Flower Power:
It's in the Genes!

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Contents

Lesson 1: A Bouquet for You!

KEY QUESTION(S):

- What are the parts of a flower?
- Do all flowers have the same number and types of parts?

SCIENCE SUBJECT:

Biology

GRADE AND ABILITY LEVEL:

9-12 Standard or Honors levels

SCIENCE CONCEPTS:

Flower anatomy, plant reproduction

OVERALL TIME ESTIMATE:

45 minutes

LEARNING STYLES:

Visual and kinesthetic

VOCABULARY:

- Petal
- Sepal
- Carpel
- Stamen
- Anther
- Style
- Stigma
- Ovary

LESSON SUMMARY:

Students will use real flowers to identify the parts of the flower. This lesson begins with the thoughts of a bored and stressed biology student at the end of the year. Her homework assignment piques her interest as it is to bring in a flower. This hands-on lab requires students to identify, measure, and count the parts of the flower. It concludes with analysis questions and a short whole-class discussion as a closure activity.

STUDENT LEARNING OBJECTIVES WITH NEXT GENERATION SUNSHINE STATE STANDARDS:

The student will be able to...

1. Identify the parts of the flower (SC.912.L.14.7)
2. Make and document observations (SC.912.N.1.6)
3. Recognize that there are differences between flower species beyond color (SC.912.N.1.6)

MATERIALS (per group of two students):

ESSENTIAL:

- Various flowers of all sizes

- A flower diagram
- A metric ruler
- Student Lab Data sheet

SUPPLEMENTAL:

- Field guide to flowers
- Computer/Internet

BACKGROUND INFORMATION:

Most flowers have male and female parts. The female parts are collectively called the **carpel** and consist of the **stigma**, **style**, and **ovary** (from top to bottom). The male parts are collectively called the **stamen** and consist of the **filaments** that hold up the **anthers**.

The main parts of the flower are:

- **Sepal:** usually green and at the base of the flower. Their function is to protect the flower as it is growing.
- **Petal:** usually the colored part of the flower. Their function is to attract pollinators to them by making nectar or scents of various types. They enclose both the male and the female parts.
- **Stamen:** this male part is usually in multiples around the female part. Its function is to hold up the anthers, which produce and display the pollen. Pollen is the plant's sperm or reproductive cell.
- **Anther:** this male part is at the end of each of the anthers. Its job is to produce pollen for the plant.
- **Filament:** this male part extends upwards to present the anthers to pollinators.
- **Carpel:** this is the female part of the plant. It houses the ovary and ends with the sticky stigma, which pollen sticks to. The pollen then grows down through the style to the carpel where fertilization of the egg by the pollen takes place to produce seeds. The carpel becomes the fruit.
- **Stigma:** this is the end of the female style, and it is sticky so that pollen sticks to it.
- **Style:** this is the female part that ends on the outside with the stigma and has the carpel at the base. It is what the pollen must grow down through in order to fertilize the egg.
- **Ovary:** This is at the base of the style of the carpel and where the egg is housed.

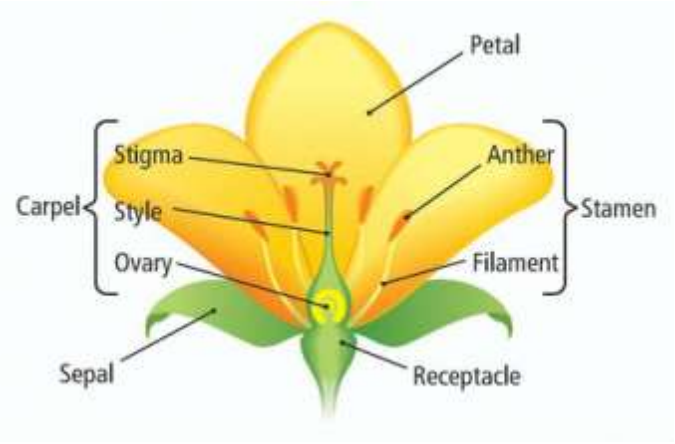


Image from leavingcertbiology.net

The usual arrangement is one style/stigma in the center of the flower and multiple stamen/anthers surrounding it. Flowers can self-pollinate usually, but cross-pollination is more common.

The flower's purpose is to attract pollinators with sweet or not-so-sweet aromas. Some flowers attract night pollinators and smell most pungent in the cooler evenings (ex. Honeysuckle). Other flowers attract daytime pollinators. Color serves as a cue to animals. White flowers are often pollinated by moths while red and orange flowers are often pollinated by hummingbirds. Pollinators span many classes of animals: bats, butterflies, birds. Animals and even humans can be pollinators as they brush up against flowers. Pollination can also be helped with the wind.

ADVANCE PREPARATION:

1. Prepare one copy of the A Bouquet for You! lab sheet per group of two students.
2. The day before this activity is to occur, assign homework to students to bring in a flower the next day.

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

1. Read the following story to the students. It can also be projected in the front of the class.

   Day 1:

   It was getting close to the end of the year at Mandarin High School in Jacksonville, Florida, and Tamara was really tired of studying and doing homework for her classes. It seemed like every day she had three hours of homework, and she couldn't wait for summer to start so she could just relax instead of being so stressed about school all the time. It felt like all she ever did was work, and most of it she forgot anyway because it seemed like everything was just isolated facts. It was Friday afternoon, last period, and she was ready to leave that place. So when her biology teacher said, "Oh, I almost forgot to give you your homework," Tamara actually groaned out loud! But then the teacher surprised the whole class. "Your homework is to bring in a flower on Monday." This seemed weird. Of course, being the overachievers that they are, everyone wanted the specifics on what was required of them so that they could make sure they didn't make a mistake and got all the points for the assignment. People were asking what color, what size, whether they could buy one, did it have to have leaves. But the answer was simple: bring in any flower, regardless of size or attachments. That was easy.

   So Tamara brought in one of her favorite flowers, a bright red hibiscus. She sometimes liked to wear a hibiscus behind her ear because it made her feel like she was from some tropical island. But she didn't usually wear it long! This was also her pet iguana's favorite food. He would see her walk into the room with one of those flowers and immediately jump down from his perch across the top of the cage. He could eat that large flower in just a few bites.

   The teacher told them to put their flowers on one of the lab tables in the back of the room. What a pretty display! And it smelled so good in class today. Tamara recognized some of the flowers: lily, rose, dandelion, daffodil, sunflower, clover, carnation, iris, and buttercup. But there were plenty that she didn't know. She wondered where they came from. And she marveled at the huge array of sizes. Some were the size of her thumbnail!

   The teacher began the lesson by sending Matthew back to choose a flower. She then began to teach the class the parts of the flower: petals, sepals, stamen, stigma, pistil, anthers, and carpel. The only problem was the flower that Johnny chose did not have sepals! But why not? Was it an important part of the flower? Was this just a mutant flower? Were there other flowers with missing parts?

