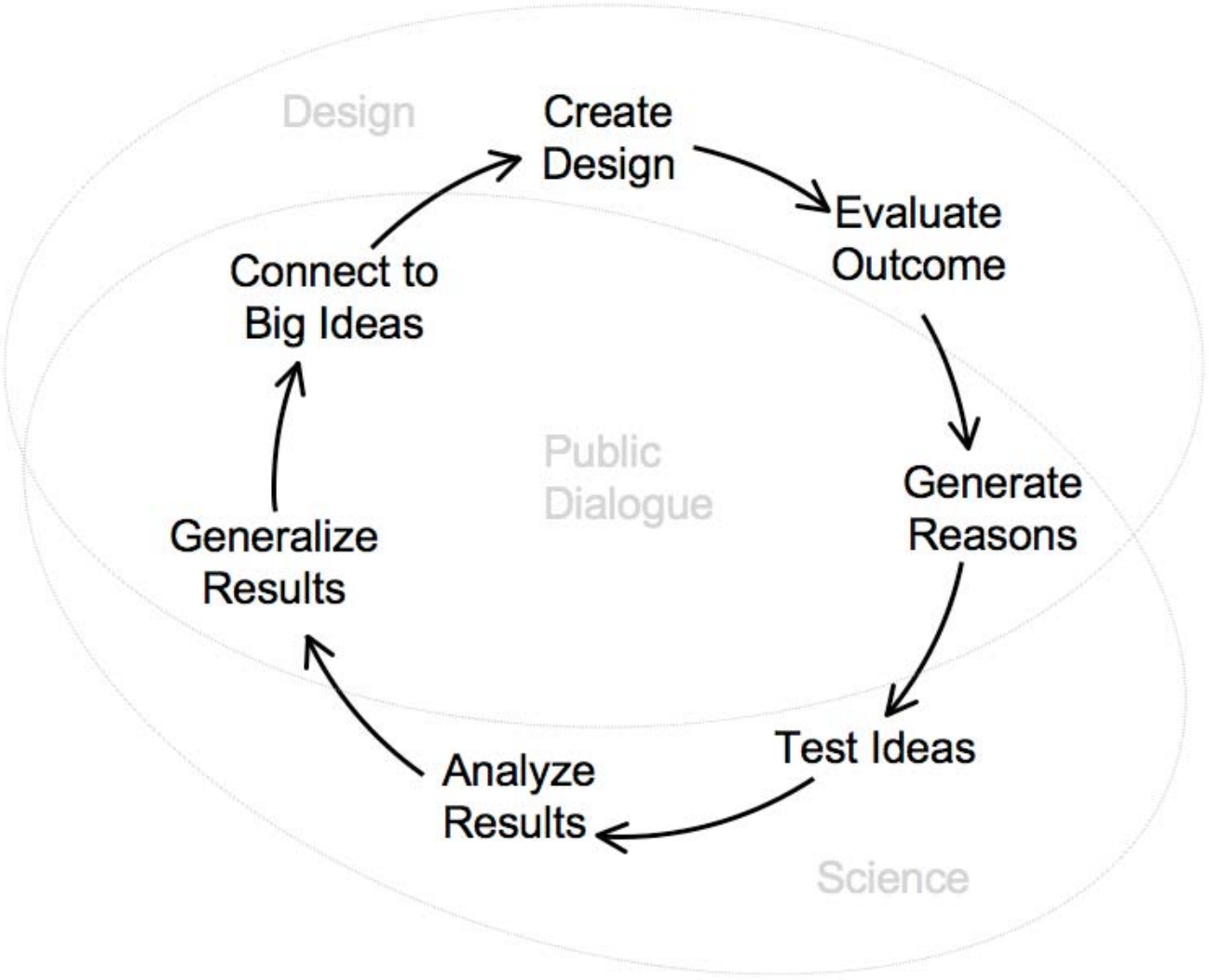
NICE-TO-HAVE REQUIREMENT
A feature that is not necessary for a prototype to work, but makes it more attractive.

DIRECTIONS

As a team, determine the requirements for your prototype. After you have selected the requirements, decide whether each is a must-have requirement or a nice-to-have requirement. Place a check mark in the appropriate column.

REQUIREMENTS	MUST-HAVE	NICE-TO-HAVE

DESIGN-SCIENCE CYCLE



Through the *Environment Subsystem*, you might notice that you completed a variety of steps. These steps make up what is called the *Design-Science Cycle*. Throughout the rest of the unit, you will continue to improve your prototype by working your way through each step in the cycle.

As you can see from the picture above, some of the work that you do is mostly about designing your prototype and other work that you do is mostly about determining the science that will make your prototype work better. In the introduction, you read that engineers learn science to help them design and that scientists work out designs to help them learn more about science. In reality, what counts as science often overlaps with what counts as design.

The space where the two overlap in this unit has been called the *Public Dialogue* space. This is the time when you and your classmates are able to share your ideas to help improve each other's prototypes.

ASEPTIC TECHNIQUE

In a microbiology lab, scientists must be careful and avoid contaminating or spreading bacteria. Scientists use aseptic technique to prevent contamination and the spread of microbes outside of the laboratory.

Aseptic technique, also known as sterile technique, involves sterilizing everything that will touch a culture of bacteria using extreme pressure and heat in a machine called an autoclave. After all of the lab supplies have been sterilized, then they may be used for lab procedures.

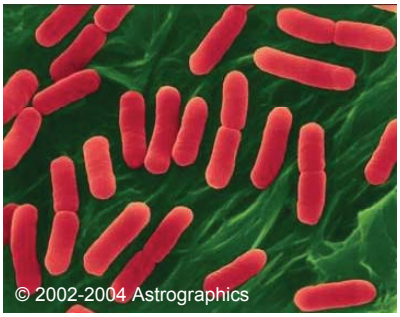
In this unit, you will be using sterilized toothpicks, pipettes, and cotton swabs. ***Sterilized items can be used only once***. It must be discarded in the biohazard waste container set out by your teacher. Do not lay a contaminated toothpick, pipette, agar plate, etc. on your lab bench.

ANYTHING THAT HAS TOUCHED THE BACTERIA MUST BE DEPOSITED IN THE BIOHAZARD WASTE CONTAINER.

If you are using more the one strain of bacteria, special care must be taken not to mix one culture with another. Mixing the bacteria can be avoided by using a different toothpick or pipette for every manipulation.

Aseptic technique also includes keeping the lab bench clean and sterile. Before you begin and after you finish each day, spray your lab area with the 10% bleach solution prepared by your teacher. Wash your hands for one minute with antibacterial hand soap both before and after each class.

Now that you are equipped with the knowledge of aseptic technique, you will be able to develop your prototype with confidence.



STERILIZE EQUIPMENT

WASH HANDS

WEAR GLOVES

DILUTION STREAK METHOD

DILUTION STREAK METHOD

Throughout this unit, you will be inoculating various agar plates. Anything that you inoculate with bacteria is called media. Agar plates are one type of media. To inoculate an agar plate, you will use the dilution streak method as described below.

Do NOT touch the agar with your fingers!

1. Glide the fat end of a toothpick back and forth across the agar surface to make several zigzags across the top of the plate. Avoid gouging the agar. Discard the toothpick in a biohazard waste container. Rotate the plate a quarter turn.

2. With a fresh sterile toothpick, draw the fat end once through the primary streak and continue streaking a zigzag across the agar surface. Discard the toothpick in a biohazard waste container. Rotate the plate a quarter turn.

3. With a fresh sterile toothpick, draw the fat end once through the secondary streak and continue streaking a zigzag across the agar surface. Discard the toothpick in a biohazard waste container. Rotate the plate a quarter turn.

4. With a fresh sterile toothpick, draw the fat end once through the tertiary streak and continue streaking a final zigzag across the agar surface. Discard the toothpick in a biohazard waste container. Replace the lid and incubate the plate upside down.

MEDIA: Liquid or solid nutrient materials used to cultivate bacteria.

AGAR: A gelatin-like substance used to culture bacteria. It is made from marine algae.

CULTURE: To grow bacteria in a medium for scientific study.

INOCULATE: To purposely infect a medium with bacteria in a controlled way.

ENVIRONMENT 1

Date:

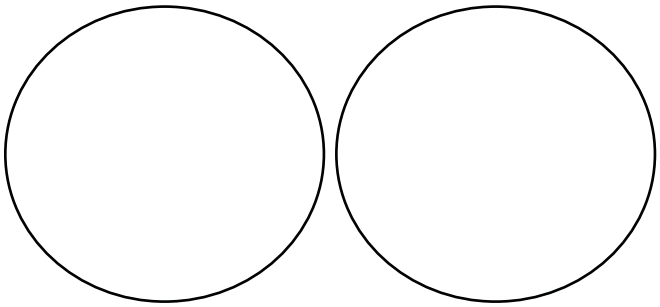
What design requirement(s) are you trying to meet with this experiment?

What variables are you testing to meet this / these requirement(s)?

Materials and Methods:

Data and Sketches

Plate 1 Plate 2



Predictions: What do you expect to ovserve at the conclusion of the experiment?

Summary and Conclusions

Think about why you were (not) able to meet your requirement(s)

ENVIRONMENT 1

Think about your conclusions. List at least three reasons why you were or were not able to meet your requirement(s).

1.

2.

3.

What are some ways that you might test these reasons to improve your prototype.

1.

2.

3.

BUGS I – CELL DNISION

Date:

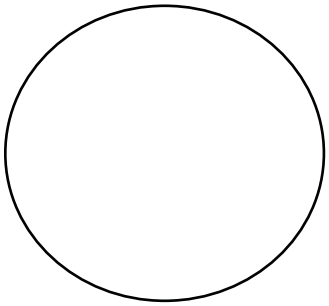
What design requirement(s) are you trying to meet with this experiment?

What variables are you testing to meet this / these requirement(s)?

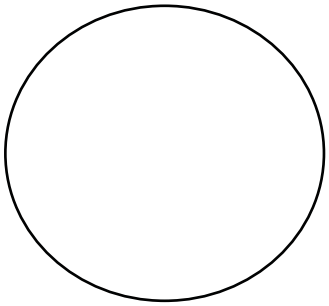
Materials and Methods:

Data and Sketches

Wet Mount



Plate



Predictions: What do you expect to ovserve at the conclusion of the experiment?

Summary and Conclusions

Think about why you were (not) able to meet your requirement(s)

BUGS I – CELL DNISION

Describe the results that were useful for your prototype?
If you are provided with a bacterial strain that needs to be cultured quickly and it does NOT grow well under condition that same conditions as *E. coli*, what steps would you take to determine the optimal growth conditions of the new strain.
Think about the results from all of the BUGs investigations. If you could observe your growing colony under a microscope, what changes would you expect to see over a 24 hour period?

BUGS I – CELL DNISION

AGAR NUTRIENT TABLE

What if your bacteria did not have the right nutrients? Look at the table below to see what agar supports *E. coli* the best.

Agar Type	Contents	Microbes Supported
LB	1% tryptone 0.5% yeast extract 1.0% NaCl 1.5% agar	*E. coli* Other enteric bacteria
LB/amp	1% tryptone 0.5% yeast extract 1.0% NaCl 1.5% agar 20 µg/mL ampicillin	Ampicillin resistant strains Strains harboring plasmids such as pBLU and pGREEN
Potato Dextrose	20% Potato infusion 2% dextrose 1.5% agar	Yeasts Molds Fungi
Nutrient	0.3% beef extract 0.5% peptone 0.8% NaCl 1.5% agar	Water and dairy bacteria that are typically hard to grow
Malt Extract	Malt extract agar plates with 1.3% maltose 0.3% dextrin 0.23% glycerol 0.08% peptone 1.5% agar	Yeasts Molds
LB/X-gal	1% tryptone 0.5% yeast extract 1.0% NaCl 1.5% agar 0.1 mM IPTG 60 µg/mL X-gal	Cloning bacteria Molds

Based on the table above, which agar(s) do you think will be the best to grow your bacteria?

WHAT IS CELL DNISION, ANYWAY?

NEW STUFF . . .

Environment

CELL DNISION: The process where a cell, called the parent cell, divides into two identical cells, called daughter cells.

