CHAPTER 1

DNA: The Hereditary Molecule
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DNA: The Hereditary Molecule

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CHAPTER 1
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SECTION A
What is DNA?
An Introduction to DNA

DID YOU REALIZE that you came with a complete set of instructions for assembly? They weren’t printed on paper like the ones that come with a new bicycle; they were written in a special code, in a substance called deoxyribonucleic acid, or DNA.

The instructions in your DNA are called your genes. Your genes told all of your cells how to grow, when to divide, and how to move. Working together with each other and many external factors (such as the amounts and kinds of foods that you have eaten), your many thousands of genes have determined all of your traits, such as the shape of your facial features, how tall you are, how much you weigh, and how healthy you are.

It is important to realize, however, that although certain bits of our DNA code for the special traits that makes each of us different, most of our DNA codes for the basic features of life that each of us shares with every other human being, as well as with every other living thing.

WHERE DID YOUR DNA INSTRUCTIONS COME FROM?

Half of your DNA came in a sperm cell from your father and half came in an egg cell produced by your mother. When those two cells fused to form a new cell — a fertilized egg — this cell used the instructions in its DNA to grow and divide many times and become you.
Because brothers and sisters each receive half of their DNA from their father and half from their mother, they often resemble one another. But because each of them gets a slightly different set of DNA instructions from each parent, no two children look exactly alike — except in the rare case of identical twins.

**JUST HOW DOES YOUR DNA DETERMINE YOUR TRAITS?**

Your DNA contains many different sections called **genes** that contain coded instructions for making different kinds of proteins. Each kind of protein has a special effect on any cell that contains it. For example, certain genes that you inherited from your mother and your father determined what kinds of proteins were made in the cells forming your hair, and thus determined what color your hair would be.

All of us are distinguishable individuals, because all of us have our own special combinations of small DNA regions that influence various aspects of our physical appearance. But as mentioned above, these regions of our DNA that distinguish us from other people are the exceptions rather than the rule. More than 99.9% of our DNA is identical to that of every other human being.

The laboratory experiences in this chapter will help you to understand more about this material called DNA and how it controls both the shared and the specialized features of yourself and every other organism on our planet.
DNA in the News

LESSON OVERVIEW

DNA GETS MENTIONED in newspaper and magazine articles with increasing frequency these days. It certainly gets more publicity than any other type of molecule. DNA evidence regularly plays a central role in highly publicized court cases involving assault, murder, or rape. There are also an increasing number of cases reported in which a person who was convicted of a violent crime many years ago has been set free due to DNA evidence. This can happen when samples collected at the time of the crime are analyzed with modern methods, and the DNA in them establishes the convict’s innocence. In news of a very different sort, newspapers regularly announce major breakthroughs in the identification of changes in DNA that are thought to be the cause of various serious human diseases. Although DNA research holds much promise for families afflicted with heritable diseases, this research is not without controversy. Many articles can be found that discuss the intense controversies that are raised by the patenting of human genes, the development of new kinds of genetically engineered plants or animals, or the issue of labeling genetically modified foods.

The purpose of this exercise is to establish a collection of such articles in your classroom that will serve as the basis for classroom discussions of various aspects of modern genetics. Starting today, you should search any newspapers or magazines that you have access to either at home, in the library or elsewhere for articles about DNA or any other topic in genetics. If you have access to the Internet, you may also wish to search it. Clip or make a copy of any such article that you find and bring it to school to share with your teacher and your classmates.

You should write a brief summary to accompany each article that you collect and then give the article and your summary to your teacher. Your summary should cover the following:

• title of the article,
• author(s) (if identified),
• name of the newspaper, magazine, or other source in which the article appeared,
• date of publication,
• volume, issue, and page number(s),
• brief summary of the main points of the article (Who did what, where, when, why, and how?).

Your teacher will give you additional instructions about how such articles will be displayed, archived, and discussed by the class.

Note: Although you should get started on this project right away, you should continue searching for and collecting relevant articles as long as you and your classmates continue to study genetics.
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SECTION B
What Does DNA Look Like?
INTRODUCTION

DNA IS THE MATERIAL that contains all of the instructions that are required for building your cells and keeping them alive. Each of the 46 chromosomes in one of your cells contains one DNA molecule that is an inch or two long, though it is far too slender to be seen with any but the most powerful electron microscopes. If we could enlarge one of these DNA molecules enough so that we could see it — let’s say so that it was about the same diameter as one of the hairs on your head — we would find that it was several miles long!

In this exercise, we will take advantage of this long, thin, “threadlike” shape of DNA molecules to “spool” them, which is to say, we will wind them up on a wooden stick like a piece of thread. As you probably know, cotton fibers (which are individually a couple of inches long and quite thin) can be combined into one long, continuous thread, because they tend to stick to one another and line up side by side. The same thing can happen when DNA molecules come out of solution — if we pull on them from one end. We will do this by slowly twisting a stick in the region where DNA is beginning to precipitate. Each DNA molecule that is initially caught and wound around the stick will catch and pull on several other molecules, each of which will then catch and pull on others. Thus, if we work carefully, we can wind all of the DNA molecules in a test tube into one long, continuous thread. Although DNA molecules are so thin that you couldn’t possibly see one of them with your naked eyes, if you wind up many such molecules together (as you will in this exercise), the DNA becomes visible, and its properties can be studied.

MATERIALS

For each group of four students:

1 test tube of DNA
1 test tube of alcohol
1 wooden stick

PROCEDURE

1. Your teacher will give you a test tube containing a solution of DNA (which was isolated from salmon sperm by a procedure rather similar to the one described in the next exercise), another test tube containing alcohol, and a wooden stick. (Fig. 1) Record your observations of the liquids in the two tubes on your Observations sheet. Can you tell which one is the DNA? How?
2. Uncap the tubes and hold the tube that contains the DNA at an angle (Fig. 2).

3. Carefully transfer the alcohol from its tube into the DNA tube (Fig. 3). Pour very slowly, so that the alcohol does not mix with the DNA solution and stir it up. What does the alcohol do? Record your observations.

4. Gently insert the wooden stick through the alcohol layer to the interface where the two liquids meet (Fig. 4). Twirl the stick gently.

5. Now slowly lift the stick from the tube and observe the material clinging to it (Fig. 5). How long a fiber can you pull from the tube?

6. Put the stick back in the tube and gently twirl it in the vicinity of the interface again. Can you get more DNA attached to the stick?

7. When you have finished with the stick, cap the tube and shake it several times (Fig. 6). Do you see more DNA in the tube now?
OBSERVATIONS

1. Describe the appearance of the liquids in the two tubes.

______________________________________________________________________

______________________________________________________________________

2. Can you tell which of the tubes contains DNA? How?

______________________________________________________________________

______________________________________________________________________

3. Describe what happened when you first twirled the stick in or near the DNA-alcohol interface.

______________________________________________________________________

______________________________________________________________________

4. When you lifted the stick out of the tube and a fiber of DNA followed, did you think that this was a single molecule of DNA? Why?