2. Distribute a copy of the A Bouquet for You! lab sheet per group of two students.
3. Tell the students they are going to be doing a lab where they compare various flowers, particularly the reproductive structures. Have students read the introduction silently. Tell them to look at the diagram of the flower on their lab sheet and the labeled part. Hold up a large flower for them to see and go through the parts with them so they know what they are looking for and where to look.
4. Have each pair of students get 5 flowers from the supply table and bring them back to their lab station. Tell them to be very careful as other students will be using these flowers later.
5. **(30-35 minutes)** Allow students to work in pairs with their flowers, completing the lab sheet as they go. They must count and measure various parts as well as answer the analysis questions at the bottom of the lab sheet.
6. Circulate through the room to help students to find the parts and identify their flowers as well as clarify any misunderstandings.

7. **(5-10 minutes)** For closure, have a mini whole-class discussion of what the students found in their observations. Sample questions are:
    a. What are the differences between the flowers and why they might be different?
    b. What could be the benefits or drawbacks of their differences?
    c. How do you think these flowers came to be so different?

ASSESSMENT SUGGESTIONS:

- For Objectives 1, 2, and 3, students will complete the A Bouquet for You! lab sheet. These can be graded.
- For Objective 1, students can be quizzed on the parts of the flower using an unlabeled flower with lines drawn to each part that they need to know.

EXTENSIONS:

ACTIVITIES: Are there other activities you know of from other resources that relate to this lesson?

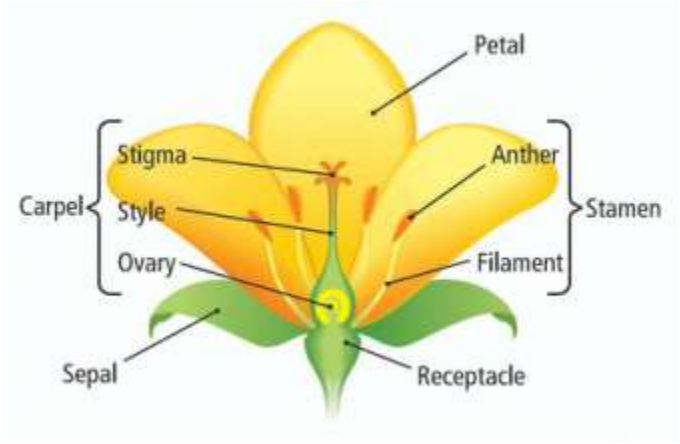
LITERATURE: Are there trade books, novels, journal articles, or other print materials that focus on the same topic(s) as this lesson?

RESOURCES/REFERENCES: List all print and/or web-based references/resources used for either lesson ideas of content background information. Provide complete citations for all references.

TEACHER PAGES: Include any material that teachers will need for the lessons. Jigsaw reading material, student reading material, worksheets for students, and answers for those student worksheets are just a few examples of material that could be included here. Be sure to label any material that is included. End this section with a lined blank page labeled notes, so teachers can jot down any helpful hints for future uses of the lesson.

A Bouquet for You! Lab Sheet **Name ____(STUDENT)_____**

This lab will show the diversity of flowers and help you to learn the names of the parts that make them up. It will also show that some flowers are not just like the diagram and have differences in parts, whether in size or number. Some flowers may even be missing parts.



[Image from leavingcertbiology.net]

1. Choose 5 different flowers from the lab table.
2. Use the diagram as a guide to identify all of the parts of each flower.
3. Make observations and collect data about the flowers. Count and measure the parts as indicated in the chart below. If a part is missing, indicate that with NA.
4. After you have completed making your observations and measurements, answer the questions below the chart.
5. Return your flowers to the main lab table.

	Drawing (Label parts)	**Number of Styles**	**Number of Sepals**	**Number of Anthers**	**Length of Sepal (cm)**	**Length of Anther (cm)**	**Length of Style (cm)**
Flower Name: **Flower Color:**							
Flower Name: **Flower Color:**							

	Drawing (Label parts)	**Number of Styles**	**Number of Sepals**	**Number of Anthers**	**Length of Sepal (cm)**	**Length of Anther (cm)**	**Length of Style (cm)**
Flower Name: **Flower Color:**							
Flower Name: **Flower Color:**							
Flower Name: **Flower Color:**							

Analysis Questions:

1. How did the flowers differ in smell?
2. How did the flowers differ in size?
3. What is the purpose of a flower for the plant?
4. Were any flowers missing parts? Which ones?
5. Why do you think they were missing parts?

6. Why were the sizes and number of anthers and stigmas different? How could that “help” or “hinder” the plant from reproducing?

7. How do plants attract pollinators?

Notes:

Lesson 2: Flower Genes: Easy as ABC!

KEY QUESTION(S):

- What are the genes that control flower development?
- What are the effects of missing genes in flowers?

***SCIENCE SUBJECT:**

Biology

***GRADE AND ABILITY LEVEL:**

9-12 Standard or Honors levels

SCIENCE CONCEPTS:

Mutations, gene expression, phenotype

OVERALL TIME ESTIMATE:

60 minutes

LEARNING STYLES:

Visual and auditory

VOCABULARY:

- Petal
- Sepal
- Carpel
- Stamen
- Anther
- Style
- Stigma
- Ovary
- Whorl
- Pollen
- Pollinator
- Gene
- Phenotype
- Fertilized
- Genome

LESSON SUMMARY:

Students will use a video on YouTube that explains the ABC Model of Flower Development to work through how this gene theory explains the presence or lack of specific flower parts. This lesson begins with the continued thoughts of the biology student from Lesson One. She is trying to make the connection between flower structures and the DNA that makes it up. This self-guided, computer-based tour of the ABC Model requires students to understand the four whorls that make up a standard flower and the gene groups that control their development. They must complete a viewing guide as they watch the video. It concludes with analysis questions and a short whole-class discussion as a closure activity.

STUDENT LEARNING OBJECTIVES WITH NEXT GENERATION SUNSHINE STATE STANDARDS:

The student will be able to...

1. Identify the four whorls that make up a standard flower (SC.912.L.14.7)
2. Describe the ABC Model of Flower Development (SC.912.L.15.15)
3. Match the flower part with the gene(s) (ABC) that codes for it (SC.912.L.16.5)

MATERIALS:

ESSENTIAL:

- Computer
- Flower Genes: Easy as ABC! Viewing Guide (one per student)

SUPPLEMENTAL:

- Add some websites here?

BACKGROUND INFORMATION:

There are a series of master genes that code for flower development, and they are known as MADS box **genes**. These genes are of the classes A, B, and C, and this model was developed in 2000 and is widely used today in many plant genomic studies. The genes code as follows:

- A: **sepals**
- B + C: **stamens**
- A + B: **petals**
- C: **carpel**

Genes A and C negatively regulate each other, which means that neither will be expressed when they are together. This is why they don't code for anything in tandem as B and C do. These genes affect the building of these four plant parts because they code for transcription factors.

Flowers normally consist of four concentric **whorls** of structures. The outermost whorl is the sepals, followed by the petals, then the stamens, with the center being the carpel.