As we have seen, when bacterial colonies grow, the cells that make up the colonies are dividing to make more cells and this causes the colonies to expand outward.

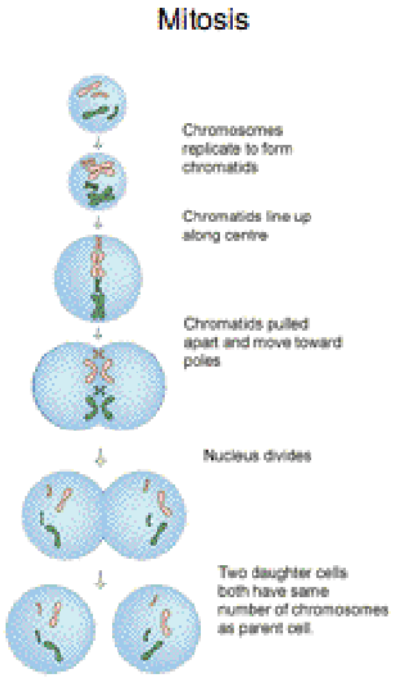
When you think of the word 'colony' perhaps the pilgrims may come to mind. When they settled in America, they formed colonies. There were many people making up each colony. Similarly, many individual bacterium make up a bacterial colony.

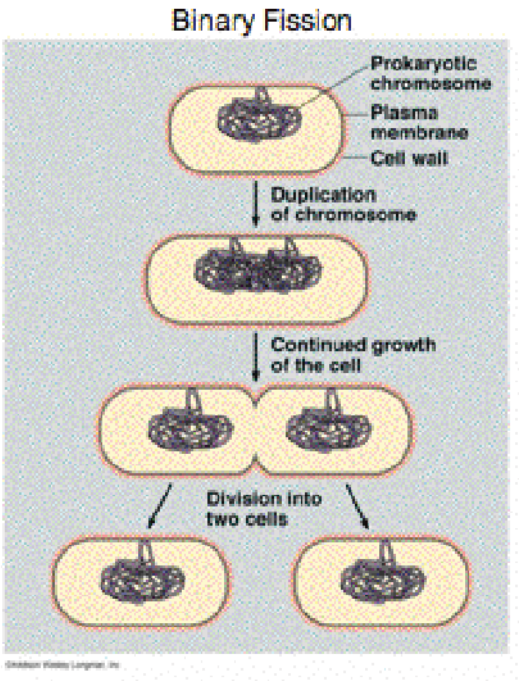
Each colony that we see on the agar, originated from a single cell! This single cell is called a parent cell. It under went a process called **cell division**. The parent cell copied everything inside its membrane so that when it split in half, each half, or daughter cell, had all that it needed to grow and divide again. This process happens over and over again, hundreds of thousands of times to make a bacterial colony.

There are different types of cell division. Bacteria undergo a process called **binary fission**. Eukaryotic cells undergo **mitosis**. Mitosis is slightly more complicated than binary fission because of the structure of eukaryotic DNA and nucleus. However, both types of cell division create genetically identical cells.

There are many things that can affect how fast this process of cell division occurs. You have already seen that temperature and nutrients are major factors that influence the rate of cell division of our bacteria.

What else do you think might speed up or slow down the rate of cell division?





SUMMARY OF SOME BIG IDEAS . . .

- To optimize cell division, bacteria must be grown under the proper conditions.

EXPONENTIAL GROWTH

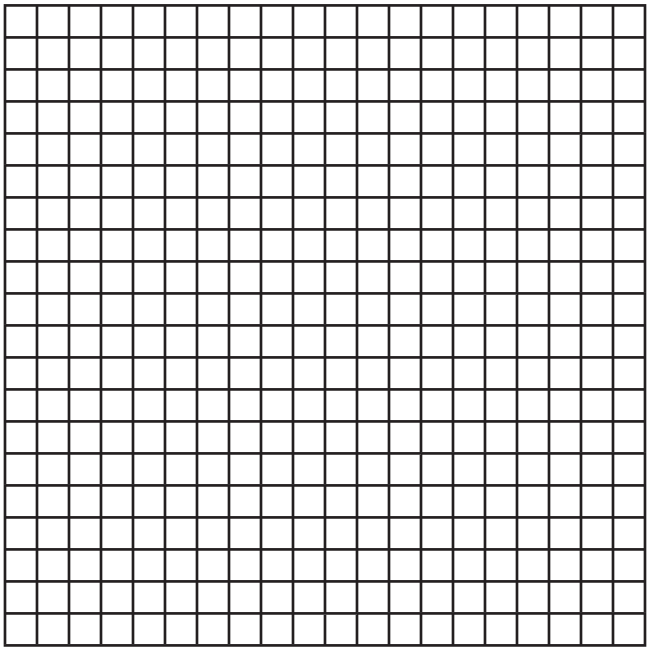
Problem: You have a type of bacteria that divides every 20 minutes. How many bacteria will you have in six hours?

Procedure: Place a bean on your desk. Pretend that twenty minutes has elapsed and double your beans by adding more. Count your beans now. Pretend 20 more minutes have elapsed and double the beans again. Count your beans. Continue this procedure until you have done this for as many 20-minute periods there are in 6 hours. What is your final count?

Remember: You are starting with 1 bacterium.

Time	# of Beans
1 hour	
2 hours	
3 hours	
4 hours	
5 hours	
6 hours	

Plot your results on the graph below.



Bacteria do not divide at this rate indefinately. If they did, bacteria would cover the earth!

Write a short paragraph explaining these questions:

- What did you notice from this exercise about the growth rate of bacteria?
- How do the bacteria divide?
- What factors would limit the growth of bacteria?
- How does this information effect your design?

BUGS PRODUCE DRUGS!

Genetic engineering (GE) has been used to create new medical treatments, vaccines, and drugs for the last 30 years. The first approved GE drug was human insulin. It was approved by the U.S.A.'s Federal Drug Administration (FDA) in 1982 for the treatment of diabetes.

Nearly 20.8 million children and adults in the United States are living with diabetes. Diabetes is a disease that affects the secretion of the hormone insulin into the bloodstream. Insulin regulates the amount of sugar in the bloodstream (blood glucose). Insulin is released to lower the blood glucose level. In a person with diabetes, the special insulin-releasing cells are not doing their job, and/or the insulin that is released is not having an adequate effect.

Early treatments were as crude as diet and exercise. In 1920, a team of scientists learned that certain cells in the pancreas of a dog produced what they called 'isletin.' This secretion appeared to be linked to sugar balance in the body. Isolating even a very small amount of isletin took six weeks! These researchers improved their method by extracting a larger amount from a fetal calf pancreas. Later this isletin was called insulin and was harvested from pig and fish, too. One downfall to this emerging treatment for diabetes was allergic reaction. Because the insulin was collected from an animal pancreas, it was full of impurities that could cause allergic reactions in the the patients using the treatment.

SECRETION: Production and release of a substance by a cell.

In 1982, a GE technique using bacteria emerged that significantly reduced the chance of allergic reactions. This GE medication was called Humulin. One of the key scientists working on the development of Humulin was Lydia Villa-Komaroff.

PRESTIGIOUS: Very important; well-known; highly regarded.

Lydia Villa-Komaroff was born a Mexican American in Santa Fe, New Mexico in 1947. She knew from an early age that she wanted to be a scientist. Her family supported and encouraged her determination to go away to college. In 1965, Villa-Komaroff enrolled at the University of Washington in Seattle, initially as a chemistry major. She was forced to change her major to biology, because at that time people believed that women had no place in chemistry. Villa-Komaroff completed her undergraduate degree (Bachelor's) at Goucher College in Maryland and went on for her Doctorate (Ph.D.) at Massachusetts Institute of Technology (MIT). At MIT, she studied under the Nobel Prize winning scientist, David Baltimore. She received her Ph.D. in 1975. Villa-Komaroff was the third Mexican American woman in the U.S. to receive such an advanced degree in the sciences. Afterwards, Villa-Komaroff worked with several prestigious scientists: Fotis Kafatos at Harvard, James Watson (one of the discoverers of DNA) at Cold Harbor Springs Lab, and Walter Gilbert (Nobel Laureate) at Cambridge, Massachusetts.

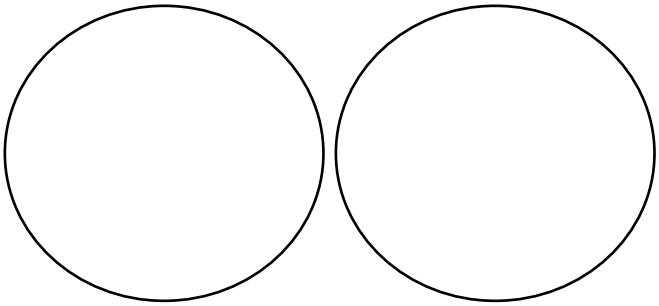
Dr. Lydia Villa-Komaroff

While at Cambridge, she developed a strain of *Eschericia coli* (*E. coli*) bacteria that was genetically engineered to produce human insulin. This was the first time a human hormone had been synthesized in bacteria. For this accomplishment, Villa-Komaroff was hired as an associate professor at Harvard Medical School and Children's Hospital in Boston.

Villa-Komaroff is one of the top 100 most influential Hispanics in America and is a member of the National Advisory of Neurological Disorders and Stroke Council, Institute of Medicine, National Research Council Committee on Assessing the Structure of NIH, and is on the board of directors of the American Association for the Advancement of Science (AAAS).

References:
Notable Hispanic American Women, Book II, Gale, 1998. Biography Resource Center, Gale, 1999
http://www.galegroup.com/free_resources/chh/bio/villa_l.htm
Wikipedia. http://en.wikipedia.org/wiki/Main_Page

GENES

Date:	Data and Sketches
What design requirement(s) are you trying to meet with this experiment?	
What variables are you testing to meet this / these requirement(s)?	
Materials and Methods:	
Predictions: What do you expect to ovserve at the conclusion of the experiment?	Summary and Conclusions Think about why you were (not) able to meet your requirement(s)

GENES

Think about your conclusions. List at least three reasons why you were or were not able to meet your requirement(s).

1.

2.

3.

What are some ways that you might test these reasons to improve your prototype.

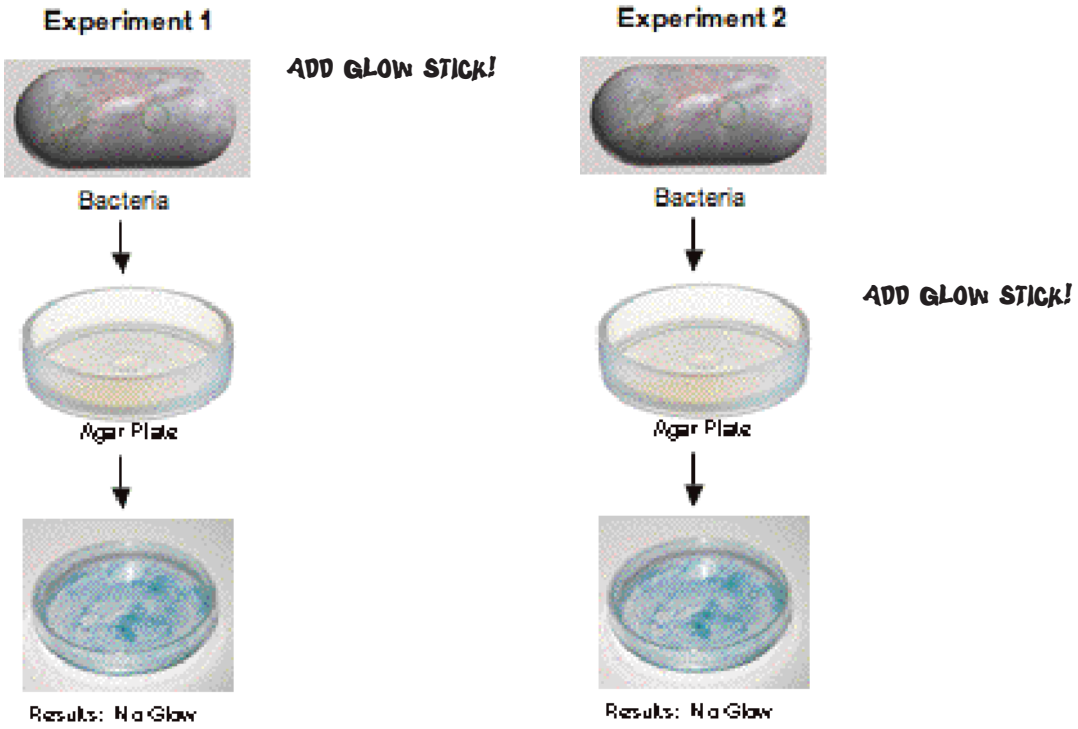
1.