______________________________________________________________________

______________________________________________________________________

5. How would you describe the appearance of DNA to someone who has never seen it?

______________________________________________________________________

______________________________________________________________________

6. What do you think it is about the biology of salmon and sperm cells that makes it easy to isolate a large quantity of DNA from salmon sperm?

______________________________________________________________________

______________________________________________________________________
Extracting DNA From Calf Thymus

INTRODUCTION

AS WE HAVE LEARNED, DNA is one of the most important substances in the world, because it carries the coded instructions for making all sorts of other molecules that are required for life. How does DNA do this? We’ll get to that part later. For now, what we want to do is to see how purified DNA, such as we examined in exercise B.1, is isolated from cells and tissues.

MATERIALS

Materials for this exercise will be found at four different distribution stations, as follows.

Station 1:
“Thymus soup”

Station 2:
Woolite or dishwashing detergent and dropping pipettes

Station 3:
Adolph’s Meat Tenderizer and small scoops or spatulas

Station 4:
Alcohol, wooden sticks and paper towels

PROCEDURE

1. At Station 1 pick up a tube of “thymus soup” that your teacher has prepared by blending a piece of calf thymus in water. Observe the thymus soup and record what you see on your OBSERVATIONS sheet.

2. At Station 2 use a dropping pipette to add a small amount of detergent (Woolite or dishwashing detergent) to the thymus soup (Fig. 1). Do not shake the tube vigorously or it will foam too much. Just swirl the tube gently to mix the two fluids. Record your observations.
3. At Station 3 use the small scoop to add a small amount of Adolph’s Meat Tenderizer to the tube (Fig. 2). Again, swirl the tube gently; do not shake. The tenderizer will not dissolve completely, but that’s fine. Record your observations.

4. Wait five minutes for the meat tenderizer to work. Use this time to finish answering questions 1-5 on your OBSERVATIONS sheet.

5. At Station 4 tip your tube slightly and then pour alcohol from the beaker so that it runs gently down the side of the tube and forms a layer 1-2 cm deep over the thymus soup (Fig. 3). Try to avoid stirring up the thymus soup as you add the alcohol.

6. Pick up a wooden stick and a paper towel at Station 4 and return to your work area.

7. Now things get more interesting! Insert the stick into the tube as far as interface where the thymus soup and alcohol meet and twirl the stick once or twice gently, just as you did in the previous exercise (Fig. 4). What happens?

8. Slowly pull the stick out of the fluid (Fig. 5). Now what is happening?.

9. Return the stick to the liquid and wind up as much DNA on the stick as you can. If you would like to save your DNA for later, you can wipe it off on the paper towel and take it with you when you leave the classroom.

The method you have just used to isolate DNA from calf thymus resembles the methods that law-enforcement agencies use to isolate DNA from samples found at crime scenes.
OBSERVATIONS

Describe what you see as you perform the steps to isolate DNA.

1. What does the “thymus soup” look like? __________________________________________________________________________
2. How does its appearance change as you add the detergent and swirl it in? __________
   __________________________________________________________________________
3. What do you think is happening at this step? __________________________________________________________________________
4. Does the appearance of the mixture change as you add the meat tenderizer and swirl it in? If so, how?
   __________________________________________________________________________
5. What do you think is happening at this step? __________________________________________________________________________
6. Describe the appearance of the mixture just after you added the alcohol. __________
   __________________________________________________________________________
7. What do you think is happening at this step? __________________________________________________________________________
8. What did you see as you twirled the stick at the interface? ________________
   __________________________________________________________________________
9. What do you think is happening at this step? __________________________________________________________________________
10. What happens as you slowly pull the stick out of the tube? ________________
    __________________________________________________________________________
11. What does DNA look like? ____________________________________________
1. Explain why DNA is so important to study.

______________________________________________________________________
______________________________________________________________________
______________________________________________________________________
______________________________________________________________________

2. Describe in your own words how one isolates DNA from animal tissue.

______________________________________________________________________
______________________________________________________________________
______________________________________________________________________
______________________________________________________________________

3. Explain the function of the following reagents: Woolite, Adolph’s Meat Tenderizer, and alcohol.

______________________________________________________________________
______________________________________________________________________
______________________________________________________________________
______________________________________________________________________

4. What does the DNA look like at the end of the procedure?

______________________________________________________________________
______________________________________________________________________
______________________________________________________________________
______________________________________________________________________
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SECTION C
What is the Structure of DNA?
The Puzzle of DNA Structure and Replication

INTRODUCTION

THE FUNCTION OF DNA is based entirely on its structure. A single strand of DNA consists of many individual building blocks called nucleotides that are connected, in a long string, by chemical bonds. Each nucleotide contains one of four possible nitrogenous bases: either adenine (A), cytosine (C), guanine (G), or thymine (T). In principle, it is possible to construct a DNA strand that contains these four types of nucleotides in any conceivable sequence. In nature, however, DNA exists as a double-stranded molecule in which the two strands that lie side by side interact in a very specific way. The nature of the interactions that occur between these two strands is such that the sequence of nitrogenous bases on one strand always has a predictable relationship to the sequence of nitrogenous bases on its partner strand. This relationship underlies the ability of DNA to serve as the hereditary material and has sometimes been called “the fundamental secret of life.”

In this exercise, you will be provided with half of a file folder and a set of puzzle pieces of different shapes and colors that represent the four kinds of nitrogenous bases that are found in all DNA molecules. You will use these pieces to solve a simple puzzle, and from the finished puzzle, you should be able to figure out the nature of the relationship that exists between opposite strands of a double-stranded DNA molecule, and why DNA is so easy to replicate when a cell gets ready to divide.

MATERIALS

For each student or pair of students:

one half of a file folder
puzzle pieces

PROCEDURE, PART A

1. Draw a straight line near and parallel to the left hand edge of your piece of file folder.

2. Select ten of your puzzle pieces at random, or in any order that you wish. Place each of these pieces on the paper so that it is letter-side-up and so that its flat end touches the line you have drawn (Fig. 1). This will be the left-hand strand of your DNA puzzle.
3. Now form the right-hand strand of the DNA by placing the remaining puzzle pieces on the paper so that the non-flat end each of them fits snugly against the non-flat end of one of the pieces in the left-hand strand.

4. Draw a straight line next to the flat ends of the pieces in the right hand strand (Fig. 2).

5. Use your finished puzzle to answer the first four questions on the next page.

**PROCEDURE, PART B**

6. Pair up with another person in your class.

7. One person in each pair should put all of his or her puzzle pieces back in the bag (Fig. 3).

8. Carefully transfer the right-hand strand of the remaining DNA puzzle to the right hand side of the other person’s piece of cardboard, leaving the left-hand strand where it is (Fig. 4).