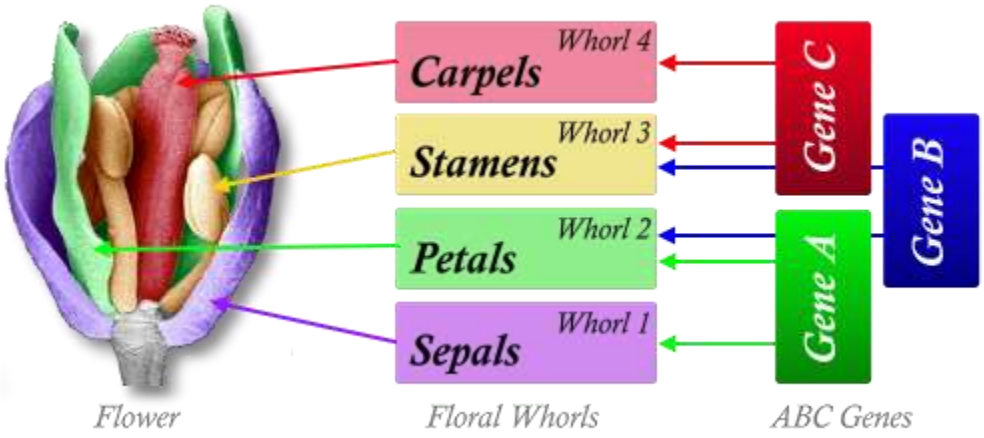


Image from Socratic.org

If there is a **mutation** in one of the gene classes, then the flower will not develop correctly. A mutation is a change in one or more of the bases in the DNA that leads to the translation into different or missing amino acids. The plant will instead make whatever it has the genes for, so essentially another whorl of petals or stamens.

- A plant lacking the A genes will produce carpels in both whorls 1 and 4 (outermost and innermost) and stamens in whorls 2 and 3. It will not produce sepals or petals at all.
- A plant lacking the B genes will produce sepals in the outermost 2 whorls and carpels in whorls 3 and 4 (innermost). Stamens and petals will not be produced.

- A plant lacking the C genes will produce sepals in both whorls 1 and 4 (outermost and innermost) and petals in whorls 2 and 3. It will not produce carpels or stamens.

A plant lacking B or C genes will not produce **pollen** at all since it is lacking the male part (stamens). A plant lacking the C genes cannot be **fertilized**, produce seeds or fruit since it is lacking the female part (carpel). A plant lacking B or C genes is therefore sterile.

Fertilization of a plant involves the male sex cell, the pollen which is given off by the anthers, landing on the sticky stigma of another flower. The pollen grows down through the style to the ovary where the DNA of the pollen enters the egg cell, the female sex cell, in the ovary of the carpel at the flower base. Fertilization occurs and mitosis commences. Seeds are produced and the carpel develops into the fruit.

ADVANCE PREPARATION:

1. Prepare copies of Flower Genes: Easy as ABC! Viewing Guide (one per student)

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

1. **(5 minutes)** Show National Geographic's "Time Lapse: Watch Flowers Bloom Before Your Eyes" at https://www.youtube.com/watch?v=LjCzPp-MK48 (3:24). Tell students that as they watch it, think about how different the flowers are before they open and after they open. Think about the parts of the flower, their purpose in life and the continuation of life.

2. Read the following story to the students. It can also be projected in the front of the class.

   Day Two

   Okay, Tamara thought. That was super easy. The parts of a flower we learned in fifth grade. We did learn some new parts, though, but they were really easy to identify. Easy stuff. But then the teacher started asking us how the flowers came to be the way they were. She reminded us that it is the genes that code for everything, so if a flower did not have sepals, then it did not have the genes that code for them. This was definitely more complicated than flower parts, and a lot of people seemed confused. So she decided to do a mini-review for us. Besides, the biology End-of-Course Exam was coming up, so we needed a review anyway.

   - DNA is how traits are coded.
   - DNA is on chromosomes.
   - Chromosomes are paired structures found in the nucleus of each cell.
   - A pair of chromosomes is called homologous chromosomes.
   - DNA is made of the nucleotides adenine, guanine, cytosine, and thymine (A,G,C,T).
   - A section of DNA is called a gene, and it codes for a protein, which is something the organism needs to build a structure or other required part (i.e., enzymes, collagen, etc.).
   - An organism's genome is the entire set of chromosomes and DNA that is present in every cell (except reproductive cells).

   Okay, so I get it. Plants are made of genes and everything that we see, or don't see, in the plant is because of the genes that are in it. But that seems very complicated! How do genes make flowers look the way they do?

3. Explain to the students that the parts of a flower are built in concentric circles known as **whorls**. These parts are coded for by specific sets of genes on specific chromosomes of plant genomes. These gene sets are known as A, B, and C. They affect each other in various ways, and if one of them is absent, then the flower is "built" differently than normal. The ABC Model of Flower Development explains why there are mutations in flowers. So if you find a flower that is missing sepals, it can be traced back to missing genes. Tell them they are going to work through a

video that discusses this and explains what the results are if a plant is missing any of these gene sets during flower development.

4. Give each student a copy of Flower Genes: Easy as ABC! Viewing Guide.
5. Tell the students to go on a computer and watch the "ABC Model of Flower Development" at the following link: https://www.youtube.com/watch?v=yHYVRJ_ORUU (Behind the Bench, 6:03). Tell them that as they watch this short video, they will probably need to pause and rewind a few times as the material is new and at a high level. In addition, they will need to pause to draw several things and complete the questions on the viewing guide sheet. Tell them that the questions are in the order of the video presentation, but some topics are difficult to understand, so please rewind a few times before you ask.
6. **(25 minutes)** Allow students sufficient time to view the video and complete the viewing guide.
7. **(5-10 minutes)** For closure, have a mini whole-class discussion to check for understanding of the concepts covered in the video. Sample questions are:
    a. How can a missing gene lead to changes in phenotype that you can see?
    b. How can mutations lead to changes in genes and make them nonfunctional?
    c. We've been talking about genes a lot, but how can we SEE genes?

    Share with students that horticulturists (plant scientists) often modify genomes of common garden and landscaping plants so they do not have the C gene. This prevents the plant from making reproductive parts and therefore fruit. Fruit is messy. It drops on the ground, rots, and must be cleaned up. It also takes resources from the plant, so plants without the C genes can grow fuller. In place of the missing parts, the plant makes extra petals, which makes the flowers bigger.

ASSESSMENT SUGGESTIONS:

- For objectives 1, 2, and 3, the viewing guide can be collected and graded.
- For objectives 1, 2, and 3 a quiz can be given to assess learning of these topics.
    - With a diagram of a flower, write in the genes that affect each part (A, B, and/or C)
    - Describe the ABC Model of Flower Development.
    - List and/or identify the 4 whorls on a diagram.

EXTENSIONS:

ACTIVITIES: Are there other activities you know of from other resources that relate to this lesson?

LITERATURE: Are there trade books, novels, journal articles, or other print materials that focus on the same topic(s) as this lesson?