2.

3.

BUGS II – TRAITS

CONTRASTING CASE 1



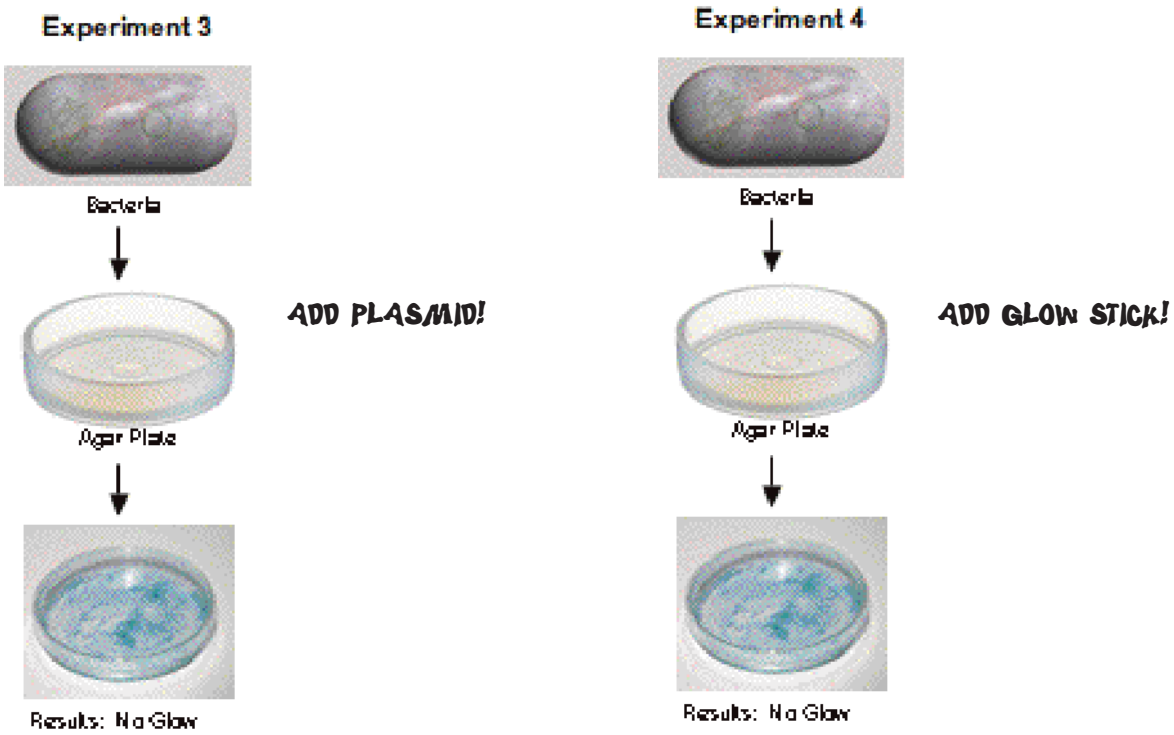
If the results are different between the experiments, explain what is different between the experimental set-ups. If the results are the same, explain what is the same between the experimental set-ups.

What claims do you feel confident in making at this point?

What claims do you think may be true but you are not yet confident about?

BUGS II – TRAITS

CONTRASTING CASE 2



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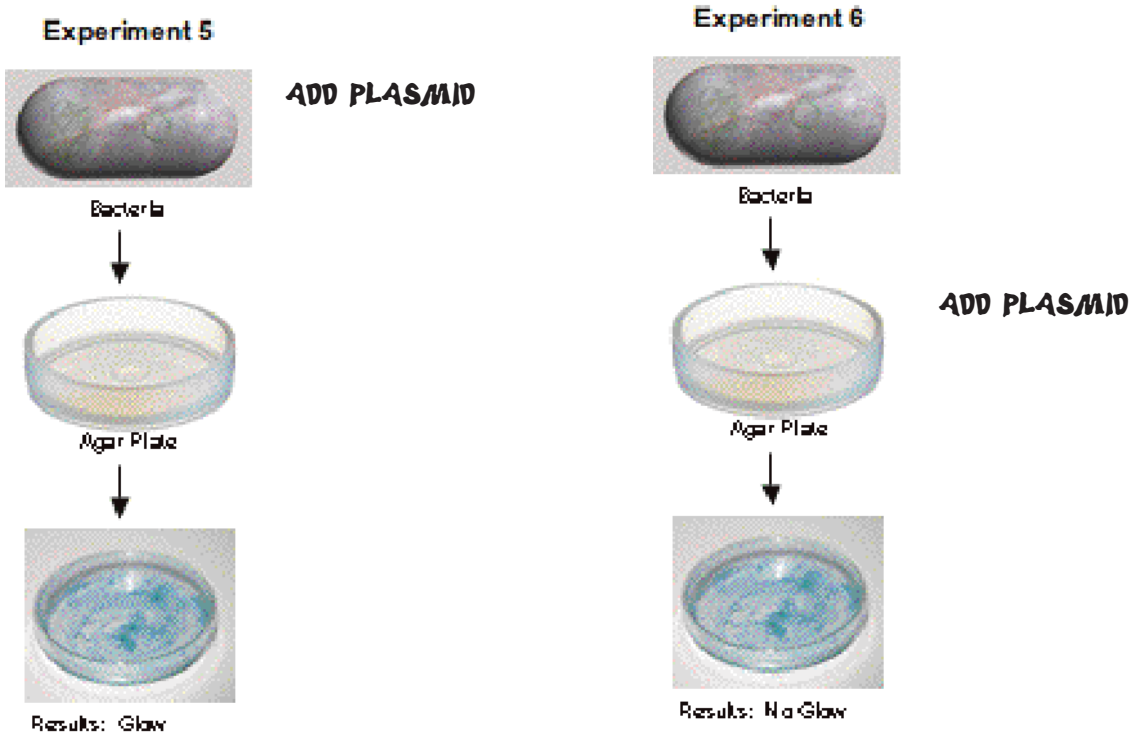
BUGS II – TRAITS

CONTRASTING CASE 3



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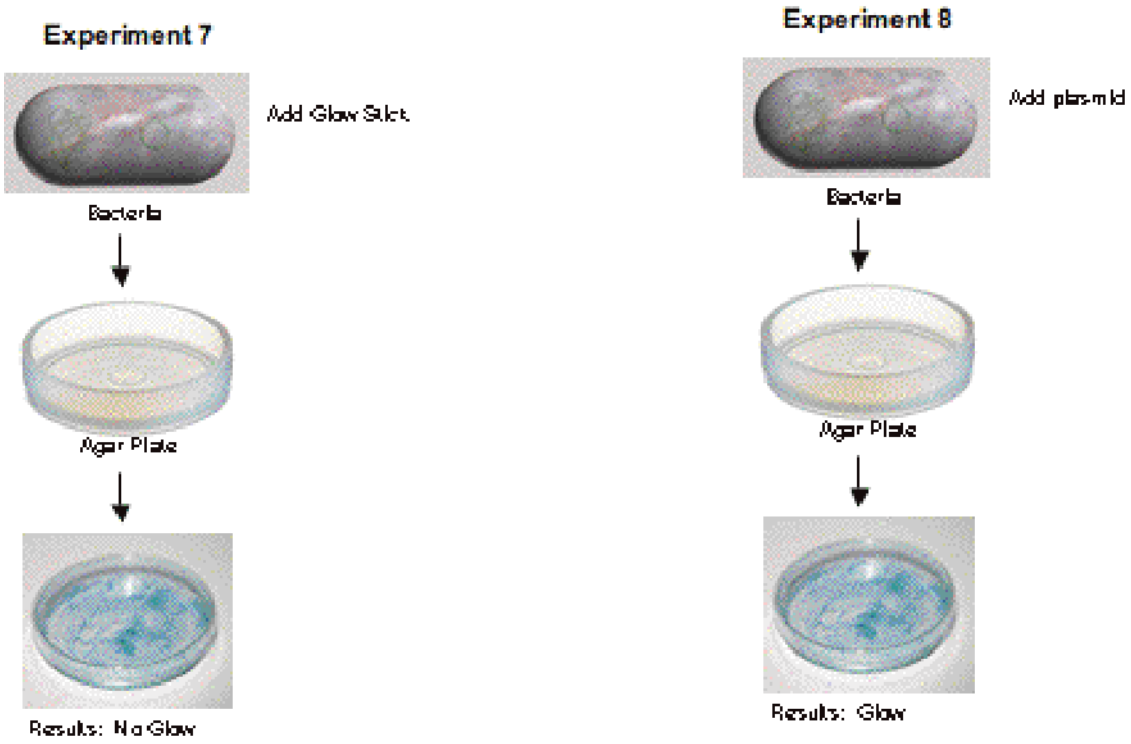
BUGS II – TRAITS

CONTRASTING CASE 4



If the results are different between the experiments, explain what is different between the experimental set-ups. If the results are the same, explain what is the same between the experimental set-ups.

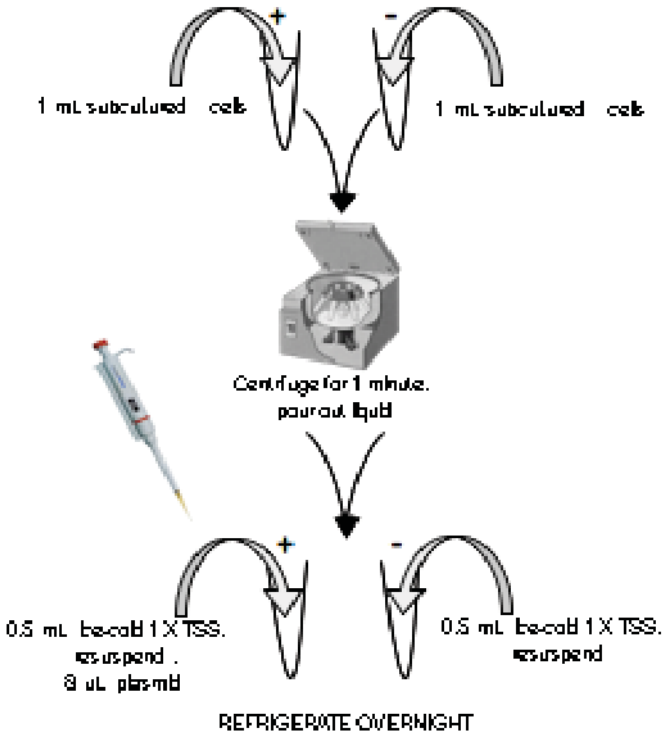
What claims do you feel confident in making at this point?

What claims do you think may be true but you are not yet confident about?

TRANSFORMATION PROCEDURES

DAY ONE

To get your bacteria to do something they normally could not do, you need to get new genetic instructions into the cells in the form of genes. There are several methods for inserting genetic information into the cells. The method you will use is called transformation.



GENE: The basic physical unit of heredity; a linear sequence of nucleotides along a segment of DNA that provides the coded instructions for synthesis of RNA that when translated into protein, leads to the expression of hereditary characters.

Open sterile pipette packages at the end with the bulbs! Do not touch the tip!