9. Use the puzzle pieces in the bag to build a second DNA strand on each piece of cardboard (Fig. 5).

10. Answer questions 5 and 6 on the next page.
ANALYSIS AND CONCLUSIONS

1. Do you see any consistent relationship between the DNA bases (puzzle pieces) in one strand of your puzzle and the bases with which they are paired in the other strand? If so, state the nature of the relationship(s) you see.

______________________________________________________________________
______________________________________________________________________

2. Half of the puzzle pieces that you were given (the A’s and G’s) were much larger than the other pieces (the C’s and T’s). Did this size difference cause your DNA model to be significantly wider in some parts than in others? If not, why not?

______________________________________________________________________
______________________________________________________________________

3. Is there any consistent difference in the way that the pieces in the right-hand strands and the left-hand strands of your model are oriented? If so, what is the difference?

______________________________________________________________________
______________________________________________________________________

4. How can you account for the fact that no matter which bases were selected for the left-hand strand of a DNA molecule, everyone had just the right pieces left over to assemble a matching right-hand strand?

______________________________________________________________________
______________________________________________________________________

Do Part B of the puzzle before you answer the next two questions.

5. Are the two DNA puzzles you now have the same or different? How can you account for this?

______________________________________________________________________
______________________________________________________________________

6. What do you suppose biologists call this process of making two identical double-stranded DNA molecules from one when it occurs in cells?

______________________________________________________________________
______________________________________________________________________
Chapter 1 • Modern Genetics for All Students

The Spiral Staircase

In 1944, a team of biologists led by Oswald Avery made a very important discovery: DNA extracted from one kind of bacterium could be used to transfer heritable traits of that bacterium to a second bacterium! This was the first clear indication that DNA was the carrier of hereditary information. Other reports drawing the same conclusion soon followed.

Before Avery, scientists had paid little attention to DNA. They knew it contained the sugar deoxyribose, plenty of phosphate and four bases.

The four bases are known as A, C, G, and T, which are short for:

- Adenine
- Guanine
- Cytosine
- Thymine

These were assumed to be present in equal proportions.

AFTER AVERY, HOWEVER, RESEARCHERS BEGAN TO LOOK MORE CLOSELY...

ERWIN Chargaff found:

1. The composition of DNA varied from one species to another, in particular in the relative amounts of the bases A, C, T, G.

2. In any DNA, the number of A's was the same as the number of T's; similarly, the number of C's was equal to the number of G's.

What did this mean? Chargaff couldn't say...

By studying X-ray pictures of DNA, Rosalind Franklin was able to show that the DNA molecule probably had the corkscrew shape of a helix with two or three chains...

But was it two or three...?
In 1952 James Watson and Francis Crick cracked the puzzle.

By playing with scale-model atoms, they observed that adenine fitted together with thymine, while guanine paired naturally with cytosine.

Each base pair would be held together by hydrogen bonding, a weak attraction that may occur between a hydrogen on one molecule and a non-hydrogen atom on another molecule.

It was also clear A did not fit with C, nor G with T.

You repel me!!
Each of these two base pairs is nearly flat:

So Watson and Crick proposed to stack them up, one after another, like stairsteps. Two sugar-phosphate strands wind around the outside.

It's a double helix!??

One complication: The two strands wind in opposite directions: the sugars on one strand are "upside down" compared with those on the other strand—

SUGAR PHOSPHATE

Etc!
**This model clearly explains Chargaff's observation that the number of T's is equal to the number of A's: T and A are always paired together!**

**Ditto for G and C!**

**This is the principle of complementarity: each base can pair with only one other, called its complement.**

**Watson and Crick got the idea!! They wrote:**

"It has not escaped our notice that the pairing... immediately suggests a possible copying mechanism for the genetic material."

**In fact, it is the key to the gene's main functions: replication and protein synthesis."**
What is a Model?  
And What is it Good For?

AS WE GREW UP, most of us played with various models: model cars, model planes, model houses (doll houses), model people (dolls), etc. Not all models are designed for childhood play, however. Models serve important functions in many areas of adult life. Engineers, for example, evaluate various possible airplane designs by building scale models and examining how they behave in a wind tunnel (which is itself a model of the atmosphere).

Models are usually highly simplified relative to the objects that they portray, and often several different models, simplified in different ways, are required to study different aspects of the same object. For example, the engineer who is designing the seats for a commercial airliner requires a very different kind of model than does the engineer who is designing the wings. Neither of these models would necessarily be more correct than the other; they would merely be simplified in different ways in order to serve different purposes.

**DNA MODELS**

Scientists find models of various kinds of molecules very useful for visualizing how their parts fit together and for predicting what their properties should be. Indeed, it was only when two biologists by the names of Watson and Crick built the first fairly accurate scale model of DNA that it became clear what the structure of real DNA molecules must be.

In the next exercise, you will build a much simpler DNA model than Watson and Crick did in order to visualize certain very simple aspects of its structure. But as you progress in the study of biology, you will probably encounter several other kinds of DNA models that are constructed so as to reveal other, more detailed aspects of DNA structure and function.
Building a Three-Dimensional DNA Model

MATERIALS

For each student or pair of students: Use the following list to make sure that your model-building kit contains the correct number of pieces of each type. Then use it to determine what each of these pieces represents.

Short straws representing the nitrogenous bases:
- 3 blue straws = A (adenine)
- 3 red straws = T (thymine)
- 3 green straws = G (guanine)
- 3 gray straws = C (cytosine)

Pieces used to build the sugar-phosphate ladders:
- 12 black connectors = sugars (deoxyribose)
- 12 red connectors = phosphate groups
- 24 yellow straws = sugar-to-phosphate bonds
- 6 white connectors = hydrogen bonds

Pieces used to build the stand for the model:
- 1 long gray straw
- 3 medium-length green straws
- 1 four-prong black or silver connector

PROCEDURE

1. Make 12 nucleotides as shown in figure 1. Each nucleotide will require one deoxyribose sugar, two yellow sugar-to-phosphate bonds, one red phosphate group, and one nitrogenous base (either red, green, blue, or gray).

Figure 1: Making a nucleotide
2. After you have made all the nucleotides you can, use the white connectors (hydrogen bonds) to join them in pairs according to the base-pairing rules (A to T and G to C), as in figure 2.

3. After you have finished forming the nucleotide pairs, use the sugar-phosphate bonds to join them (fig. 3), in any order you want.

4. Note that the sugar-phosphate chain on the left side has a red phosphate group at the bottom (its 5' end) but not at the top (its 3' end) and that the sugar-phosphate chain on the right side is just the opposite. This is one of the ways that enzymes that interact with DNA molecules can tell the two strands apart.
5. Build the stand for your model using the one long gray straw, the three medium-length green straws and the four-pronged black connector.