RESOURCES/REFERENCES: List all print and/or web-based references/resources used for either lesson ideas of content background information. Provide complete citations for all references.

TEACHER PAGES:

Flower Genes: Easy as ABC! Worksheet **Name ___(STUDENT)_____**

Watch National Geographic's "Time Lapse: Watch Flowers Bloom Before Your Eyes" (3:24)
https://www.youtube.com/watch?v=LjCzPp-MK48
As you watch it, think about how different the flowers are before they open and after they open. Think about the parts of the flower and their purpose in life and the continuation of life.

The parts of a flower are built in concentric circles known as **whorls**. These parts are coded for by specific sets of genes on specific chromosomes of plant genomes. These gene sets are known as A, B, and C. They affect each other in various ways, and if one of them is absent, then the flower is "built" differently than normal. The ABC Model of Flower Development explains why there are mutations in flowers. So if you find a flower that is missing sepals, it can be traced back to missing genes.

Watch "ABC Model of Flower Development" (Behind the Bench, 6:03) at the following link:
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As you watch this short video, you will probably need to pause and rewind a few times as the material is new and at a high level. In addition, you will need to pause so that you can draw several things and complete the questions below. The questions are in the order of the video presentation. Some topics are difficult to understand, so please rewind a few times before you ask.

Questions to answer as you view the video:

1. What does floral mean?

2. *Arabidopsis* is the name of the plant the scientist is using to draw. Why did he choose this one?

3. Which part is on the outermost whorl?

4. What is the function of the structures in the outermost whorl?

5. What part is in the second whorl?

6. What is the function of the structures in the second whorl?

7. How do pollinators help aid in reproduction of the plant?

8. What part is in the third whorl?

9. Is the third whorl a male or female structure?

10. What do stamens have on them ("possess")?

11. Which part is in the center whorl?

12. Is the part in the center a male or female structure?

13. What are two things that the carpel possesses?

14. Which whorl gives the flower its color?

15. Draw and label the diagram in the video showing the four whorls and what they are.

16. How many gene classes (sets) code for the four different whorls and what are they called?

17. Complete the following chart showing what each class of genes codes for.

Gene Class	Structure It Codes For	Whorl Number
A		
A + B		
B + C		
C		

18. What does it mean that Class A and C genes are "mutually antagonistic?"

19. Why doesn't Gene Class B code for any structure?

20. Complete the following chart by putting in which whorls, if any, the following structures would be found in if the listed gene class were absent. If a structure is not produced, write 'absent' under it.

Missing Gene Class	Stamens	Carpels	Petals	Sepals
A				
B				
C				

21. Can a flower that is missing gene class A or B make petals?

22. Which gene class is missing if a flower does not have sepals?

23. Can a flower that is missing gene class B or C make pollen? Why?

24. Which missing gene class will lead to a flower not being able to make seeds and fruit? Why?

25. Which of the three mutants would result in a sterile plant, meaning it cannot function as a male or a female? Why?

Flower Genes: Easy as ABC! **Name ____(TEACHER)_____**

Watch National Geographic's "Time Lapse: Watch Flowers Bloom Before Your Eyes" (3:24)
https://www.youtube.com/watch?v=LjCzPp-MK48
As you watch it, think about how different the flowers are before they open and after they open. Think about the parts of the flower and their purpose in life and the continuation of life.

The parts of a flower are built in concentric circles known as **whorls**. These parts are coded for by specific sets of genes on specific chromosomes of plant genomes. These gene sets are known as A, B, and C. They affect each other in various ways, and if one of them is absent, then the flower is "built" differently than normal. The ABC Model of Flower Development explains why there are mutations in flowers. So if you find a flower that is missing sepals, it can be traced back to missing genes.

Watch "ABC Model of Flower Development" (Behind the Bench, 6:03) at the following link:
https://www.youtube.com/watch?v=yHYVRJ_ORUU

As you watch this short video, you will probably need to pause and rewind a few times as the material is new and at a high level. In addition, you will need to pause so that you can draw several things and complete the questions below. The questions are in the order of the video presentation. Some topics are difficult to understand, so please rewind a few times before you ask.

Questions to answer as you view the video:

1. What does floral mean? (flower)
2. *Arabidopsis* is the name of the plant the scientist is using to draw. Why did he choose this one? (It is commonly studied)
3. Which part is on the outermost whorl? (sepals)
4. What is the function of the structures in the outermost whorl? (protect the flower while it is developing)
5. What part is in the second whorl? (petals)
6. What is the function of the structures in the second whorl? (attract pollinators)
7. How do pollinators help aid in reproduction of the plant? (they carry the pollen onto other flowers of plants)
8. What part is in the third whorl? (the stamens)
9. Is the third whorl a male or female structure? (male)
10. What do stamens have on them ("possess")? (anther, pollen)

11. Which part is in the center whorl? (carpel)

12. Is the part in the center a male or female structure? (female)

13. What are two things that the carpel possesses? (the ovary and the developing seeds)

14. Which whorl gives the flower its color? (the second one, the petals)

15. Draw and label the diagram in the video showing the four whorls and what they are.

16. How many gene classes (sets) code for the four different whorls and what are they called? (3; A, B, & C)

17. Complete the following chart showing what each class of genes codes for.

Gene Class	Structure It Codes For	Whorl Number
A	sepals	1
A + B	petals	2
B + C	stamens	3
C	carpels	4

18. What does it mean that Class A and C genes are "mutually antagonistic?" (they cancel the expression of each other, so that is why there is a clear separation of A & C genes and they don't get expressed at the same time)

19. Why doesn't Gene Class B code for any structure? (it cannot code for a structure on its own, it must be expressed with A or C)

20. Complete the following chart by putting in which whorls, if any, the following structures would be found in if the listed gene class were absent. If a structure is not produced, write 'absent' under it.

Missing Gene Class	Stamens	Carpels	Petals	Sepals
A	2,3	1,4	Absent	Absent
B	Absent	3,4	Absent	1,2

C	Absent	Absent	2,3	1,4

21. Can a flower that is missing gene class A or B make petals? (no)

22. Which gene class is missing if a flower does not have sepals? (A)

23. Can a flower that is missing gene class B or C make pollen? Why? (no, because it does not make stamens)

24. Which missing gene class will lead to a flower not being able to make seeds and fruit? Why? (C, because the carpel is what becomes these when it is fertilized with pollen)

25. Which of the three mutants would result in a sterile plant, meaning it cannot function as a male or a female? Why? (C, because it would not produce stamens or carpels)

Notes:

Lesson 3: Flower DNA: Deciphering the Order

KEY QUESTION(S):
- How is DNA sequenced?