MATERIALS

- Subcultured cell suspension
- Transformation kit
- Centrifuge
- Transfer pipette

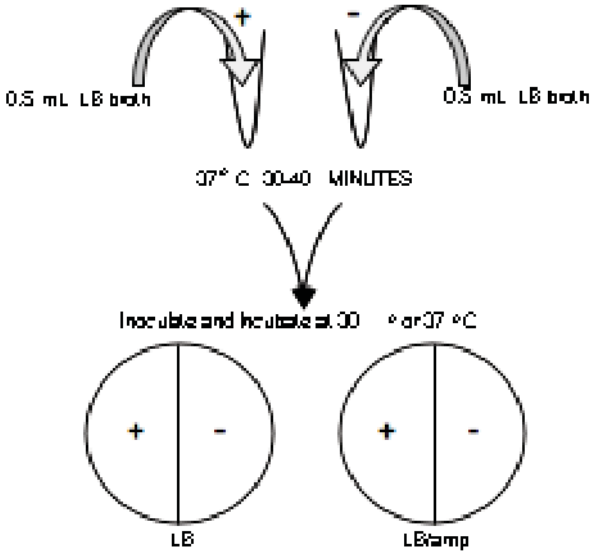
METHODS

1. Label two 1.5 mL tubes: (strain)+ and (strain)-, where (strain) is the type of bacteria you are using [DH5, or XL1]
2. Add 1 mL subcultured cells to the (+) and (-) tubes and load them in the centrifuge with the hinge of the tube toward the outside. Turn the centrifuge on and spin for one minute to pellet the cells.
3. Pour out all of the liquid (supernatant) from both tubes in the biohazard container. (Note. The cells are now stuck to the bottom of the tubes).
4. Add 0.5 mL ice-cold 1XTSS solution to both tubes. Gently resuspend the pellet by flicking the tube with your finger.
5. Add contents of the plasmid tube to the **(+) TUBE ONLY.**
6. Place both tubes in refrigerator overnight.

Remember! Use a new pipette after each manipulation!

TRANSFORMATION PROCEDURES

DAY TWO



GENE: The basic physical unit of heredity; a linear sequence of nucleotides along a segment of DNA that provides the coded instructions for synthesis of RNA that when translated into protein, leads to the expression of hereditary characters.

MATERIALS

- Transformation kit
- Transfer pipette
- 30º C incubator
- 37º C incubator

Open sterile pipette packages at the end with the bulbs! Do not touch the tip!

METHODS

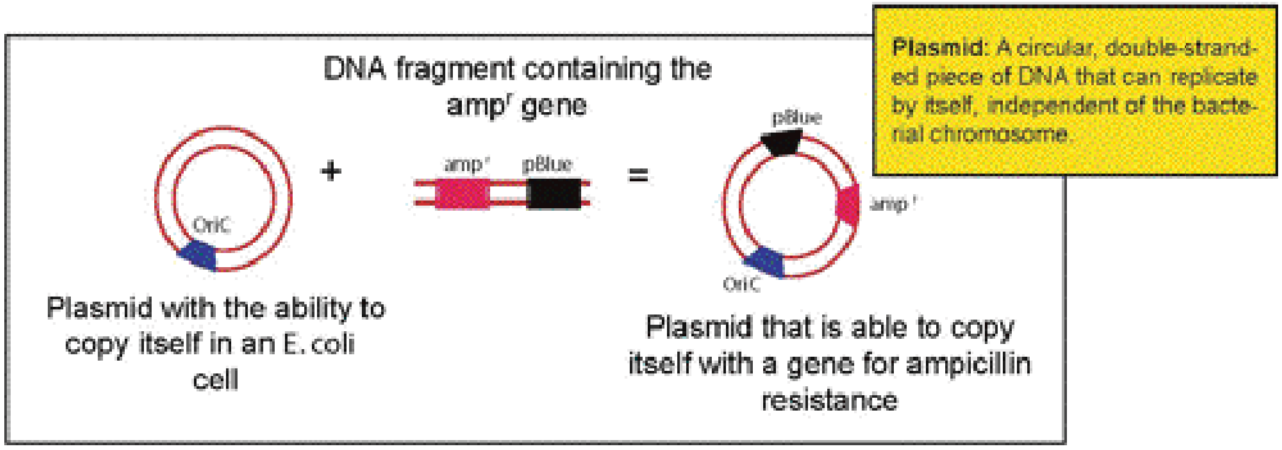
1. Add 0.5 mL LB broth to both tubes
2. Incubate at 37º C for 30-40 minutes.
3. Label the control plates (like the control plate on the left).
4. Label the experimental plates (like the experimental plate on the right).
5. Inoculate.
    - Spread ~150 µL from the (+) tube on the half of the control and experimental plates labeled '+'.
    - Spread ~150 µL from the (-) tube on the half of the control and experimental plates labeled '-'.
6. Incubate both plates at 30º C or 37º C overnight.

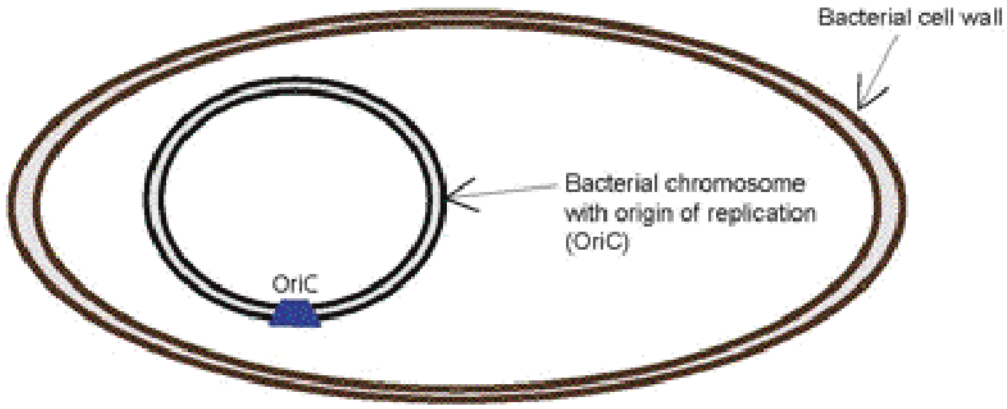
Remember! Use a new pipette after each manipulation!

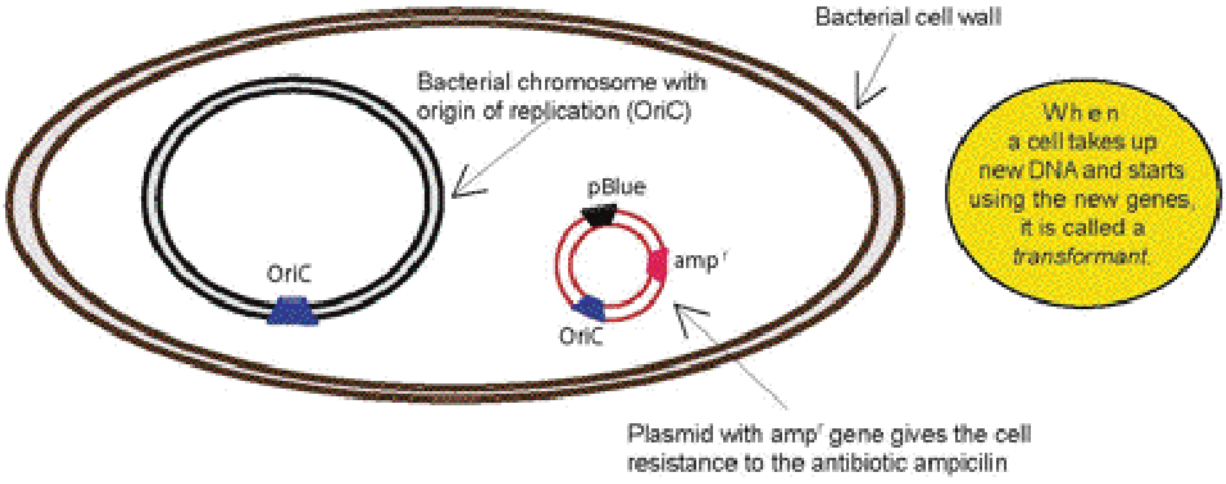
TRANSFORMATION PROCEDURES

A NOTE ABOUT THE NEW GENES ...

The genes you are inserting are located on a circular piece of DNA called a plasmid. Once inside the cell, the plasmid is able to copy itself without any help from the cell.







BUGS III – TRANSFORMATION

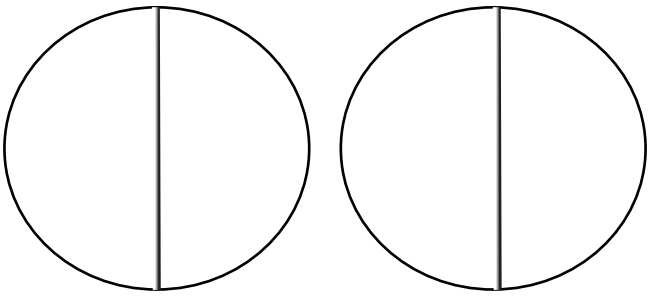
Date:

What design requirement(s) are you trying to meet with this experiment?

What variables are you testing to meet this / these requirement(s)?

Materials and Methods:

Data and Sketches



Predictions: What do you expect to ovserve at the conclusion of the experiment?

Summary and Conclusions

Think about why you were (not) able to meet your requirement(s)

BUGS III – TRANSFORMATION

Think about your conclusions. List at least three reasons why you were or were not able to meet your requirement(s).

1.

2.

3.

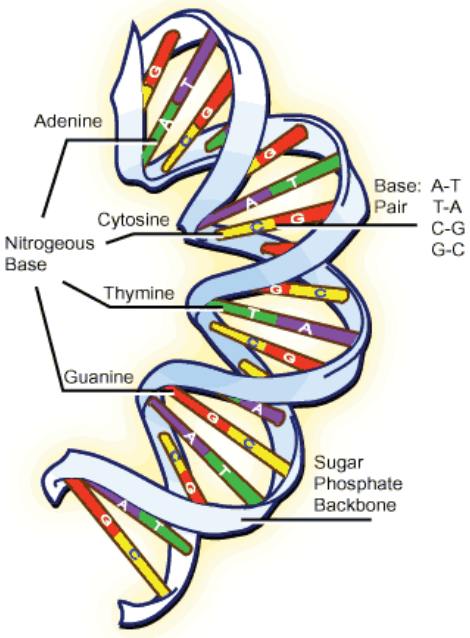
What are some ways that you might test these reasons to improve your prototype.

1.

2.

3.

BUGS N – DNA



WHAT IF THE ENVIRONMENT IS NOT THE PROBLEM?

DNA CODING

So your bacteria are not cooperating and not doing what you want them to do? Maybe something was wrong with the genetic instructions you gave to them. Try working with your genetic instructions to find out what these instructions look like and how they work. Using a DNA model, you will make a double-stranded copy of your gene.

MATERIALS

- Your gene sequence
- DNA models

METHODS

1. Make a single strand of DNA.
   a. Locate the sequence of your gene.
   b. Lay out a strip of masking tape with the sticky side up. This tape will represent the 'backbone' or the 'sides of the DNA ladder'.
   c. Use the key to line up the nucleotides. Each nucleotide should be lined up 5' to 3'. The 5' end should closer to the start of the segment.
2. Make a complementary strand of DNA.
   a. Match up each nucleotide on the single strand of DNA with its complementary base pair.
      - 'A' always pairs with 'T'.
      - 'G' always pairs with 'C'.
3. Connect the single strand of DNA with its complement by using the wooden dowels. These dowels represent the 'rungs of the DNA ladder'.
4. Twist your DNA segment so that it looks like the picture above.

DNA: (DEOXYRIBONUCLEIC ACID) The hereditary material of most organisms. It makes up genes.

NUCLEOTIDE: The building block of DNA. Nucleotides include the compounds adenine, thymine, guanine, and cytosine.

GENE: The basic physical unit of heredity.

HEREDITY: The genetic transmission of characteristics from parent to offspring.

COMPLEMENTARY BASE PAIRS

Adenine ⇆ Thymine

Guanine ⇆ Cytosine

WRITE OUT YOUR DNA SEQUENCE

WRITE OUT YOUR DNA COMPLEMENT

BUGS N - DNA

Describe the results that were useful for your prototype?

Think about the results from all of the BUGs investigations. What do you think these results say about genes?

BUGS V – WHERE IS THE DNA?

WET MOUNTS VS. ROOT TIPS

What if the genetic instructions did not get to where they need to be in the cell? Where is DNA kept in the cell? Make a wet mount of a transformed colony and compare that to a prepared slide of an onion root tip.