6. Slide the white hydrogen bonds that run down the middle of your model onto the stand.

7. To get all of your model to fit on the stand, you will need to twist it counterclockwise at the top (fig. 4). Now you have a representation of the famous double-helix of DNA.

8. Answer the questions on the next page.
ANALYSIS AND CONCLUSIONS

YOU HAVE NOW built two models of double-stranded DNA: the flat one in the puzzle at the beginning of this exercise that you used to deduce the base-pairing rules and how DNA is replicated (which we will call “model A”), and the one you have just built with straws and connectors (which we will call “model B”). As mentioned earlier, often two models of the same thing will be simplified in different ways to emphasize different features of the object they are representing.

1. What feature or features of a double-stranded DNA molecule are represented better in model A than in model B?

______________________________________________________________________
______________________________________________________________________
______________________________________________________________________

2. What feature or features of a double-stranded DNA molecule are represented better in model B than in model A?

______________________________________________________________________
______________________________________________________________________
______________________________________________________________________

3. What feature or features of a double-stranded DNA molecule that you read about in the excerpt from the Cartoon Guide to Genetics are not well represented in either model A or model B?

______________________________________________________________________
______________________________________________________________________
______________________________________________________________________
DNA Model Questions

THE DIAGRAM ON THE left represents an untwisted, double-stranded DNA molecule.

1. Label each sugar group on the diagram with a letter S.
2. Label each phosphate group with a letter P.
3. One adenine (A) and one guanine (G) have already been labeled. Label the rest of the nitrogenous bases.
4. Circle one nucleotide. What three things go together to make a nucleotide?
   _____________________________________
   _____________________________________
   _____________________________________
5. The sides of the DNA ladder are made up of alternating ________________________
   and ________________________ groups.
6. The rungs of the DNA ladder are made up of _______________________________
7. A is always paired with ________________
8. G is always paired with ________________
9. Paired bases are held together by weak bonds called __________________ bonds.
10. When the DNA ladder twists the way it normally does, the shape of the molecule is called a ___________________________
DNA Word Search

FIND AND CIRCLE the words that go with each of the clues given below. Then write the answers on the lines next to the clues.

I J Y R L W X B V X M I U R W Y T Q W D
U S P G P Y J W L S V Q E H B K D N I I
B E L D K E Z P H O S P H A T E L C T U
N S N P E E D Y W B L T T Q O U A S H K
B A E I N Y E O X I F C H X N C Q Y J R
E B D A S Z K M C R R Q L S I D D G A T
O S D B R O H A K C P R M E P R N G D I
C U A W M O T C J H I X L G O L U L E S
Z O L W N I V Y N B N C N G V S B F N L
H N D V O O L D C U U K E N E W I S I Q
T E E N R X O S C N Y N U N I S N N N L
Q G T A E B E L O F B Q I V E B O Q E I
T O S D O G E B J O E N I M Y H T B N X
X R I S A O I W N P A L B S P D Q Q N R
C T W B T R S D G U A H B J Z Z Y F O P
V I T I Y S S T G M T S R A U H S X A V
E N D X B A S E P A I R X Y Z O C Y N Y
D E O Y E W I L E H E L B U O D Q
I E J S E A I E Q V W Q X M L C B X P V
D A X F M N H G K N X I I A F R R G M Y

1. The nitrogenous base A ____________
2. The nitrogenous base C ____________
3. The nitrogenous base G ____________
4. The nitrogenous base T ____________
5. The genetic material inside all cells ____________ (abbreviation)
6. The full name for DNA _____________________________
7. The scientific name for the shape of the DNA molecule ____________________________
   (two words)
8. The arrangement of two bases in the DNA molecule forms a ____________
   ____________________________ (two words)
9. The name of the bonds that hold the two strands of DNA together (between the bases) ____________ (two words)
10. Pairs of these molecules form the steps or rungs in the DNA molecule ____________
   ____________________________ (two words)
11. This subunit of DNA has three parts: a phosphate, a sugar and a nitrogenous base _________________
12. The long backbones of the DNA molecule are made of alternating sugar and _________________ bonds.
13. This process occurs when DNA makes a copy of itself _________________
CHAPTER 1
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SECTION D
What Does DNA Do?
DNA Codes For Proteins

PROTEINS DO THE nitty-gritty jobs of every living cell. Proteins are the molecules that give structure and shape to living cells and that carry out all of the chemical reactions necessary for life. The importance of DNA is that it contains the information that is used to make all of the proteins on which life depends.

The proteins whose structures are specified by our DNA do more than carry out all of the reactions necessary to keep our cells alive; they also digest our food for us, influence how we will respond to infections, and determine what color our eyes, hair and skin will be.

Proteins are made of long strings of individual building blocks known as amino acids. Amino acids come in 20 different kinds that are all slightly different from one another chemically. It is not important for us to understand the details of the chemical differences that distinguish these 20 kinds of amino acids. But it is important to realize that because of these differences, the structure and function of every protein depends on the sequence in which the various kinds of amino acids are strung together. A protein may contain many hundred amino acids. But if just one of these amino acid is changed, the function of the protein might change drastically.

A gene, which is a functional unit of DNA, carries coded information indicating the precise sequence in which amino acids should be strung together to make one particular kind of protein that will play one particular role in the life of the cell.

The elongated shape of collagen protein, shown in this molecular model, allows collagen to provide structural support to cells and organs. Antibodies, such as this human immunoglobulin, recognize and attack viruses, bacteria, and other foreign substances.
There are several different categories of proteins. Most proteins function as enzymes to regulate the speed of particular chemical reactions. Every cell contains hundreds of different enzymes that work together to release energy from food molecules and to use energy to build new cellular materials. All enzymes are proteins. But not all proteins are enzymes. **Structural proteins**, as the name implies, provide structural support inside and outside cells and for the body as a whole. **Defense proteins** are an assortment of proteins in the blood that recognize and fight off foreign invaders, such as bacteria and viruses. **Transport proteins** are used to carry various molecules into and out of cells as well as through the body. The table on the next page gives a few examples of these and other kinds of proteins.