***SCIENCE SUBJECT:**
Biology, Biotechnology

***GRADE AND ABILITY LEVEL:**
9-12 Honor's Biology

SCIENCE CONCEPTS:
- DNA
- Replication

OVERALL TIME ESTIMATE:
90 minutes

LEARNING STYLES: Visual and auditory

VOCABULARY:
- DNA sequencing
- DNA polymerase
- DNA Primer
- Terminator bases
- Adenine
- Guanine
- Cytosine
- Thymine
- Plasmid
- DNA base
- Denature
- Enzyme
- Electrophoresis
- Fluorescent
- Sanger sequencing
- Nucleotide
- Shotgun sequencing
- Deep sequencing
- Alignment
- Consensus sequence
- PCR
- Base pairing rules

LESSON SUMMARY:
Students will use several videos on YouTube that explain the methods that are used to sequence DNA. This lesson begins with the continued thoughts of the biology student from Lesson One. She is still trying to make the connection between flower structures and the DNA that makes it up. The first video is a short, simple analogy of the Sanger Method of DNA sequencing to give students the overview before going into the specifics of sequencing. The second video contains more specific steps to the Sanger Method, and they will complete a viewing guide with questions to make sure they follow along. The third video goes into

other, more recent DNA sequencing methods, and students will complete a viewing guide as well. It concludes with a short whole-class discussion as a closure activity.

STUDENT LEARNING OBJECTIVES WITH NEXT GENERATION SUNSHINE STATE STANDARDS:

The student will be able to...

1. Describe how the Sanger Method of DNA sequencing is performed (SC.912.L.16.3, SC.912.L.16.12)
2. Describe newer methods of DNA sequencing (SC.912.L.16.3, SC.912.L.16.12)
3. Describe how PCR is used in DNA sequencing (SC.912.L.16.3, SC.912.L.16.12)

MATERIALS:

ESSENTIAL:

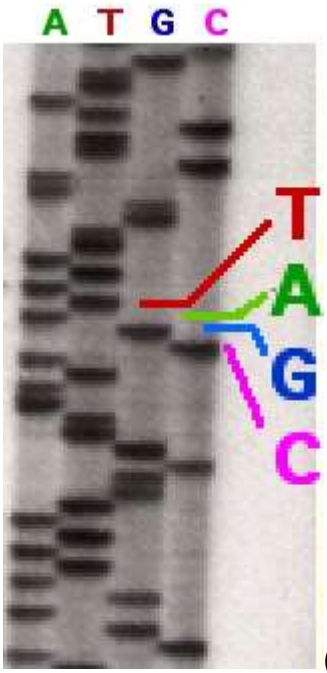
1. Computer
2. Flower DNA: Deciphering the Order Viewing Guide (one per student)
3. DNA Sequence Assembly—Student Worksheet (HHMI) (one per student)

SUPPLEMENTAL:

BACKGROUND INFORMATION:

The **Sanger Method** of **DNA Sequencing** was developed in 1977 by Frederick Sanger and was the most widely used sequencing method for about 40 years. It is the classic method of sequencing and how it all began. But it was slow and expensive because of the multiple copying or PCR steps involved. New methods, such as the NextGen, are larger scale, cheaper, and automated by computers. They sequence DNA by synthesizing a new complementary strand, one base at a time. When it adds a **DNA base**, it gives off a signal which the computer reads. Since we know the complementary strand, we can infer the original strand. An interesting fact is that the bases that are added are tagged with the same **fluorescent** compound (luciferin) that lightning bugs use to make light! Fluorescent compounds give off a glow in ultraviolet light. The Sanger method is still used today to validate other sequencing procedures, for small scale projects, and to get extremely long DNA segments.

The Sanger Method is also known as the chain-termination method since it works by adding chain-terminating fluorescent bases. It requires a single-stranded DNA template. This can be achieved by heating to a high temperature to denature and separate the two DNA strands. A **DNA primer** is required to bind to a known section of the DNA and to get the adding of bases (elongation) started. The bases that are required are normal deoxynucleosidetriphosphates (dNTPs) and modified di-deoxynucleotidetriphosphates (ddNTPs). ddNTPs terminate DNA strand elongation and can be tagged with radioactive or fluorescent labels. Each DNA sample must undergo four separate reactions. Each reaction contains all four of the standard nucleotides (dATP, dGTP, dCTP and dTTP) and the DNA polymerase. One of the four dideoxynucleotides (ddATP, ddGTP, ddCTP, or ddTTP) is added to each reaction while the other added nucleotides are ordinary ones. When a ddNTP is added to the elongating strand, it stops. At this point, the mixtures are heated to denaturing again to separate the elongated fragment from the original template DNA. Then the resulting DNA fragments are separated by size using **gel electrophoresis**. This involves passing an electrical charge through the DNA fragments loaded into a gel medium. Each reaction is run into a separate lane in the gel (lanes A, T, C, G). Shorter fragments will travel further through the gel since they weigh less. The bands can then be seen using UV light, and the sequence can be read right off the gel. The bands in the gel show the terminator bases since they are tagged, so depending on position, you can read the entire sequence.



(Image from Wikipedia)

The sequence can also be read using an optical system that is automated to save time and money.

PCR (Polymerase Chain Reaction) is used to make many copies (thousands to millions) of DNA quickly. It was developed in 1983 by Kary Mullis and is a cheap and reliable way to repeatedly replicate a specific segment of DNA. It is probably the most widely used technique in molecular biology since it is applicable to many fields in biology. It is used regularly in biomedical research, criminal forensics, and molecular archaeology. There are multiple PCR methods, but most use exposing the reactants to cycles of repeated heating and cooling. The heating is used to denature the DNA and separate the two strands. **Primers** (short DNA fragments) that are complementary to the target DNA are used to start the process, and **DNA Polymerase** is the enzyme that puts the correct base in place. Base pairing rules are that **Cytosine** and **Guanine** are **complementary** to each other and pair up, while **Adenine** and **Thymine** are complementary. Once the first DNA is cloned, another series of PCR steps is performed, and this is repeated over and over again. The effect is to exponentially increase the number of copies of the DNA segment as everything "in the pot" is copied.

ADVANCE PREPARATION:

2. Prepare copies of Flower DNA: Deciphering the Order Viewing Guide (one per student)
3. Prepare copies of DNA Sequence Assembly—Student Worksheet (HHMI) (one per student)

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

1. **(10 minutes)** Read the following story to the students. It can also be projected in the front of the class.

   Day 3:

   Jeez! That was intense! My teacher said she learned about that at the University of Florida (Go Gators!) this past summer when she worked with a college researcher in his lab. But why should we have to learn about it? It seems way too high a level for a high school freshman, even if we are in the "smartest" classes. And it is the end of the year. We should be winding down now. But I do get that things are the way they are because of their DNA and how it is coded. Kinda like my name is my name because of the way the letters are arranged.