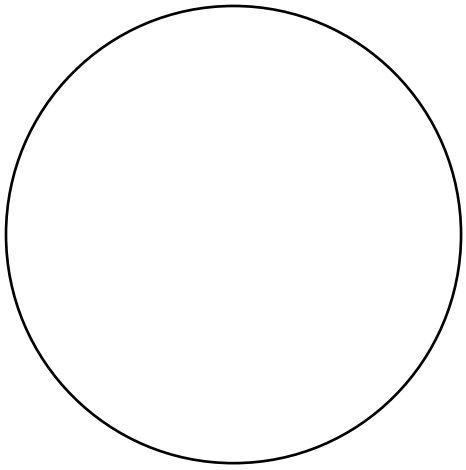
MATERIALS

- Plates from last class
- Prepared slide of an onion root tip
- 1 microscope slide
- 1 cover slip
- 1 pack of sterile toothpicks
- Water dropper
- Compound light microscope
- Methylene blue stain
- Paper towels
- Goggles and gloves

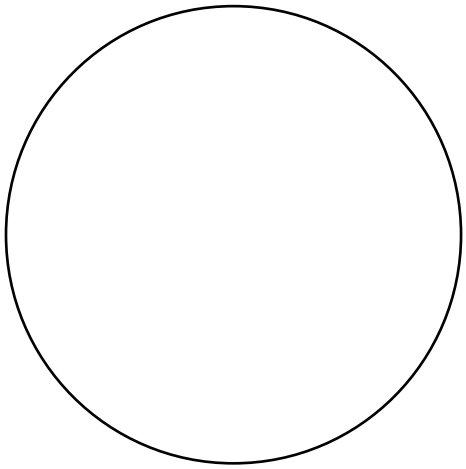
METHODS

1. Set the slide on a paper towel face-up on your bench. Place a single small drop of water near the center of a clean microscope slide.
2. Touch one small colony, lightly, with a sterile toothpick. BE CAREFUL NOT TO SCRATCH THE AGAR.
3. Swirl the toothpick in the water drop on your microscope slide in a circular motion.
4. Keep your slide flat on the bench. Hold a cover slip at a 45^{o} angle to the slide.
5. Slide the edge of the cover slip up the slide until it just touches the edge of the water droplet.
6. Slowly lower the cover slip onto the water droplet, to avoid air bubbles. Repeat steps 1-6, if you see large air bubbles.
7. Place one drop of methylene blue stain on the microscope slide next to, NOT ON TOP OF the cover slip. The drop of stain must touch the edge of the cover slip.
8. Tear off a small piece of paper towel. Place the ripped edge on the side of the cover slip OPPOSITE the drop of stain. The paper towel will start to soak up the water and draw the stain under the cover slip. This will stain the bacteria so you can see them under a microscope.
9. Look at the prepared slide of the onion root tip.
10. Compare the wet mount you made with the onion root tip. Clean slides as before.

Sketch a few cells from the onion root tip.



Sketch a few cells from the wet mount of bacteria.



What are some of the differences you noticed between the onion root tip and your bacteria?

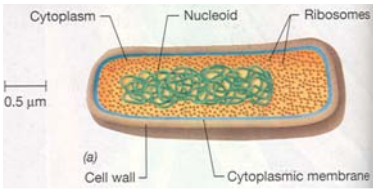
BUGS V – WHERE IS THE DNA?

ARE ALL CELLS LIKE E. COLI?

Organisms are made up of cells. Some organisms like bacteria are made up of a single cell. Other organisms are made up of multiple cells. Not all cells are the same. Cells are classified into two main groups: prokaryotes and eukaryotes.

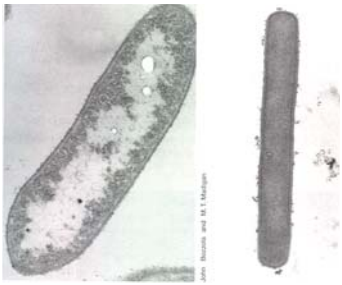
WHAT ARE PROKARYOTES?

Most prokaryotes are bacteria. *E. coli* are classified as prokaryotes. They are very, very small cells. Prokaryotes do not have a nucleus. Typically, the genetic material of a prokaryote is a single ball of double-stranded DNA stored in the nucleoid. Various small circular pieces of DNA, called plasmids are distributed throughout the cell and give the cell other traits not encoded in the nucleoid. In fact, the only type of organelle inside a prokaryote is a ribosome where proteins are made. A cell wall protects the prokaryote from the environment.



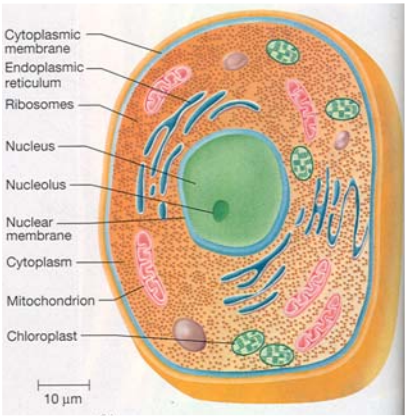
Most of the time, prokaryotes use asexual reproduction. During this asexual reproduction, the DNA of a parent cell is duplicated and divided between two daughter cells. This process is called binary fission.

PROKARYOTE: A cell or organism that lacks a nucleus and other membrane-boundorganelles that has a single ball of double-stranded DNA.

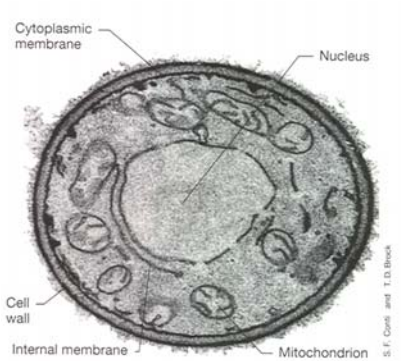


WHAT ARE EUKARYOTES?

There are many types of eukaryotes. For example, all of the cells in a mammalian body are eukaryotic. Other types of eukaryotes are mold, yeast, fungi, amoebae, and the cells of plants, reptiles, amphibians, birds, and you!



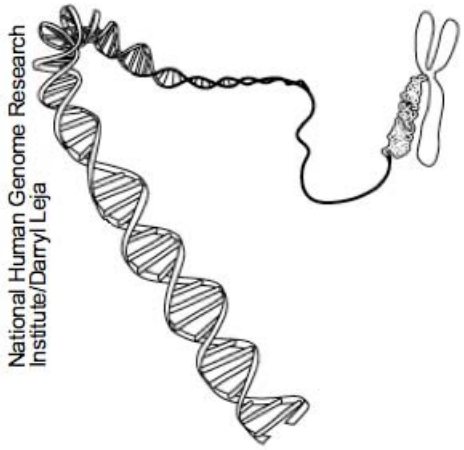
Generally, eukaryotic cells are much larger than prokaryotes, typically a thousand times by volume. Eukaryotes have a true nucleus and membrane-bound organelles. There are many types of organelles, such as vacuoles, endoplasmic reticula, mitochondria and chloroplasts. Organelles are held in place by the cytoskeleton. The cytoskeleton supports a eukaryote's cell like your bones support your body.



EUKARYOTE: A cell or organism whose cells have a membrane-enclosed nucleus and organelles.

BUGS V – WHERE IS THE DNA?

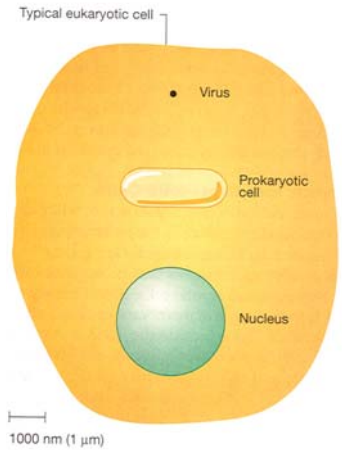
Typically, the genetic material, or DNA, of a eukaryote is located inside the cell nucleus. Eukaryotic DNA is double-stranded and packed like thread on a spool. Many spools of double-stranded DNA make up a chromosome.



Most of the time, the replication of eukaryotic cells is asexual. This process is called mitosis. During mitosis a full copy of the DNA is created by the parent cell. When the parent cell divides, one complete copy of the DNA is given to each daughter cell in a way similar to binary fission in prokaryotes. New DNA is not introduced during mitosis.

WHAT CAME FIRST, THE EUKS OR PROKS?

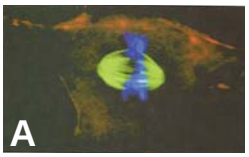
Some organelles inside eukaryotes perform metabolic functions that might have originated from endosymbiotic bacteria. Endosymbiotic theory proposes that a bacterium (prokaryote) cell was eaten by a much larger eukaryote. Instead of being used as food, the bacterium improved how the eukaryote functioned. For example, the bacterium might have enabled the eukaryote to perform photosynthesis or cellular respiration that helped the larger cell produce more energy. In return for the bacterium's help, the eukaryote provided food and shelter for the bacterium.

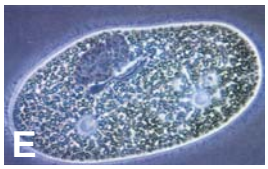


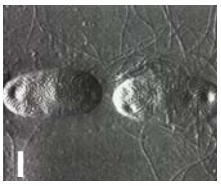
PROKARYOTES VS. EUKARYOTES

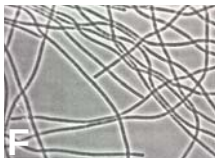
CHARACTERISTIC	PROKARYOTES	EUKARYOTES
Size	1-10 µm	10-100 µm
Nuclear envelope	Absent	Present
Chromosomes	Singular Circular Packaged without histones	Multiple Linear Packaged with histones
Golgi apparati	Absent	Present
Endoplasmic reticula	Absent	Present
Mitochondria	Absent	Present
Chloroplasts	Absent	Present
Ribosomes	Present (smaller)	Present (larger)
Cytoskeleton	Absent	Present

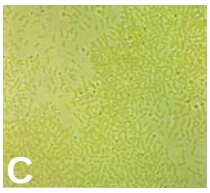
BUGS V – WHERE IS THE DNA?

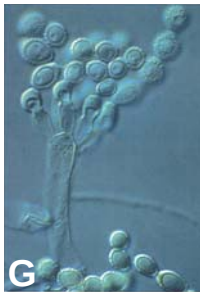


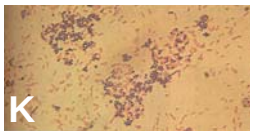


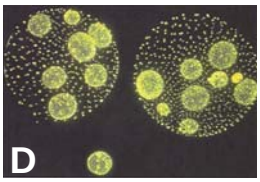












Which cells are eukaryotic?

Which cells are prokaryotic?

Circle where you think the DNA is located in each cell.

Choose an image of prokaryotic cells and an image of eukaryotic cells. What are some of the differences between the two?

What are some of the similarities?

BUGS VI – REPLICATION

REPLICATING DNA

So, your bacteria are not cooperating and not doing what you want them to do. Maybe something was wrong with how the cells were copying the genetic instructions you gave them. Try working with your genetic instructions to get a better understanding of what these instructions look like and how they work. Using the DNA model, make a double-stranded copy of your gene and replicate it.

MATERIALS

- Your gene sequence
- DNA models

METHODS

1. Make a single strand of DNA (follow step 1 on page 29).
2. Make a complementary strand of DNA (follow step 2 on page 29).
3. Connect the single strand of DNA to its complement (follow steps 3 and 4 on page 29).
4. Replicate your DNA.
   a. Open the DNA at one end by pulling one strand off the wooden dowels. This represents the breaking of hydrogen bonds between the nucleotide strands.
   b. Make a complementary strand of DNA for the DNA strand that you have pulled off the wooden dowels.
   c. Match up each nucleotide on the single strand of DNA with its complementary base pair.
      - 'A' always pairs with 'T'.
      - 'G' always pairs with 'C'.