Every aspect of life depends on proteins. In the next set of activities, you will learn about the processes a cell uses to convert the coded information in a bit of DNA (a gene) into a particular kind of protein.
### A Few Human Proteins and Their Functions

<table>
<thead>
<tr>
<th>Protein</th>
<th>Type</th>
<th>What it does</th>
</tr>
</thead>
</table>
| hemoglobin | a red, iron-containing, transport protein | • makes your blood red  
• picks up oxygen from the air in your lungs and releases it to cells elsewhere in your body  
• if you don’t make enough hemoglobin, or you make a type that doesn’t carry oxygen well, you will be anemic |
| insulin | a hormone | • tells your cells when to remove sugar from your blood after a meal  
• if you don’t make enough insulin, or if your cells do not respond to it well, the concentration of sugar in your blood will get too high, and you will have diabetes |
| antibody | a defense protein | • fights infections from bacteria and viruses  
• vaccination triggers production of antibodies that will fight a particular kind of infection  
• if you couldn’t make any antibodies, you would have been dead long ago! |
| lactase | an enzyme in your digestive tract | • digests lactose (milk sugar) in milk and dairy products into simpler sugars  
• if you don’t make enough lactase, you get diarrhea when you drink milk or eat ice cream or other dairy products  
• but you can buy lactase pills to swallow before eating dairy products |
| collagen | a structural protein | • provides the tough structural framework of your skin, bones, tendons, and cartilage |
| keratin | a structural protein | • provides the resistant outer layer of your skin and the tough structural material of your hair and nails |
| myosin | a structural protein that is also an enzyme | • uses energy derived from food — and works together with actin — to cause muscles to contract  
• you can increase the myosin content of your muscles with exercise |
How DNA Codes For Proteins

The molecule is the message

Enzymes and other proteins come in many shapes, but in an important respect they are all alike.

Unfold any protein, and you'll find its simply a chain of amino acids.

Let go of the ends, and the protein will be fold itself, owing to the mutual attractions among the components.

Actually, many proteins need help from another "chaperone" protein to fold up.

That is: the sequence determines the structure.

IN VIEW OF THE RELATIONSHIP BETWEEN GENES AND PROTEINS, THIS SUGGESTS THAT THE SEQUENCE OF DNA MUST SOMEHOW PARALLEL OR REFLECT THE SEQUENCE OF THE PROTEIN.

The sequence of base pairs may be thought of as a series of "words" specifying the order of amino acids in each protein.
Why does life have to be so complicated?

To make the translation from DNA "words" to amino acids, some sophisticated molecular machinery comes into play...

A "Messenger molecule" copied from DNA.

A family of "translator" molecules to connect "message" to amino acids.

A large body which holds things in place and helps form the bond between two amino acids.

All three of these agents are partly or entirely made of that other nucleic acid:

RNA—**RIBONUCLEIC ACID**—RESEMBLES DNA: A SUGAR-PHOSPHATE BACKBONE WITH A SERIES OF BASES ATTACHED.

**The Differences:**

Its sugar is RIBOSE, rather than DEOXYRIBOSE; RNA is usually SINGLE-STRANDED; and it is much shorter—50 to 1000 NUCLEOTIDES, compared with a million or more in DNA.

And finally, while the bases A, C, and G are the same as in DNA, RNA has in place of T another base called **URACIL** ("U").

Which, like THYMINE, is complementary to ADENINE:

Now let's see how RNA works!!
Protein synthesis begins when a region of DNA is teased apart and a molecule of RNA is built along one strand by an enzyme called RNA polymerase. This process is called transcription.

It happens as in DNA replication: each base of the RNA is complementary to the corresponding base on the DNA.

This RNA is called the messenger, or mRNA, because it carries the genetic message from the DNA to the protein factory.

The "words" of the message are triplets of bases – A-U-G, A-C-A, etc. The technical name for one of these groups is a codon.
The actual translators of the genetic code are a group of RNA molecules called transfer RNA, or tRNA. Owing to pairing among its bases, tRNA’s twist themselves into this key shape.

The loop end of tRNA has three unpaired bases. This “anticodon” may bind with the complementary codon of mRNA. At the “tail” end of tRNA is a site for attaching a single amino acid.
For each anticodon, there is an enzyme which recognizes it and attaches the appropriate amino acid to its tRNA.

Once they are linked, the anticodon binds to the complementary codon of message.

Schematically, this is the way a string of bases is translated into a sequence of amino acids. However, the cell needs one more piece of equipment to make it work: the ribosome.
HOW PROTEINS ARE MADE

The final ingredient in the protein-making apparatus is an object that holds everything in place.

This is the ribosome, a double ball of about 50 proteins wrapped up with RNA. This RNA is called ribosomal RNA, or rRNA for short.

The ribosome has two slots in which molecules of tRNA can fit snugly.
Now to make a protein:
When the mRNA reads out the DNA sequence,
it enters a sea of ribosomes.

One half at a time, a ribosome binds onto the mRNA.

The binding site is located at or near the codon A-U-G.

Thus, A-U-G is always the first "word" of every message.

A-U-G and the next codon each bond with complementary tRNA's,
which fit into the slots on the ribosome.
Each tRNA carries an amino acid (AA), the first one always being methionine, which goes with AUG.

An enzyme in the ribosome links the two amino acids, and the first tRNA floats away.

The ribosome then moves down three more bases.

Another tRNA and amino acid bind on.

The amino acids are linked; the "empty" tRNA is discarded; and so the ribosome moves along the message, piling up amino acids, which fold themselves into a protein.
QUESTIONS ON “HOW DNA CODES FOR PROTEINS”

AFTER YOU HAVE read about protein synthesis in your excerpt from the Cartoon Guide to Genetics, answer the following questions.

1. What is the relationship between genes and proteins? ____________________________
   _________________________________________________________________________

2. How does RNA differ from DNA? _____________________________________________
   _________________________________________________________________________

3. What is the molecule that carries the information from a gene to the place
   where a protein will be made? _______________________________________________

4. What is the process by which such a molecule is made? _________________________

5. What is the enzyme that mediates the process named above? _____________________

6. What is the structure on which proteins are made? _____________________________

7. How many bases form one “word” of the RNA message? ________________________

8. What is the technical name for such a group of bases found on mRNA? ___________

9. What is another term for protein synthesis? _________________________________

10. What is the group of molecules that translates the genetic code? _______________

11. What is an anticodon? ___________________________________________________

12. At the tail end of each tRNA molecule, an ______________ attaches the appropriate
    ________________________ molecule to the tRNA.

13. What happens when two tRNAs are side by side on a ribosome? _______________
    _________________________________________________________________________

14. The first codon on an mRNA always is _________________________________.

15. This codes for the amino acid called _________________________.

Name __________________________________________________
Date ____________________________ Hour ________________
The Gene Expression Dance

AS YOU HAVE READ in your excerpt from the Cartoon Guide to Genetics, three kinds of RNA molecules cooperate to convert the coded information in DNA (a gene) into a protein with a particular sequence of amino acids.

The process in which the nucleotide sequence of a gene (DNA) is used to specify the amino acid sequence of a protein is called gene expression, and it consists of two major phases: 1) transcription, in which a messenger RNA (mRNA) molecule that is complementary in nucleotide sequence to the gene is synthesized, and 2) translation, in which the message carried by that mRNA molecule is used to synthesize the corresponding protein. In this exercise, your class will perform a simulation of these two processes.

MATERIALS

Your teacher will hand out the gene-expression flash cards that you will use in this dance.

PROCEDURE

Your teacher will instruct you how to perform the dance.
Paper Proteins: Models for Simulating Gene Expression

MATERIALS

For each student or pair of students:
1 set of paper-protein puzzle pieces

PROCEDURE

Follow the directions below to model the processes of transcription and translation, and to make a paper protein.