   Some kids started complaining about how hard it was and how it really didn't have to do with what we HAD to know, so she started backtracking. She reminded us that mutations are changes in the DNA, and

that they could be good, bad, or have no effect on the organism. She also reminded us of the types of mutations that could happen in DNA:

- **Substitution:** one base is changed for another
- **Insertion:** one or more bases is inserted into the DNA strand
- **Deletion:** one or more bases is deleted from a DNA strand
- **Missense:** a change in one base that leads to a different amino acid coded for
- **Nonsense:** a change in one base that signals a STOP
- **Frame-Shift:** an insertion or deletion (unless it is a multiple of 3) will cause everything after that point to move forward, which changes all of the amino acids that are coded for
- **Silent:** A mutation that doesn't change which amino acid is coded for

So with all that can go wrong with DNA, it's no wonder there are such weird things in nature. But how do they figure that stuff out? How do they KNOW what the DNA looks like or what the sequence of A, T, C, and G's is? I raised my hand and asked my teacher that question. She said that there are several different ways to do it, but they all involve using a little bit of logic and puzzle skills.

She said that she learned a little about it at UF. Apparently, they can take DNA and blast it with sound waves that will break it up into random small pieces, kindof like taking a done puzzle and randomly breaking it so that there are chunks of done sections. This can be done several times with a species' DNA, then you sequence those short pieces of DNA by sending it off to a company. It is very expensive to do, and they are limited to the size of the DNA strand they can sequence, so that is why it has to be chopped up.

2. **(5 minutes)** Show HHMI's "Sanger Method of DNA Sequencing" (:51) for an overview of how DNA is sequenced at https://www.hhmi.org/biointeractive/sanger-method-dna-sequencing. Tell students that as they watch it, think about how DNA is being deciphered.

3. Tell students that the Sanger Method of DNA Sequencing was developed in 1977 by Frederick Sanger and was the most widely used sequencing method for about 40 years. It is the classic method of sequencing and how it all began.

4. **(20 minutes)** Give each student a copy of the Flower DNA: Deciphering the Order Viewing Guide. Tell them to watch "DNA Sequencing – 3D" (YourGenome, 4:54) at the following link: https://www.youtube.com/watch?v=ONGdehkB8jU. Tell them the video is more specific on the Sanger method of DNA sequencing, so they will need to pay close attention to it. As they watch it, they should follow along on the viewing guide and answer the questions about how DNA is sequenced. Allow them time to view the video and answer the questions.

5. **(5 minutes)** Bring the students back together and tell them that the Sanger Method was slow and expensive because of the multiple copying or PCR steps involved. New methods, such as the NextGen, are larger scale, cheaper, and automated by computers. They sequence DNA by synthesizing a new complementary strand, one base at a time. When it adds a base, it gives off a signal which the computer reads. Since we know the complementary strand, we can infer the original strand. An interesting fact is that the bases that are added are tagged with the same fluorescent compound that lightning bugs use to make light! The Sanger method is still used today to validate other sequencing procedures, for small scale projects, and to get extremely long DNA segments.

6. Give each student a copy of the DNA Sequence Assembly—Student Worksheet (HHMI).

7. **(5 minutes)** Tell students that the next video is actually a PowerPoint, but it has video clips embedded in it which come from HHMI's holiday lecture series of "Viral Outbreak: The Science of Emerging Disease." It

goes into more detail on the different kinds of DNA sequencing that can be done and why each technique is helpful to the overall picture or sequencing of the genome. Tell them that as they go through the slides and watch the short video clips, follow along and answer the questions on their worksheet. Make sure they view all of the embedded videos in order to understand and answer all of the questions (slides 1,2,6,17,20, and 26).

8. Tell students to view HHMI's BioInteractive: DNA Sequence Assembly slide presentation at https://www.hhmi.org/biointeractive/dna-sequence-assembly. Tell them to complete the viewing guide questions that go along with it.
9. **(30 minutes)** Allow students sufficient time to view the slides and video clips and complete the viewing guide.

10. **(5-10 minutes)** For closure, have a mini whole-class discussion to check for understanding of the concepts covered in the videos. Sample questions are:
    d. How does a computer "read" what a base is?
    e. How do we make bases "visible" to the computer?
    f. What other biotechnology process is required to do Sanger Method?
    g. How does PCR fit into the role of DNA sequencing?

ASSESSMENT SUGGESTIONS:

- For objectives 1, 2, and 3, the viewing guides can be collected and graded.
- For objectives 1, 2, and 3 a quiz can be given to assess learning of these topics.
    - Describe how the Sanger Method is used to sequence DNA
    - Describe how one of the newer methods of DNA sequencing differs from the Sanger Method
    - Describe the role that PCR plays in DNA sequencing

EXTENSIONS:

ACTIVITIES: Are there other activities you know of from other resources that relate to this lesson?

LITERATURE: Are there trade books, novels, journal articles, or other print materials that focus on the same topic(s) as this lesson?

RESOURCES/REFERENCES: List all print and/or web-based references/resources used for either lesson ideas of content background information. Provide complete citations for all references.

TEACHER PAGES:

Flower DNA: Deciphering the Order Viewing Guide

Name_____(Student)_____

Watch "DNA Sequencing – 3D" (YourGenome, 4:54) at the following link:
https://www.youtube.com/watch?v=ONGdehkB8jU

This video is more specific, so you will need to pay close attention to it. As you watch it, answer the following questions about how DNA is sequenced.

1. What is the cut up DNA put into?

2. Why is the plasmid put into a bacterial cell?

3. What are the four things that are added to the cut up DNA when it is put into the plate wells?

-
-
-
-

4. Why is the DNA heated to 96 °C?

5. What happens when the temperature is lowered to 50 °C?

8. Which enzyme attaches to the primer when the temperature is raised to 60°C?

7. This enzyme adds bases to the DNA strand until what is randomly added?

8. Why is the DNA then heated to 96°C again?

9. What determines the length of the DNA fragment?

10. What is the name of the process that separates the DNA fragments by length?

11. How can a capillary tube with gel separate the DNA fragments by length?

12. What "reads" the color of the terminator base?

13. How does this method tell you what the sequence of DNA is?

Flower DNA: Deciphering the Order Viewing Guide

Name_____(Teacher)_____

Watch "DNA Sequencing – 3D" (YourGenome, 4:54) at the following link:
https://www.youtube.com/watch?v=ONGdehkB8jU

This video is more specific, so you will need to pay close attention to it. As you watch it, answer the following questions about how DNA is sequenced.

1. What is the cut up DNA put into? (plasmid DNA)
2. Why is the plasmid put into a bacterial cell? (so that it can multiply over and over again)
3. What are the four things that are added to the cut up DNA when it is put into the plate wells?
    - Free DNA bases
    - DNA primers
    - DNA Polymerase
    - Terminator bases
4. Why is the DNA heated to 96 °C? (to separate the two DNA strands, or denature it)
5. What happens when the temperature is lowered to 50 °C? (the primer joins to the plasmid DNA)
6. Which enzyme attaches to the primer when the temperature is raised to 60°C? (DNA Polymerase)
7. This enzyme adds bases to the DNA strand until what is randomly added? (a terminator base, which signals for it to stop adding bases)
8. Why is the DNA then heated to 96°C again? (it causes the DNA to separate into two strands, which releases the short segment that was just made)
9. What determines the length of the DNA fragment? (when the terminator got added)
10. What is the name of the process that separates the DNA fragments by length? (electrophoresis)
11. How can a capillary tube with gel separate the DNA fragments by length? (a charge is applied to it, and the negatively-charged DNA moves through it. Shorter fragments travel further, so you get the fragments sorted by size, or base-pair lengths)
12. What "reads" the color of the terminator base? (a laser reads the fluorescent color)
13. How does this method tell you what the sequence of DNA is? (the laser reads the color of the last terminator base of each DNA fragment. Since they are read in order of shortest to longest, and each color is a different base (A,T,C,G), the sequence can be determined.)