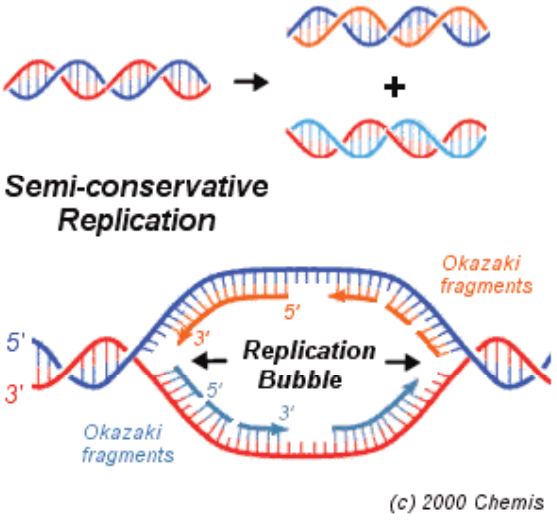
COMPLEMENTARY BASE PAIRS

Adenine ⇆ Thymine

Guanine ⇆ Cytosine

What did you notice about the strands as you were replicating them?

What does this tell you about how DNA is replicated in the cell?



WHAT ARE GENES, ANYWAY?

LINK BACK TO . . .

Environment

In the last subsystem, you saw how temperature and nutrients in the agar greatly impact the number and size of the colonies you saw.

During cell division, each cell is rapidly copying its DNA to pass on to each daughter cell. Encoded in this DNA are many genes.

DNA: (DEOXYRIBONUCLEIC ACID) The hereditary material of most organisms. It makes up genes.

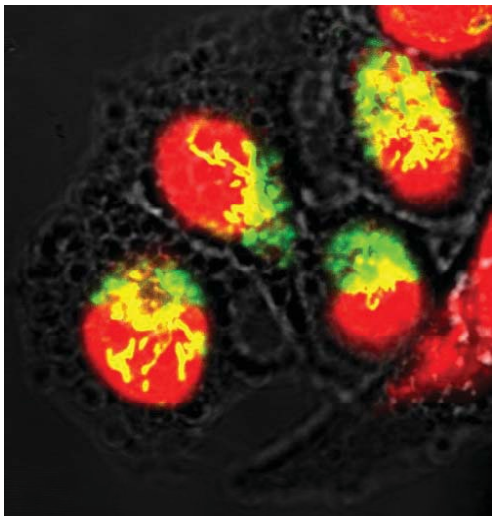
NEW STUFF . . .

DNA Structure and Location

You have learned that DNA is the material that composes the genetic instructions you are giving to your bacteria. This DNA has genes encoded in it. Genes are the instructions your bacteria use to take on a new characteristic.

THE DNA IS LIKE A BOOK AND THE GENES ARE LIKE THE CHAPTERS.

Also, you have learned that this DNA is located within the cell membrane. It is double-stranded and composed of nucleotides.



The picture above shows four eukaryotic cells. The nuclei are the four gray areas and inside each nucleus is the DNA shown in white.

NEW STUFF . . .

Genes and Heredity

GENE: The basic physical unit of heredity.

When you looked at your plates, you noticed that whatever was added really did not help the bacteria do what you wanted them to do. New characteristics are obtained only through the genes.

Remember that each colony originates from a single cell. The single cell copies everything, including its DNA, and passes that on to the next generation of cells, called daughter cells. Did you notice how when the whole colony was glowing or pigmented it continued to have that characteristic? Not only parts of the colony had the characteristic, but the whole colony had it! This result tells us that every cell has the same genes and that each cell in the colony inherited those genes from the previous generation.

HEREDITY: The genetic transmission of characteristics from parent to offspring.

The nature of heredity has been in place since the evolution of the earliest life forms on earth. The same method of heredity, passing genes from one generation to the next, extends all the way the up tree of life to higher mammals like you and I.

HUMANS SHARE THE SAME METHOD OF HEREDITY AS BACTERIA!

SUMMARY OF SOME BIG IDEAS . . .

- To optimize cell division, bacteria must be grown under the proper conditions.
- DNA is a double-stranded, anti-parallel molecule located in the bacterial cytoplasm that codes for genes.
- Genes are inherited from previous generations and transmit a characteristic.

REFLECTION

What do you think was the hardest part about introducing new genes into your bacteria?

How are genes and DNA the same? How are they different?

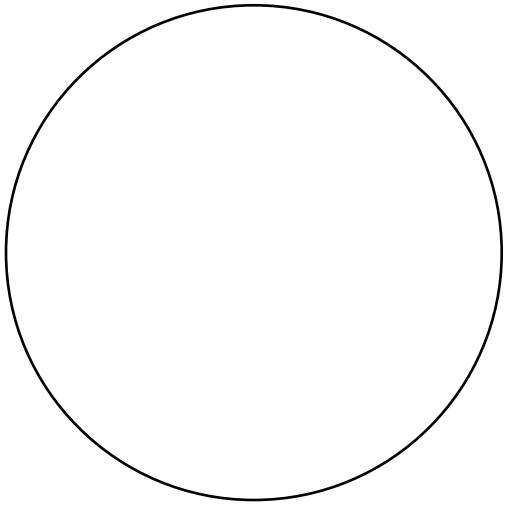
What have you learned about genes that will help you improve your prototype? What more do you want to know about genes?

How do you think a cell uses a gene to get to a trait?

EXPRESSION 1

Date:

Record and sketch what your colonies look like after they have been refrigerated for a few days.



Summary and Conclusions

Think about why you were (not) able to meet your requirement(s)

Think about your conclusions. List at least three reasons why you were or were not able to meet your requirement(s).

1.

2.

3.

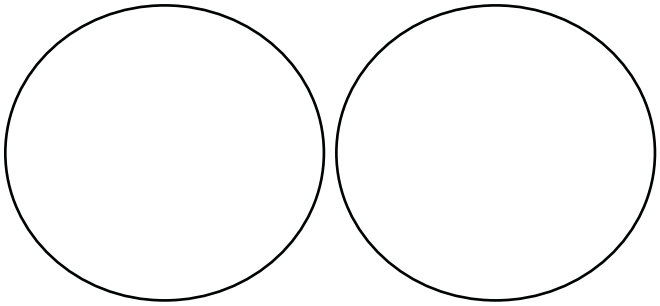
What are some ways that you might test these reasons to improve your prototype.

1.

2.

3.

BUGS VII – RESCUE

Date:	Data and Sketches
What design requirement(s) are you trying to meet with this experiment?	
What variables are you testing to meet this / these requirement(s)?	
Materials and Methods:	
Predictions: What do you expect to ovserve at the conclusion of the experiment?	Summary and Conclusions Think about why you were (not) able to meet your requirement(s)

BUGS VII – RESCUE

Describe the results that were useful for your prototype?

Think about the results from all of the BUGs investigations. What do you think these results say about how genes are expressed?

WHAT IS EXPRESSION, ANYWAY?

LINK BACK . . .

Environment

We have seen that bacteria respond directly to their environment. For instance, if they are placed in an environment that is too warm, then they will not grow. Similarly, the bacterial cells will not divide and the colonies will not grow if they are incubated at temperatures that are too cold. In addition to temperature, we saw in the *Environment Subsystem* that bacteria respond to the available nutrients.

How did the fresh or enhanced agar change your bacteria?

Bacteria in nature use various molecules from their environment to tell them what genes need to be turned on and off. Not all genes can be turned on or off at the same time. Your bacteria ran out of a particular nutrient that they require in order to express the new gene. The nutrients that you added to the media of your bacteria are what caused the gene to be turned on again and the desired characteristic to return.

Genes

Recall that the new genes you have given to your bacteria are encoded in a plasmid that is a circular and double-stranded piece of DNA. Once these genes are inserted into the bacterial cell, the cell can begin using them.

Why might a cell not use a gene?

What might cause a gene to be turned on?

NEW STUFF . . .

Expression

PROTEIN: a gene product that the cell uses to display a trait.

When you inoculated fresh plates with your transformants, you changed the growing environment of your bacteria. Introducing your bacteria to a more favorable environment caused a new set of genes to be expressed or turned on and off. When certain genes are turned on, then other genes might be turned off, and vice versa.

The environment affects the genes that are expressed. When a gene is turned on, it produces a gene product. Gene products are proteins that the cell uses to display a trait or characteristics.

There are over 4,000 genes that are encoded on the *E. coli* chromosome and over 30,000 genes in the human genome! Amazingly, each gene provides instructions for making a single unique protein.

GENES CAN BE TURNED ON AND OFF, DEPENDING ON THE ENVIRONMENT, TO PRODUCE GENE PRODUCTS PROTEINS THAT GIVE RISE TO A CHARACTERISTIC.

SUMMARY OF SOME BIG IDEAS . . .

- To optimize cell division, bacteria must be grown under the proper conditions.
- DNA is a double-stranded, anti-parallel molecule located in the bacterial cytoplasm that codes for genes.
- Genes are inherited from previous generations and transmit a characteristic.
- Genes can be turned on and off, depending on the environment, to produce gene products (proteins) that give rise to a characteristic.

REFLECTION

Explain 3 ways that the environment influiences gene expression. At least one of these ways must be supported by data from your experiments.
"The environment can create and influence genetic traits." Is this statement true or false? What experimental evidence do you have to support your claim?
What have you learned about expression that will help you improve your prototype?
How do you think a cell makes a trait from a gene?

WHAT IS THE CENTRAL DOGMA, ANYWAY?

LINK BACK . . .

Environment

CENTRAL DOGMA: The information flow from DNA to RNA to protein through the processes of transcription and translation.

In the *Expression Subsystem*, you saw that bacteria use various molecules from their environment to tell them what genes need to be turned on and off. Not all genes are turned on or off at the same time. Your bacteria ran out of a particular nutrient that they required to express the new gene. The nutrients that you added to the media are what caused the gene to be turned on again and the desired characteristic to return.You were able to change the environment to rescue the desired characteristic in your bacteria.

Can you manipulate the environment in such a way to get your bacteria to express more of the gene?

Genes

Recall that the new genes you have given your bacteria are encoded in a plasmid that is a circular and double-stranded piece of DNA. Once these genes have been inserted into the bacterial cell, then the cell can pass them on and begin to use them, depending on the environment.

When is a gene made of when it is turned off?

Does that gene get passed on to the next generation the same way as if it were turned on?

NEW STUFF . . .

Expression

In the *Expression Subsystem*, when you inoculated fresh plates with your transformants, you changed the growing environment of your bacteria. Introducing your bacteria to a more favorable environment caused a new set of genes to be expressed, or turned on and off. When certain genes are turned on, then other genes might be turned off, and vice versa.

By culturing more bacteria are you expressing a single gene more, or more single genes?

When a gene is expressed, it produces a protein. This gene expression occurs through several processes that are linked by the central dogma. The central dogma consists of two processes: transcription and translation.

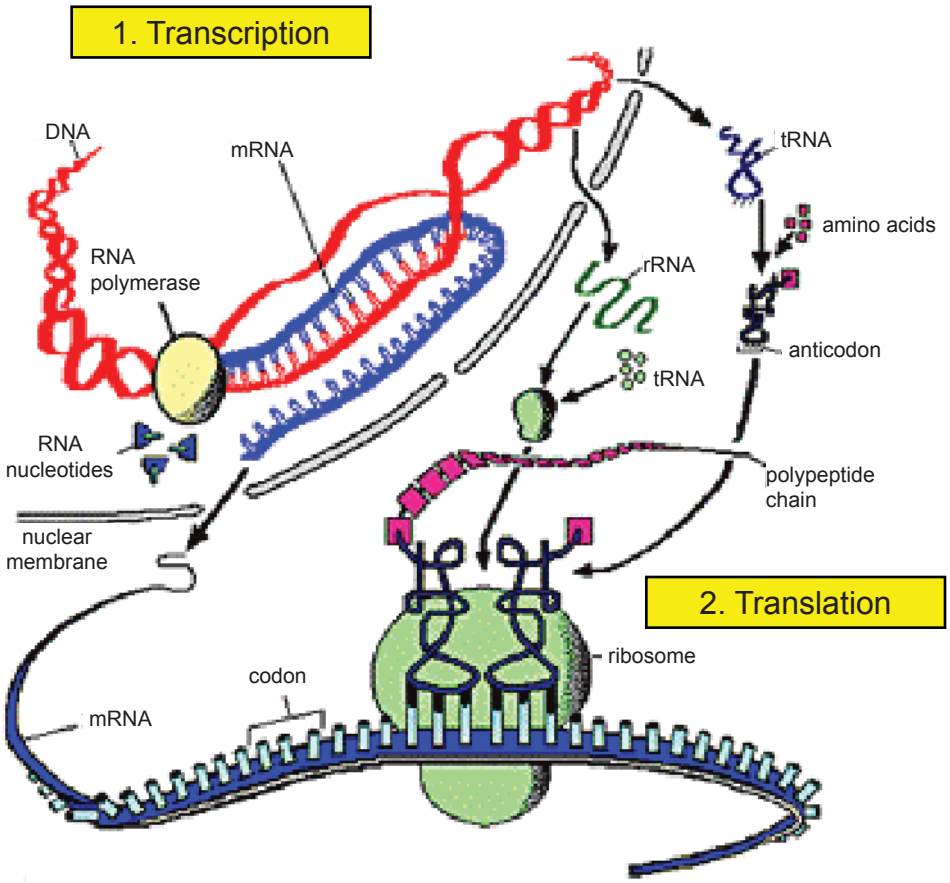
In **transcription**, the gene you inserted into your bacteria will be decoded into a piece of RNA.