1. Place the DNA strip on the desk so that the letters read properly for you (fig. 1).

2. Working from left to right, find the mRNA pieces that match the DNA and line them up (fig. 2). What is the process in which an mRNA molecule that is complementary to a DNA molecule is produced?

(Answer question 2 here)

3. Separate the mRNA from the DNA to simulate the mRNA moving out of the nucleus to a ribosome in the cytoplasm of the cell. Leave the mRNA pieces lined up next to one another (fig. 3).

4. Match each of the tRNA pieces to the amino acid piece that fits with it (fig. 4). Lay them out so that all of them are visible.
5. Search for the tRNA that will base pair with the first codon of the mRNA (the one on the left-hand end). Move the tRNA with its attached amino acid into place in the mRNA (fig. 5). Continue with the second codon, and so forth. What is the name for this process in which a protein is produced that has an amino acid sequence specified by an mRNA molecule?

(Answer question 5 here)

6. Now pull the string of six amino acids away from the tRNA (fig. 6). These six amino acids represent a new protein. (However, real proteins always contain many more amino acids than this, sometimes more than a thousand.)

After you have completed these steps, use the model pieces to explain the two component processes of gene expression to another student. Then write out the steps in your own words. You may refer to your notes or a book to check for scientific accuracy.
Using the Genetic Code to Translate an mRNA

AT THE HEART OF the regular and predictable relationship between the sequence of nucleotides in any gene and the sequence of amino acids present in the protein for which that gene is said to “code” is a fixed set of nucleotide-to-amino acid relationships that is known as the genetic code. Just as the Morse code can be printed in the form of a table indicating which letter of the English alphabet is specified by each combination of dots and dashes, the genetic code is usually printed in the form of a table indicating what kind of amino acid is specified by each possible mRNA codon.

To the right is a DNA coding sequence that codes for part of the hemoglobin molecule. Complete the mRNA strand following base-pairing rules. Then use the mRNA Genetic Code Table on the next page to determine the amino acid sequence for which this piece of a gene codes.

<table>
<thead>
<tr>
<th>DNA</th>
<th>mRNA</th>
<th>Amino Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>G</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>G</td>
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<tr>
<td>G</td>
<td>G</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>C</td>
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<td>T</td>
<td>G</td>
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</tr>
<tr>
<td>A</td>
<td>G</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>G</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>A</td>
<td></td>
</tr>
</tbody>
</table>

Congratulations! You have just “synthesized” the first part (12 amino acids) of the protein called β-globin, which is part of the hemoglobin molecules that make your blood red and carry oxygen to cells throughout your body. (Each real β-globin molecule actually consists of a string of 147 amino acids.)
### mRNA GENETIC CODE TABLE

<table>
<thead>
<tr>
<th>1st base</th>
<th>2nd base</th>
<th>3rd base</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>U</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U</td>
<td>UUU = phe</td>
<td>UAU = tyr</td>
</tr>
<tr>
<td></td>
<td>UUC = phe</td>
<td>UAC = tyr</td>
</tr>
<tr>
<td></td>
<td>UUA = leu</td>
<td>UAA = stop</td>
</tr>
<tr>
<td></td>
<td>UUG = leu</td>
<td>UAG = stop</td>
</tr>
<tr>
<td><strong>C</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>CUU = leu</td>
<td>CAU = his</td>
</tr>
<tr>
<td></td>
<td>CUC = leu</td>
<td>CAC = his</td>
</tr>
<tr>
<td></td>
<td>CUA = leu</td>
<td>CAA = gln</td>
</tr>
<tr>
<td></td>
<td>CUG = leu</td>
<td>CAG = gln</td>
</tr>
<tr>
<td><strong>A</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>AUU = ile</td>
<td>AAA = asp</td>
</tr>
<tr>
<td></td>
<td>AUC = ile</td>
<td>AAC = asp</td>
</tr>
<tr>
<td></td>
<td>AUA = ile</td>
<td>AAA = lys</td>
</tr>
<tr>
<td></td>
<td>AUG = met</td>
<td>AAG = lys</td>
</tr>
<tr>
<td><strong>G</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>GUU = val</td>
<td>GAU = asp</td>
</tr>
<tr>
<td></td>
<td>GUC = val</td>
<td>GAC = asp</td>
</tr>
<tr>
<td></td>
<td>GUA = val</td>
<td>GAA = glu</td>
</tr>
<tr>
<td></td>
<td>GUG = val</td>
<td>GAG = glu</td>
</tr>
</tbody>
</table>

### Amino Acid Abbreviations

- ala = alanine
- arg = arginine
- asn = asparagine
- asp = aspartic acid
- cys = cysteine
- gln = glutamine
- glu = glutamic acid
- gly = glycine
- his = histidine
- ile = isoleucine
- leu = leucine
- lys = lysine
- met = methionine
- phe = phenylalanine
- pro = proline
- ser = serine
- thr = threonine
- trp = tryptophan
- tyr = tyrosine
- val = valine
CHAPTER 1
DNA: The Hereditary Molecule

SECTION E
How Does DNA Determine a Trait?
An Introduction to the Connections Between Genes and Visible Traits

BECAUSE EVERY ASPECT OF OUR LIFE depends on proteins, which are encoded by DNA, proteins act as the intermediaries between our DNA and our heritable traits. By now, we are beginning to understand how particular genes determine the amino acid sequences of particular proteins. But how do these proteins determine our various distinctive visible traits?

Most of us have more difficulty understanding this aspect of the gene-to-trait relationship than the DNA-codes-for-protein part. This is not surprising, because most of our obvious traits (such as height, weight, facial features, and skin color) are the results of interactions of many different gene-encoded proteins with one another and with a number of environmental factors. Differences in skin color, for example, are the result of the combined actions of various inherited versions of at least seven different gene-encoded proteins with one another and with the amount of sunshine to which the individual has been exposed. The range of differences that exist in the curliness and color of human hair have an even more complex basis.

To simplify things, the gene-to-trait relationship can be thought of as occurring in steps like this:

- Our genes (which are DNA) specify the structure of our proteins.
- Our proteins, interacting with one another and various environmental factors, determine the chemical and physical properties of each of our cells.
- The combined chemical and physical properties of all of our cells determine the shape, appearance, and behavior of our body as a whole.

In the exercise that follows, we will perform an experiment that illustrates the first two steps of this DNA-to-trait relationship in a very dramatic way. (The third step is omitted, because the organism we will be using for the demonstration is a one-celled organism: a bacterium.) The experiment should also reinforce the idea that each visible trait of an organism is usually the result of several gene-encoded proteins working together. This is because the piece of DNA that we will use to make a bacterium glow in the dark carries seven genes that encode seven different proteins. All seven of these proteins must work together to make a bacterium glow in the dark. If any one of the proteins fails to do its job, no light is generated.
Shine On!