Howard Hughes Medical Institute
2010 Holiday Lectures on Science

DNA Sequence Assembly—Student Worksheet

About This Worksheet

This worksheet complements the Click and Learn "DNA Sequence Assembly" developed in conjunction with the 2010 Holiday Lectures on Science, "Viral Outbreak: The Science of Emerging Disease."

Author: Ann Brokaw, Rocky River High School, Rocky River, OH

Answer the following questions as you proceed through the slides.

1. Read the information on slide 1 and watch the video clip. Explain what the amount of DNA sequence that can be generated for a dollar reveals about sequencing technology?

_____

_____

_____

2. Summarize how *Sanger sequencing* works.

_____

_____

_____

_____

3. What is the upper limit for the number of nucleotides that can be read using Sanger sequencing? Explain why this is the limit.

_____

_____

4. Read slide 4 and provide an example of an *overlap sequence* in the space below.

5. Using your own words, describe *shotgun sequencing*.

6. Describe *deep sequencing*.

7. Read slides 7 through 16 and answer the following questions as you proceed through the slides.
   a. How is overlapping text used in this example?

   b. Explain in your own words what it means to "align" text or sequence. (You may use an example if it helps.)

   c. How can discrepancies be reconciled?

   d. What are the first eight words of the sample assembly on slide 16?

8. What is a major challenge in deep sequencing?

9. Read slides 18 through 27 and answer the following questions as you proceed through the slides. Be sure to watch the embedded video clips also.
   a. What is a *consensus sequence*?

b. How are gaps in a sequence filled in? Explain your answer. (Be sure to include the terms "PCR" and "Sanger sequencing" in your explanation.)

_____

_____

_____

c. Make a list of the technologies used to sequence the genome of a newly discovered virus.

_____

_____

_____

10. More and more research labs are becoming "interdisciplinary." In your own words, explain why it is important to have computer scientists who understand biology and biologists who understand computer science.

_____

_____

_____

_____

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Notes:

Lesson 4: Sequencing DNA II (hands-on)

KEY QUESTION(S):

How does cycle sequencing work?

How can scientists use problem solving and biotechnology techniques to sequence DNA?

Lesson Summary:

Students will work through the Cycle Sequencing animation (dnai.org) while answering questions and labeling diagrams.

- They will then do a paper activity to simulate the steps of Cycle Sequencing.
- All DNA fragments will be organized on the board as an electrophoresis apparatus in the order they would run through the chamber.

Finally, they will use colored pencils to represent the fluorescent tags and infer the DNA sequence

Sequencing Lesson Outline

DNA www.dnai.org

Name: Date:

Sequencing

Materials

- Scissors
- clear cellophane tape
- 4 paper cups
- 4 colored pencils (red, blue, green and yellow)
- 1 copy of nucleotides (A, T, C, G) and dideoxynucleotides (ddA, ddT, ddC, ddG) sheet
- 1 copy of the DNA template and primer sheet
- a computer with Internet access

Procedure

1. Cut out the following:
   - 20 of each free nucleotide (A, T, C, G) = 80 dNTPs;
   - 5 of each dideoxynucleotide (ddA, ddT, ddC, ddG) = 20 ddNTPs;
   - 1 template DNA (10 nucleotides long); and
   - 5 primers (5 nucleotides long).
2. Label each of the four paper cups: A, T, C, and G.
3. Place the dideoxynucleotides (ddNTPs) and the nucleotides (dNTPs) into the appropriate cups. For example, the A's and ddA's should be mixed together in cup "A."
4. Go to www.dnai.org > **Manipulation > Techniques > sorting and sequencing.**

   Click on the 2-D animation called "Cycle sequencing." This animation shows how a section of DNA is sequenced.
5. Work through this animation to find out the reagents required to perform a cycle sequencing reaction. (Hint: you'll need to hit "continue" three times to see all the reagents).

   You should have paper versions of most of these reagents in front of you.

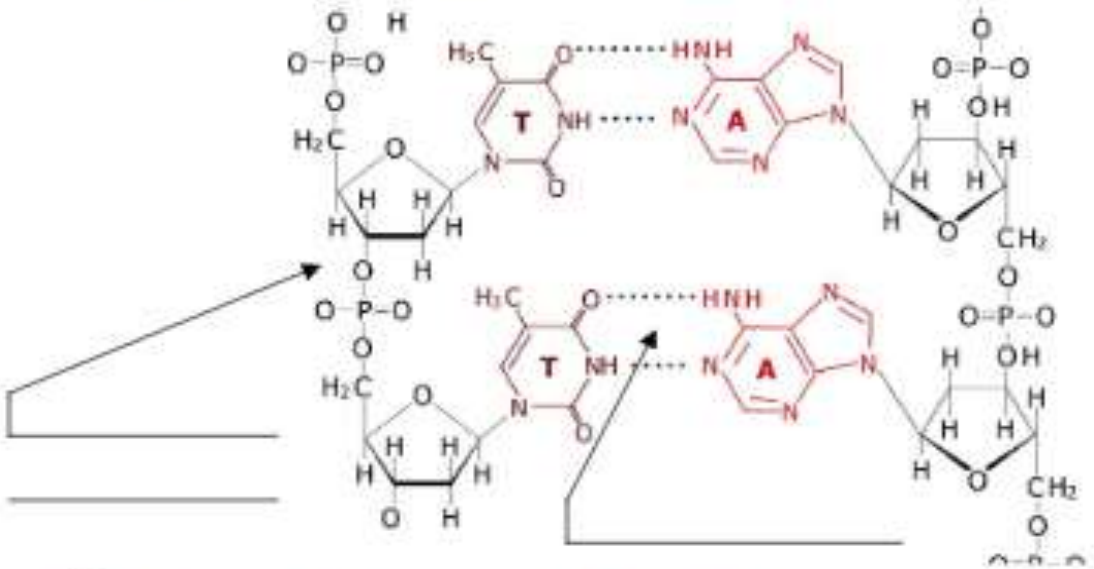
   Which reagent don't you have? _____

   You will act out the part of the missing reagent during this exercise.

The sequencing reaction

In this "reaction," you will be simulating making and breaking two types of bonds between the nucleotides.

On the diagram below, label the chemical bonds (hydrogen or phosphodiester) that hold the molecules in DNA together.



6. Each cycle of the sequencing reaction has distinct three steps, each performed at a different temperature. What are those three temperatures? Briefly state which action is performed at each temperature.