In **translation**, the piece of RNA that was transcribed from the DNA is decoded into a protein product.

THE CELL USES DNA AS THE BLUEPRINT FOR THE PROTEINS THAT GIVE RISE TO A CHARACTERISTIC OR TRAIT.

TO MAKE A PROTEIN THE CELL TRANSCRIBES A GENE FROM THE DNA INTO RNA AND TRANSLATES THAT RNA INTO THE NEEDED PROTEIN.

WHAT IS THE CENTRAL DOGMA, ANYWAY?



Protein Synthesis

TRANSCRIPTION: The process that decodes a gene into a piece of RNA.

TRANSLATION: The process that decodes a piece of RNA into a protein.

RNA: (RIBONUCLEIC ACID) A single-stranded molecule made from DNA during transcription.

- Single - stranded
- Uracil replaces Thymine
- Ribose replaces Deoxyribose
- 3 types:
  - Messenger (mRNA); translated into protein
  - Transfer (tRNA)
  - Ribosomal (rRNA)

SUMMARY OF SOME BIG IDEAS . . .

- To optimize cell division, bacteria must be grown under the proper conditions.
- DNA is a double-stranded, anti-parallel molecule located in the bacterial cytoplasm that codes for genes.
- Genes are inherited from previous generations and transmit a characteristic.
- Genes can be turned on and off, depending on the environment, to produce gene products (proteins) that give rise to a characteristic.
- The cell uses DNA as the blueprint for the proteins that give rise to a characteristic or trait.
- To make a protein the cell transcribes a gene from the DNA into RNA and translates that RNA into the needed protein.

BUGS VIII – DECODING I

DECODING DNA

Do you want to get your bacteria to express your new gene more? Because expression enhancement is a new technology and many researchers are currently working on it, we cannot play with gene expression directly. However, why not look at your gene sequence and see how it gets transcribed and translated into the protein that makes the desired characteristic? Using the DNA model, transcribe and translate your gene.

MATERIALS

- Your gene sequence
- DNA models
- Central Dogma kit

METHODS

1. Make a single strand of DNA (follow step 1 on page 29).
2. Make a complementary strand of DNA (follow step 2 on page 31).
3. Connect the single strand of DNA to its complement (follow steps 3 and 4 on page 31).
4. Transcribe a single string of DNA into RNA.
   a. Open the DNA at one end by pulling one strand off the wooden dowels. This represents the breaking of hydrogen bonds between the nucleotide strands.
   b. Begin transcribing that strand of DNA into a single-strand of RNA. Remember, RNA differs from DNA in several ways.
      - 'A' always pairs with 'U'.
      - 'G' always pairs with 'C'.
5. Translate the RNA RNA molecule on your ribosome into a protein chain using tRNA's.
   a. Use your Central Dogma kit to locate the ribosomes and proteins. The chips represent the amino acids.
   b. Join each amino acid to the next with tape.
   c. As the ribosome moves along the mRNA strand, pull the attached amino acid off the card.
   d. This chain of amino acids later makes a protein.

What are some of the differences you saw between DNA and RNA?

WRITE OUT YOUR DNA SEQUENCE

WRITE OUT YOUR RNA SEQUENCE

WRITE OUT YOUR PROTEIN CHAIN

BUGS IX – DECODING II
WITH MUTATIONS

DECODING DNA

Remember that your gene is a piece of RNA that is decoded from the DNA. What if the cell does not make the piece of RNA correctly? This type of mistake occurs in the cell when there is a 'typo' in the DNA sequence. A 'typo' in the DNA is called a mutation. Mutations are very rare because most cells have built in proof-reading machinery to correct mistakes made during replication. Sometimes mutations do not change anything. At other times, mutations can cause a wide range changes in the cell. Sometimes mutations can kill the cell. What happens to the protein if there is a 'typo' in the original DNA instructions? Using the DNA model, transcribe and translate your gene with a mistake in the sequence.

MUTATION: An incorrect nucleotide ('typo') in the DNA sequence.

MATERIALS

- Your gene sequence
- DNA models
- Central Dogma kit

METHODS

1. Examine the two sequences in your kit. One is mutated the other is not. Locate the mutation.
2. Write the mutated DNA base(s) on the masking tape of your model.
3. Transcribe the mutation and write the mutated base(s) on your RNA molecule's masking tape.
4. Translate your mutated RNA into protein.
5. Share the differences between your mutated protein and the first protein you made with the class.

WRITE OUT YOUR DNA SEQUENCE

WRITE OUT YOUR RNA SEQUENCE

WRITE OUT YOUR PROTEIN CHAIN

What are some of the differences you saw between your DNA, RNA, and protein molecules in *BUGs VIII* vs. *BUGs IX*?

TYPES OF MUTATIONS

Wild Type – the normal DNA sequence for a gene.

Ex. THE BIG FLY HAD ONE RED EYE TOO

1. Silent mutation: one DNA base pair is changed resulting in no change in the protein
   - Ex. THE BIG FLY HAD ONE RED EYE TWO
2. Missense mutation - one DNA base pair is changed resulting in a substitution for an amino acid.
   - Ex. THE BOG FLY HAD ONE RED EYE TOO
3. Nonsense mutation – one DNA base pair is changed resulting in a premature signal for the cell to stop building a protein.
   - Ex. THE BIG STOP
4. Insertion – the number of DNA bases has been increased by adding a piece of DNA.
   - Ex. THE BIG WET FLY HAD ONE RED EYE TOO
5. Deletion- the number of DNA bases is reduced by removing a piece of DNA
   - Ex. THE BIG FLY HAD ONE EYE TOO
6. Duplication – a piece of DNA is abnormally copied one or more times.
   - Ex. THE BIG FLY FLY HAD ONE RED EYE TOO
7. Frameshift mutation- an addition or loss of DNA bases causes the gene to be read from the wrong amino acid. Insertions, deletions, and duplications can all result in frameshift mutations.
   - Ex. THB IGF LYH ADO NER EDE YET OO

TYPES OF MUTATIONS

Identify the type of mutation for each one done in class.

Group	Type of mutation	Rank protein change: 0 = no change 1 = some change 2 = drastic change 3 = no protein	Do you think the bacteria will be glowing / blue?
		0 1 2 3	Yes No Unknown
		0 1 2 3	Yes No Unknown
		0 1 2 3	Yes No Unknown
		0 1 2 3	Yes No Unknown
		0 1 2 3	Yes No Unknown
		0 1 2 3	Yes No Unknown
		0 1 2 3	Yes No Unknown
		0 1 2 3	Yes No Unknown
		0 1 2 3	Yes No Unknown
		0 1 2 3	Yes No Unknown
		0 1 2 3	Yes No Unknown
		0 1 2 3	Yes No Unknown
		0 1 2 3	Yes No Unknown
		0 1 2 3	Yes No Unknown
		0 1 2 3	Yes No Unknown

Explain why some mutations cause drastic changes in traits and others do not.

BUGS X – ENHANCEMENT

IS IT POSSIBLE TO GET MORE OF THE TRAIT?

You know that transcription and translation cause a gene to be expressed. Do you think it might be possible to make a cell produce more of a trait by enhancing one or both processes? Currently, scientists and engineers are working hard to figure out how to enhance both transcription and translation. They find it difficult and very expensive to study the effects of enhancement. Because of these limits, you will not be able to conduct enhancement tests yourself. However, your teacher will give you the results of some tests so that you can investigate what happens in cells when transcription and/or translation are enhanced.

WHAT HAPPENED IN YOUR SAMPLES?

	Increase	Decrease	No Change
DNA			
mRNA			
Protein A			
Protein B			
Protein C			

Data Summary

DID THE ENHANCEMENT WORK?

PROCESS	ENHANCED?	WHY? OR WHY NOT?
None		
Transcription Only		
Translation Only		
Both Transcription and Translation		

Based on your data, do you think that sometimes we should be concerned about the effects of genetic engineering?

MINI-SYMPOSIUM II

BEFORE MINI-SYMPOSIUM II

- Complete the Mini-Symposium II sheet on the following page.
- Complete the Mini-Symposium II transparency.
- Prepare your questions for other teams.

SUCCESS RATE

0 - Not tried yet
1 - Tried with little success
2 - Tried with some success
3 - Tried with good success

DURING MINI-SYMPOSIUM II

- Present your prototype.
- Ask each group one of your questions.
- Record their response.

AFTER MINI-SYMPOSIUM II

- Rate each team's response to your questions.
- Consider revising your prototype based on comments during the mini-symposium.

MINI-SYMPOSIUM II

Team Name: Prototype:

MUST-HAVE REQUIREMENTS	SUCCESS RATE

The hardest part so far . . .

We have been most successful at . . . (Hint. This should be the requirement with the highest success rate)

We could improve our prototype by . . . (Hint. This should be the requirement with the lowest success rate)

MINI-SYMPOSIUM II

Question 1:

Name of team asked this question:

That team's response to this question:

Our rating of their response:

0	1	2	3	4	5
No response	Poor	Fair	Good	Excellent	Superior

Question 2:

Name of team asked this question:

That team's response to this question:

Our rating of their response:

0	1	2	3	4	5
No response	Poor	Fair	Good	Excellent	Superior

Question 3:

Name of team asked this question:

That team's response to this question:

Our rating of their response:

0	1	2	3	4	5
No response	Poor	Fair	Good	Excellent	Superior

MINI-SYMPOSIUM II

Question 4:

Name of team asked this question:

That team's response to this question:

Our rating of their response:

0	1	2	3	4	5
No response	Poor	Fair	Good	Excellent	Superior

Question 5:

Name of team asked this question:

That team's response to this question:

Our rating of their response:

0	1	2	3	4	5
No response	Poor	Fair	Good	Excellent	Superior

Question 6:

Name of team asked this question:

That team's response to this question:

Our rating of their response:

0	1	2	3	4	5
No response	Poor	Fair	Good	Excellent	Superior

MINI-SYMPOSIUM II

Question 7:

Name of team asked this question:

That team's response to this question:

Our rating of their response:

0	1	2	3	4	5
No response	Poor	Fair	Good	Excellent	Superior

Question 8:

Name of team asked this question:

That team's response to this question:

Our rating of their response:

0	1	2	3	4	5
No response	Poor	Fair	Good	Excellent	Superior

Question 9:

Name of team asked this question:

That team's response to this question:

Our rating of their response:

0	1	2	3	4	5
No response	Poor	Fair	Good	Excellent	Superior

ENVIRONMENT II

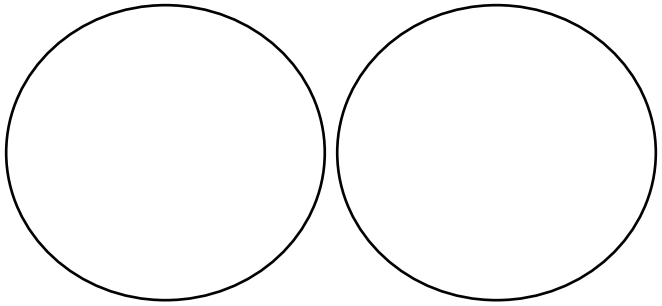
Date:

What design requirement(s) are you trying to meet with this experiment?