A. How to Read a Micropipettor
   (1000 µl size)

IN MODERN BIOLOGY LABS, it is often necessary to measure extremely small volumes of liquid. The measurements are usually in microliters (µl). One microliter is 1/1,000,000 of a liter. Scientists use instruments called micropipettors to measure these small volumes. The first step in learning to use a micropipettor is to learn how to set it for the exact volume you want to transfer. To do this, you need to look at the window on the micropipettor. The numbers in the window represent the volume of liquid, shown in µl (microliters), that will be measured. The window looks like the ones pictured below. Due to differences in models, your micropipettor may be different than the one pictured.

The numbers in the window will look like these when the micropipettor is set to measure the volumes shown in microliters.

\[
\begin{array}{ccc}
0 & 0 & 1 \\
1 & 5 & 0 \\
0 & 0 & 0
\end{array}
\]

100 µl 500 µl 1000 µl

In the windows below, write the numbers that would indicate that the micropipettor was set properly to measure the given volumes.

\[
\begin{array}{cccc}
\_ & \_ & \_ & \_ \\
\_ & \_ & \_ & \_ \\
\_ & \_ & \_ & \_ \\
150 µl & 980 µl & 240 µl & 556 µl
\end{array}
\]

Now that you have filled in the windows, practice setting a micropipettor to the above volumes. Let your teacher check your work, especially when you set the volume of 556 µl. This is a little tricky.
Micropipetting Practice

MATERIALS

For each group of four students:
1 1000 µl micropipettor
2 sterile large tips
1 marking pen
1 tube of colored water (food coloring)
3 pieces of filter paper or white paper towel
1 container for waste disposal

PROCEDURE

1. Check the size of your micropipettor. It should say either 100-1000 or P1000 on the circle on top of the plunger (fig. 1).

2. Set the numbers in the window to read 015 (fig. 2). This represents 150 microliters. Remember that a microliter is 1/1,000,000 of a liter. Write 015 and your name on the filter paper.

3. Next, carefully place a tip firmly on the end of the micropipettor (fig. 3). Do not touch the pointed end of the tip with your fingers. This is to prevent your samples from becoming contaminated.

4. To take up a volume of liquid (fig. 4):
   a. Depress the plunger to the first stop. You can feel a resistance. Do not push the button down as hard as you can.
   b. Hold the tube and the micropipettor at eye level. Put just the point of the tip into the liquid found in the tube labeled “CW” for colored water.
   c. Slowly release the plunger button and suction up liquid.
   d. Look at the tip to check for bubbles. If you have bubbles, dispense the liquid back into the tube. You will need to start over.
5. To expel the volume of liquid onto a piece of filter paper (fig. 5).
   a. Hold the tip of micropipettor directly above the labeled filter paper.
   b. Slowly push the plunger button all the way down (past the first stop to the second stop). Look at the tip to make sure all of the liquid came out.

6. To eject the tip:
   a. Hold the tip over a waste disposal container.
   b. Push the eject button.

7. Reset the numbers in the window to read 020 (fig. 6). Write 020 and your name on another piece of filter paper. Follow steps 3-5 to transfer this volume of liquid to the filter paper. What volume in \( \mu l \) does 020 represent?

   (Answer question 7 here)

8. Now reset the numbers in the window to read 024 (fig. 7). Write 024 and your name on another piece of filter paper and follow steps 3-6. What volume in \( \mu l \) does 024 represent?

   (Answer question 8 here)
B. Practicing Microbiological Techniques

IN THIS LABORATORY, we will be using the bacteria *E. coli*. To avoid contamination of your experiment with unwanted bacteria, it is necessary to use sterile techniques and work with all reagents carefully. Wipe your work area with bleach before and after your laboratory and dispose of used pipette tips, tubes, etc. in the containers provided by your teacher. Always wash your hands thoroughly after each laboratory exercise.

MATERIALS

For each group of four students:

- 4 pairs of safety goggles
- 1 petri dish with *E. coli*
- 2 inoculating loops
- 1 tube of sterile nutrient broth (labeled “NB”)
- 1 waste container with bleach
- 1 small beaker of 70% alcohol
- 1 petri dish with nutrient agar
- 1 1000 µl micropipettor
- 1 sterile pipette tip
- 1 piece of plastic wrap
- 1 spray bottle of disinfectant
- 1 marking pen

PROCEDURE

1. Put on your safety goggles. Observe your plate of bacteria. To avoid contamination, do not open the lid yet. Notice that the bacteria grow in clumps called **colonies** (fig. 1). Each colony started as one bacterium that then multiplied many times. All of the bacteria in a colony usually are identical. Bacteria grow and divide quite rapidly. Under the best conditions, *E. coli* cells can divide every 20 minutes or so.

2. Transfer a colony of bacteria into the tube of nutrient broth. Follow these steps:

   a. Carefully remove an inoculating loop (fig. 2) from the package. Do not touch the loop to anything.

   b. Carefully tilt the lid of the petri dish containing the bacteria, without moving the lid away from the dish (fig. 3). This protects the agar from contamination with organisms floating in the air.
c. Insert the loop end of your inoculating loop and touch a single colony of the bacteria with the tip (fig. 3). Withdraw the loop and replace the lid.

d. Place the loop containing the bacteria into the tube of nutrient broth. Twist the loop to mix the E. coli with the broth (fig. 4). Check to be certain the bacteria have come off the loop. Remove the loop. Recap the tube. Dispose of the loop in the bleach solution provided at your lab station.

3. Next, you will practice transferring a bacterial suspension from a tube to a petri dish.

a. Label the bottom of a petri dish containing sterile nutrient agar with your name and the date.

b. Set the micropipettor to 100 µl. Using a sterile large tip, transfer from the tube 100 µl of the bacterial suspension that you just made onto the agar in the petri dish (fig. 5). Dispose of the tip in the waste container.

c. Using a new sterile loop, spread the broth containing the bacteria over the surface of the agar. Make a zigzag motion across the agar (fig. 6). Turn the plate 90° and repeat the zigzag motion. Dispose of the loop in the waste container. Put the cover on the plate and seal it with a piece of plastic wrap.

d. Return your plate to your teacher.
C. Part One
Shine On! Engineering Glow-in-the-Dark Bacteria

MATERIALS

For each group of four students:
- 4 pairs of safety goggles
- 1 spray bottle of disinfectant
- 1 cup with ice
- 1 tube of sterile water (labeled “W”)
- 1 marking pen
- 1 tube of plasmid DNA (labeled “DNA”)
- 1 tube of CaCl₂ (Labeled “C”)
- 1 petri dish with E. coli
- 2 sterile inoculating loops
- 1 1000 µl micropipettor

Per class:
- 1 water bath at 42° C

PROCEDURE

1. Put on your safety goggles. Spray and wipe your work area with disinfectant.

2. Get a cup with ice in it. Locate the tube labeled “W.” This tube contains a very small amount of water. Put your initials on the tube. Gently tap the tube on the counter to shake the drop of water to the bottom of the tube. This tube will be your control tube. Place it in your cup of ice (fig. 1).

3. Locate the tube labeled “DNA.” This tube contains the DNA with the genes that will cause your bacteria to glow in the dark. Put your initials on the tube. Place it in your cup of ice (fig. 1).

4. Locate the tube labeled “C.” It contains a chemical called calcium chloride, CaCl₂. Calcium chloride makes bacteria “leaky,” so that big molecules like DNA can get inside the cells. Put your initials on the tube and place it in the ice (fig. 1).

5. Locate the petri dish culture of E. coli. (You may be sharing this with one or more groups.)
6. Use the inoculating loop to transfer a mass of bacteria to the tube labeled “C.” Then use the micropipettor to mix the bacteria with the liquid in tube “C.” To do this:

a. Tilt the lid of the petri dish, holding it over the dish.

b. Pick a colony off the surface of the agar with the loop, then close the dish (fig. 2).

c. Open tube “C,” put the loop with bacteria into the liquid, and gently spin the handle of the inoculating loop to knock off the clump of bacteria (fig. 3). Be sure the bacteria came off the loop! Dispose of the loop.

d. Set your micropipettor to 250 µl.

e. Place a sterile tip on the micropipettor.

f. Pick up the tube that now holds the bacteria and gently pipette the fluid in and out to break up the clump of bacteria (fig. 4). Cap the tube and dispose of the tip.

g. Put the tube with the bacterial mixture into the ice (fig. 5).
7. Add 250 µl of the bacterial suspension you just made to the tube containing water. To do this:

   a. Set your micropipettor to 250 µl.

   b. Place a sterile tip on it.

   c. Transfer 250 µl of the bacterial suspension from tube “C” to tube “W.” Pipette in and out several times to mix (fig. 6).

   d. Place tube “W” on ice.

   e. Dispose of the tip.

8. Repeat the steps in 7 to add 250 µl of the fluid in tube “C” to tube “DNA” (fig. 7).

9. Start timing both tubes (“W” and “DNA”) at this point. Leave them on ice for 15 minutes (fig. 8).
10. At the end of 15 minutes, bring the tubes (still on ice) to the water bath. Check the temperature in the water bath to make sure it is 42°C. Transfer the two tubes from the ice to the water bath (fig. 9). Leave the two tubes in the water bath for exactly 90 seconds.

11. Remove the tubes from the water bath and put them back on ice for one minute (fig. 10). After that, they can be at room temperature.

12. Set the micropipettor for 250 µl. Place a new tip on the micropipettor. Locate the tube marked “NB.” This tube contains nutrient broth and provides nutrients for the bacteria. Add 250 µl from the tube marked “NB” to each of the two tubes (tube “W” and tube “DNA”) (fig. 11). Dispose of the tip.

13. You can stop here and store your tubes in the refrigerator until tomorrow, or you can go on to Part Two. Your teacher will direct you.
C. Part Two
Shine On! Engineering Glow-in-the-Dark Bacteria

1. If you stopped at the end of Part One, obtain your “W” and “DNA” tubes from your teacher. If you did not stop, simply continue.

2. Gently shake or invert each tube (fig. 1).

3. Locate the two petri dishes, one labeled “WATER,” the other labeled “DNA.” Put your group name and hour on the bottom of each dish.

4. Set the micropipettor at 100 µl. Place a new tip on the micropipettor and transfer 100 µl from tube “W” onto the agar in the plate dish marked “WATER” (fig. 2). Dispose of the tip in the waste container.

5. Using a sterile loop, spread the bacteria across the surface of the agar. Remember to zigzag, then rotate the dish and repeat (fig. 3). Dispose of the loop.
6. Place a new tip on the micropipettor and transfer 100 µl from tube DNA onto the plate marked DNA (fig. 4).

7. Using a sterile loop, spread the bacteria (fig. 5) across the surface of the agar. Remember to zigzag, then rotate the dish and repeat. Dispose of the loop. Seal both dishes with plastic wrap and place them where your teacher directs. Incubate at room temperature.
Part One

Pipette 250 µl from tube C into tube W

Pipette 250 µl from tube C into tube DNA

Plasmid with “light switch gene”

Part Two

Pipette 100 µl from tube W onto an agar plate labeled WATER

Pipette 100 µl from tube DNA onto an agar plate labeled DNA

Incubate 24-48 hours at room temperature

STOP POINT

PART TWO

Part Three

Observe

Observe
C. Part Three  
Shine On! Viewing Plates

IT MAY TAKE SEVERAL days before the bacteria that you have genetically engineered begin to glow in the dark. Observe both of your agar plates each day and record what you see on the lines below. Be sure to record when bacterial colonies can first be seen on each plate. Meanwhile, begin filling out the Laboratory Write Up on the next two pages.

Once colonies have appeared on your “DNA” plate, observe both plates each day in a very dark room to see if they have begun to glow. Record the day that this happens. Continue observing the plates daily, and record whether the bacteria are still glowing. When your bacteria have stopped glowing in the dark, ask your teacher for instructions about how to dispose of your plates. Then complete your Laboratory Write Up.

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LABORATORY WRITE UP

1. Purpose: Describe in your own words the reason for performing this experiment.

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2. Background Information: Give information about the idea of adding genes to bacteria in order to change their traits.

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______________________________________________________________________

3. Hypothesis: State the expected outcome of the experiment.

______________________________________________________________________
______________________________________________________________________

4. Independent Variable: State the independent variable.

______________________________________________________________________
______________________________________________________________________

5. Dependent Variable: State the dependent variable.

______________________________________________________________________
______________________________________________________________________

6. Controls: List the controls.

______________________________________________________________________
______________________________________________________________________

7. Procedure: Briefly describe in your own words the steps you took to perform the experiment.

______________________________________________________________________
______________________________________________________________________
8. Data/Observations: Organize your data and observations into a neat, meaningful chart.

9. Conclusions and Recommendations for Future Experiments: Tell what the data mean. Were the results what you expected? Was your hypothesis on target? In addition, write a paragraph telling your ideas for future experiments (how to improve, what other things to try, any mistakes to correct, etc.).
ALTERNATIVE ASSIGNMENT: NEWS ARTICLE

YOU ARE TO WRITE an article that is suitable to be printed in a newspaper and describes the Shine On experiment. Make your article interesting, informative, and scientifically accurate. Include the overall idea of the experiment as well as a brief description of the protocol and a conclusion. Your grammar and spelling need to be correct. The best articles may be submitted to the school newspaper.

NEWS ARTICLE GRADING SHEET

___ Scientific accuracy

___ Complete: Included introduction, brief procedure, description of the plates, conclusion

___ Creativity/Interest

___ Grammar/Spelling

___ Total