First Temperature: _____ Action: _____

Second Temperature: _____ Action: _____

Third Temperature: _____ Action: _____

7. Perform one cycle of the sequencing reaction. All three steps make one cycle.

Use the animation and the information below (Steps I, II, and III) as a guide to modeling this process with your paper cutouts.

Name: Date:

Step I. Denaturing

Use the scissors to cut the double-stranded template DNA along the dotted line.

What causes the DNA to denature in a sequencing reaction?

_____

Step II. Annealing

Line up one of the primers with its complementary sequence on a strand of the template DNA.

What type of bonds form between the complementary bases?

_____

Step III. Extending

The enzyme DNA polymerase adds complementary nucleotides to a growing strand of a partially double-stranded DNA molecule in the 5' to 3' direction. Here, you will mimic the action of that enzyme.

A. Choosing the appropriate nucleotide

Start at the end of the double-stranded region (where the primer is now bound) and read the next base on the single stranded DNA template.

Decide which of the four nucleotides is complementary to this base.

B. Forming a phosphodiester bond

Shake the cup labeled with this letter and randomly pick out a slip of paper.
Like DNA polymerase, you shouldn't be able to distinguish between free nucleotides (dNTPs) and dideoxynucleotides (ddNTPs) before you pull them out of the cup.

Place your new nucleotide at the end of your growing strand. Eventually, you will use a piece of scotch tape to attach the new slip of paper, simulating a phosphodiester bond.

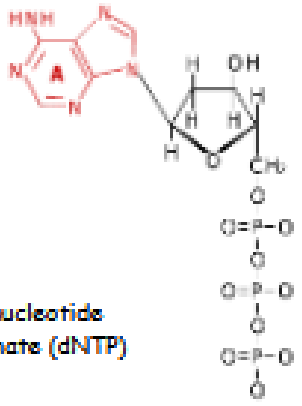
Was the nucleotide you just added a free nucleotide or a dideoxynucleotide?

Name: Date:

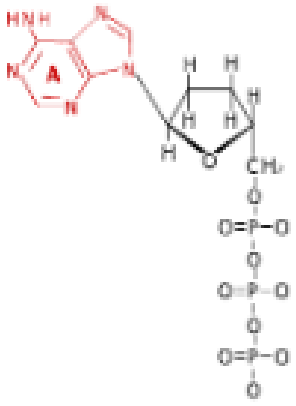
Look at the structures of a free nucleotide (also called deoxynucleotide triphosphates or dNTP) and a dideoxynucleotide (also called dideoxynucleotide triphosphate or ddNTP) below.

Circle the difference(s) between the structures.

Direction of DNA synthesis



A deoxynucleotide triphosphate (dNTP)



A dideoxynucleotide triphosphate (ddNTP)

Why do you think a ddNTP would be unable to form a phosphodiester bond?

_____

C. Determine whether or not to terminate the cycle

If you added a free nucleotide (A, C, G, or T), then DNA polymerase will continue to synthesize the growing strand of DNA. Repeat Step III: Extending.

If you are able to complete the strand before encountering a ddNTP, just empty the pieces back into the cups and start step III again.

If you added a dideoxynucleotide (ddA, ddC, ddG, or ddT), the synthesis of the new strand has ceased. The missing hydroxyl group on a ddNTP will not allow the DNA polymerase to add a new nucleotide to the growing strand.

Use pieces of scotch tape to stick the primer and nucleotide(s) together, and put the strand you made aside.

8. Perform another cycle.

Step I: Denaturing	Begin again with your original template.
Step II: Annealing	Use a new primer to bind to the single stranded template DNA.
Step III: Extending	Follow the same decision process, adding free nucleotides until you encounter a ddNTP that stops the synthesis.

9. Complete a total of five cycles. Follow the above steps (I, II, and III) until all five primers have had new nucleotides added to them (taped on). Other groups in your class will also do five cycles each, making a total of approximately 25 cycles. Scientists typically use 25 cycles to sequence a segment of DNA.

10. Place all the different size fragments of DNA produced into a single container for the class.

Sorting the pieces

11. As a class, perform an electrophoresis that models the workings of automated sequencing machine. Electrophoresis sorts different sized fragments of DNA.

    To develop a better understanding of how electrophoresis works, go to www.dnai.org > **Manipulation > Techniques > sorting and sequencing.**

    Click on the *Gel Electrophoresis* 2-D animation.

12. Use the blackboard or an empty section of wall to simulate the electrophoresis process.

    The top of the board = the positive pole of an electrophoresis setup.
    The bottom of the board = the negative pole.

    In a sequencer, all of the fragments produced by the 25 cycles are run in a single lane.

    Organize the fragments on the board (use masking tape to hold them up) in the order that they would run through the gel in a DNA sequencer.

    (Hint: Smaller fragments will move through the gel more quickly than longer fragments. If there are multiple fragments of the same size, place the same size fragments on top of each other. In a real gel there are thousands of fragments of the same size.)

Seeing the sequence

13. In a sequencing reaction, the dideoxynucleotides (ddA, ddT, ddC, and ddG) are labeled with four different-colored fluorescent dyes.

    Use the "Cycle Sequencing" animation to learn which colors represent each nucleotide.

    List the colors that correspond with each dideoxynucleotide.

Dideoxynucleotide	Color
ddG	_____
ddA	_____
ddT	_____
ddC	_____

14. Imagine that you are the laser capable of distinguishing between the different wavelengths of light (colors) for each of the four different nucleotides. You will always read only the dideoxynucleotide (the last one on the fragment).

    Create a graph that shows how your fragments would read. (The computer attached to an automated DNA sequencer usually performs this task.) Remember the smallest fragments will run past the laser first; so read the fragments from smallest to largest. Draw a peak in the color that corresponds with that letter along the timeline below.

    For example, if the dideoxynucleotide on the shortest fragment is ddA, then draw a peak from the "NORMAL" line at the bottom to the "PEAK" line at the top at point "1" in ddA's color (green).

    Use a different colored pencil for each letter and work on only one letter at a time.

    0 1 2 3 4 5

    PEAK_____

    NORMAL_____

    0 1 2 3 4 5

    The graph you have drawn is called an electropherogram.

    Using the electropherogram, you can determine the sequence of your template DNA.

15. Based upon your sequencing data above, what is the sequence?

    _____

16. If your template strand is 10 nucleotides long, how long should your sequence be?

17. Why is your final sequence different from your original template sequence?

Lesson 5: Fish Protein Fingerprinting

Key Questions

- **How is protein analysis performed?**
- **How does gel electrophoresis aid in protein analysis?**
- **What information about the evolution of a species can we get from protein analysis?**

Lesson Summary

Students will use Carolina Biological Supply Company's Fish Protein Fingerprinting on Polyacrylamide Gels Kit to:

- **Explore the evolution of fish species through protein analysis.**
- **Perform gel electrophoresis on extracted muscle protein mixtures from 7 different types of fish.**
- **Compare the protein fingerprints from the 7 different types of fish, hypothesize on the degree of relatedness of the fish, and compare their ideas to a standard evolutionary tree of fish.**