What variables are you testing to meet this / these requirement(s)?

Materials and Methods:

Data and Sketches



Predictions: What do you expect to ovserve at the conclusion of the experiment?

Summary and Conclusions

Think about why you were (not) able to meet your requirement(s)

ENVIRONMENT II

Have you met all the requirements for your prototype? What, if any, are the remaining requirements?	List at least three reasons why you not able to meet these remaining requirement(s). 1. 2. 3.

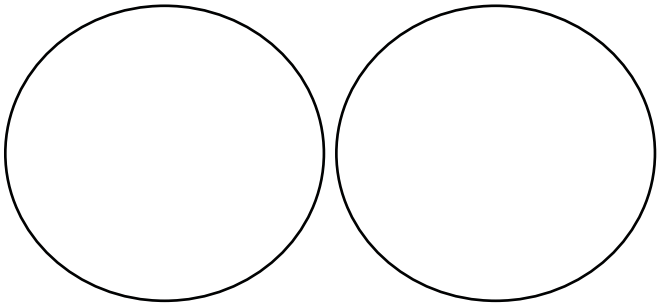
What are some ways that you might test these reasons to improve your prototype.

1.

2.

3.

BUGS X1 – REAL-WORLD

Date:	Data and Sketches
What reason are you trying to test?	
What measures will you take to test this reason?	
Predictions	
Notes	Summary and Conclusions Think about why you were (not) able to meet your requirement(s

BUGS XI – REAL-WORLD

Describe the results that were useful for your prototype?

Think about the results from all of the BUGs investigations. What do you think these results say about how cells replicate in the real world?

How do genes and the environment interact, anyway?

Link back . . .

Environment

You saw that bacteria in nature use various molecules from their environment to tell them what genes need to be turned on and off. Not all genes are turned on or off at the same time. Your bacteria ran out of a particular nutrient that they required to express the new gene. The nutrients that you added to the media are what caused the gene to be turned on again and the desired characteristic to return.

How do you increase the rate of cell division?

Genes

The new genes that you have given your bacteria are encoded in a plasmid that is a circular and double-stranded piece of DNA. Once these genes were inserted into the bacterial cell, the cell passed them on and could begin to use them, depending on the environment. In order to pass the genes on, the DNA had to be replicated.

How does DNA replication occur?

What does this mean for your prototype?

New stuff . . .

Expression

When you inoculated fresh plates with your transformants, you changed the growing environment of your bacteria. Introducing your bacteria to a more favorable environment caused a new set of genes to be expressed, or turned on and off. When certain genes are turned on, then other genes might be turned off, and vice versa.

How do you think you can enhance gene expression?

Can you inhibit or slow gene expression? How?

How Do Genes and the Environment Interact, Anyway?

More New Stuff . . .

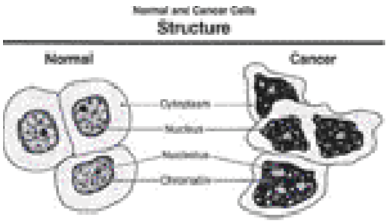
Expression

When a gene is expressed it produces a protein. This gene expression occurs through several processes that are linked by the central dogma. The central dogma consists of two processes: transcription and translation.

In **transcription**, the gene you inserted into your bacteria will be decoded into a piece of RNA. In **translation**, the piece of RNA that was transcribed from the DNA is decoded into a protein product.

A 'typo' in the DNA is called a mutation. Mutations are very rare because most cells have built in proof-reading machinery to correct mistakes made during replication. However, when mutations occur sometimes they do not change anything. A mutation can kill the cell or sometimes it can even cause a wide range of changes.

Mutations are mistakes in the DNA sequence that result in an incorrect protein blueprint and a range of cellular effects.



One class of mutations can cause cancer. Cancer cells have mutated DNA. This incorrect DNA is unable to code for the correct protein that tells these cells to stop replicating. Because these cells are unable to code for the correct protein, they are unable to do their intended jobs and these cells grow out of control causing tumors.

Important: Although you have learned that the environment cannot give an organism traits, genes and the environment interact in complicated ways. For instance, cancers can be caused by environmental factors such as UV radiation. The environment can also influence the expression of genes. For instance, a human genetically programmed to grow to 6 ft may be shorter because of malnutrition, disease, or injury. Certain traits may be selected for by the environment allowing particular genes to be passed on to the next generation.

Mutation: An incorrect nucleotide ('typo') in the DNA sequence.

Summary of Some Big Ideas . . .

- To optimize cell division, bacteria must be grown under the proper conditions.
- DNA is a double-stranded, anti-parallel molecule located in the bacterial cytoplasm that codes for genes.
- Genes are inherited from previous generations and transmit a characteristic.
- Genes can be turned on and off, depending on the environment, to produce gene products (proteins) that give rise to a characteristic.
- The cell uses DNA as the blueprint for the proteins that give rise to a characteristic or trait.
- To make protein the cell transcribes a gene from the DNA into RNA and translates that RNA into the needed protein.
- In order for the cells to divide and the colony to grow, the DNA in each parent cell must be replicated and a cop given to each daughter cell.
- Mutations are mistakes in the DNA sequence that result in an incorrect protein

WHAT IS A PATENT APPLICATION?

It can take a long time for designs to be ready to sell. Once scientists, engineers, or companies have developed a successful prototype they apply for a patent. A patent insures that no one else will be able to develop an identical product. In this way, companies and individuals are able to earn money on a product that they spent many hours and dollars developing. A patent guarantees that the scientist or engineer (or the company that he or she works for) is the legal owner of the design.

Dr. Jeffrey Hillman, the developer of the anti-cavity bacteria, has been awarded many patents over the last 25 years. These patents insure that he and his company, Oragenics, are the only ones who can develop this special type of bacteria.

Other people may develop bacteria designed to perform different functions or anti-cavity bacteria based on different technology, but no one can steal his design.

The U.S. Patent Office has awarded over one million patents. Thomas Edison has often been called 'America's Great Inventor'. He has 1,093 patents for products like the light bulb and the phonograph (the century-old precursor to the MP3 player). The most patents have been awarded to Shunpei Yamazaki for his work in computer and video technology. He has 1,432 patents. With over 3,000 patents, IBM has the most patents of any company. Their top patent holder is Ravi Arimilli. At age 42, he has been awarded more than 300 patents, 78 of them during 2002 alone! That's three patents every two weeks!

PATENT APPLICATION

Date:
Prototype Name:
Inventors (ID numbers only):
What problem does the prototype solve?
How does the prototype work?
When (what situations) is the prototype most useful?

PATENT APPLICATION

What are the must-have requirements for the design?
What is the function for each of the subsystems?
How does the environment influence gene expression in the prototype?

GALLERY WALK

BEFORE THE GALLERY WALK

Prepare your prototype and your team's poster. Be ready to explain what you did during this unit. You should talk about the parts of your design that were successful and the parts that were not as successful. Also, be ready to tell people about the changes you made along the way. Expect to get lots of questions.

YOUR GALLERY WALK POSTER SHOULD INCLUDE . . .

- The name of your prototype
- Your team members
- The needs and new ideas that you discussed at the beginning
- The requirements for your prototype
- How you did or did not meet each of your requirements
- The function for each of the subsystems
- How the prototype works
- When and where the prototype should be used
- The various tests and experiments that you ran while developing your prototype
- How the bacteria is able to express the new trait
- Draw a sketch of your gene
- Draw a sketch of your gene becomes a trait
- What steps could be taken to improve the prototype

DURING THE GALLERY WALK

Visit the posters of two groups in your class. Ask lots of questions so that you learn many details about how each team developed and created their prototype. To complete your evaluation sheets, you will need to summarize the prototype, identify at least one strength and one weakness of the prototype, and make recommendations for improving the prototype.

GENETICS JARGON

Agar	A gelatin-like substance used to culture bacteria. It is made from marine algae.
Base	5 nitrogen-containing compounds that are arranged in a particular order in DNA and RNA; A, C, T, G, U; nitrogen bases.
Base Pairs	Complimentary base pairs; A-T, C-G, A-U; pairing rules of bases in DNA and RNA.
Cell Division	The process where a cell, called the parent cell, divides into two identical cells, called the daughter cells.
Central Dogma	The information flow from DNA to RNA to protein through the process of transcription and translation.
Colonize	Bacteria become established in a habitat; this habitat could be an agar plate, a host, or a wound.
Culture	To grow bacteria in a medium for scientific study.
DNA (Deoxyribonucleic Acid)	The hereditary material of most organisms. It makes up the genes.
Engineering	The application of scientific knowledge and technical knowledge to solve human problems.
Eukaryote	An organism whose cells have a membrane-enclosed nucleus and organelles.
Function	How a prototype, or the subsystems of a prototype, work.
Gene	Unit of heredity;a section of DNA that codes for RNA and / or protein that causes physical characteristics.
Genetic Engineering	Scientific alteration of the structure of genetic material in a living organism. It involves the production and use of recombinant DNA and has been employed to create bacteria that synthesize insulin and other human proteins.

GENETICS JARGON

Genotype	Genetic description of a trait.
Heredity	The genetic transmission of characteristics from parent to offspring.
Inoculate	To purposely infect a medium with bacteria in a controlled way.
Media	Liquid or solid nutrient materials used to cultivate bacteria.
Must-Have Requirement	A feature that is necessary for a prototype to work.
Mutation	An incorrect nucleotide (‘typo’) in the DNA sequence.
Need	A problem that requires a solution.
Nice-to-Have Requirement	A feature that is not necessary for a prototype to work, but makes it more attractive.
Nucleotide	The building block of DNA. Includes the compounds adenine, thymine, guanine, and cytosine.
Phenotype	Visible appearance or description of a trait.
Plasmid	A circular, double-stranded piece of DNA that can replicate by itself, independent of the bacterial chromosome.
Prokaryote	An cell or organism lacking a nucleus and other membrane-bound organelles that has a single ball of double-stranded DNA
Protein	A gene product that the cell uses to display a trait.

GENETICS JARGON

RNA (Ribonucleic Acid)	A single-stranded molecule transcribed from DNA that is composed of a sequence of nucleotides that is complementary to the transcribed DNA strand. RNA's composition is similar to DNA except that instead of thymine, RNA has uracil.
Subsystem	The parts of a prototype.
Trait	Visible appearance or description of a gene; characteristic; phenotype
Transcription	The process that decodes a gene into a piece of DNA.
Transformant	A cell that has taken up a new gene and has started to use that new gene.
Translation	The process that decodes a piece of RNA into protein.

TEAM MEMBER RATINGS

Rating Scale 0 Absent 1 Poor 2 Fair 3 Good 4 Excellent 5 Superior

Activity	Lead Biologist	Analytical Biologist	General Biologist	Other	Quality Assurance Biologist
Needs and New Ideas	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID:
Require-ments	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID:
Mini-Sympo-sium I	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID:
Environment I	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID:
BUGs I: Cell Division	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID:
Genes	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID:
BUGs II: Traits	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID:
BUGs III: Transforma-tion	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID:
BUGs IV: DNA	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID:
BUGs V: Where is the DNA?	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID:
BUGs VI: Replication	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID:

TEAM MEMBER RATINGS

Rating Scale 0 Absent 1 Poor 2 Fair 3 Good 4 Excellent 5 Superior

Activity	Lead Biologist	Analytical Biologist	General Biologist	Other	Quality Assurance Biologist
Expression I	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID:
BUGs VII: Rescue	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID:
BUGs VIII: Decoding I	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID:
BUGs IX: Decoding II with mutations	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID:
Types of Mutations	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID:
BUGs X: Enhancement	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID:
Mini-Symposium II	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID:
Environment II	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID:
BUGs XI: Real-World	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID:
Patent Application	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID:
Gallery Walk	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: 0 1 2 3 4 5	ID: