

DNA Replication Activity Guide

Student Handout

Deoxyribonucleic Acid (DNA)



Engaging in a Discussion of DNA

1. List at least three reasons why a cell must undergo division.

2. Imagine you are cutting a bagel (one of the most common household injuries) and you get a cut. The cut heals. How do the new cells compare to the original (pre-cut) cells?

3. How does your body ensure that the new cells are the same?

4. How does DNA get into the new cells?

Introduction

No molecular structure has gained more world-wide recognition than the DNA double helix. The famous *Nature* paper written by James Watson and Francis Crick in 1953 entitled, *Molecular Structure of Nucleic Acids*, ends with, "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material." Since then, many scientists have focused on researching the mechanism of DNA replication.

DNA Replication Continued

Modeling DNA Replication Activity

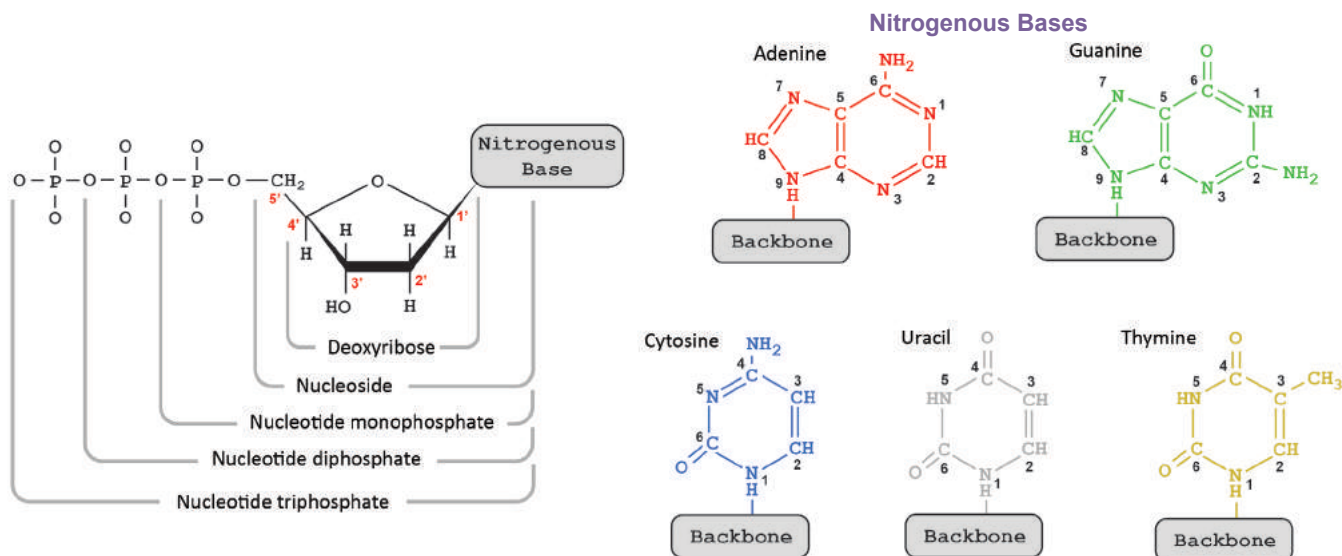
In this lesson you will learn how a copy of DNA is replicated for each cell. You will model a 2D representation of DNA replication using the foam nucleotide pieces.

Assemble the non-template strand of the DNA sequence according to the pattern shown below.



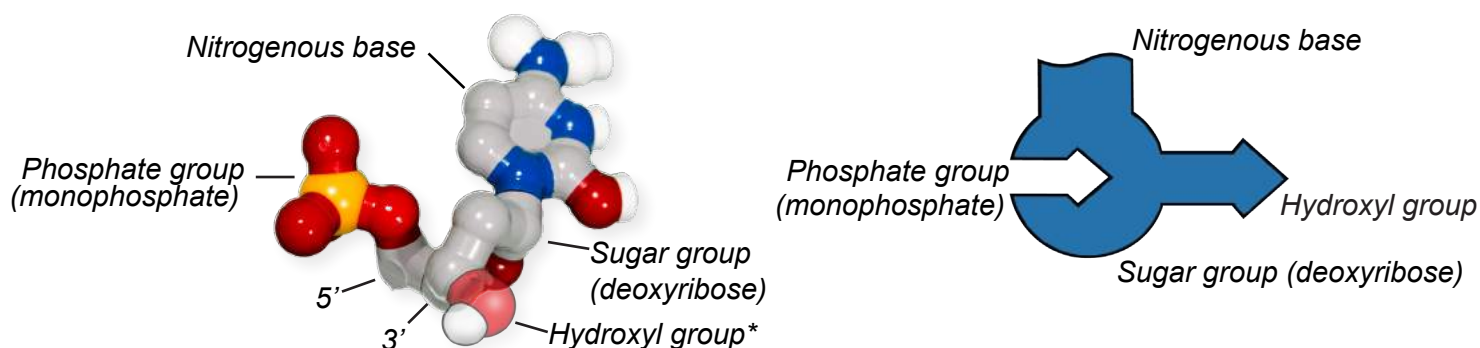
Pair the nucleotides of the template strand to the non-template strand of DNA you constructed, to create a double-stranded DNA model.

5. Record the template strand bases in the blank spaces provided above.
6. Examine the strands of DNA. What do you observe about the **arrow** ends of the model?
- _____
7. What does the arrow indicate?



DNA Replication Continued

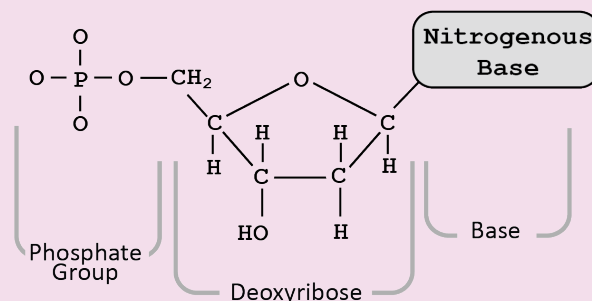
Compare the foam nucleotide to the plastic model on left and chemical drawings on the previous page.



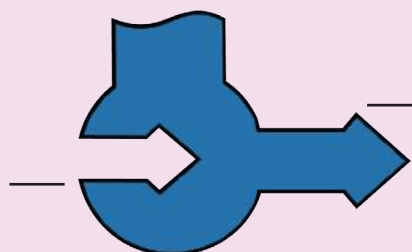
*The hydroxyl group (OH) shown in the above illustration has been added to the photo of the nucleotide, since this particular model of a nucleotide doesn't show the hydroxyl group.

Examine the diagrams to the right.

8. Circle and label the 3' carbon and the 5' carbon in the DNA nucleotide shown in the diagram to the right. **Primes are used in numbering carbons on the sugar (deoxyribose) portion of the nucleotide to distinguish them from the nitrogenous base carbons.**

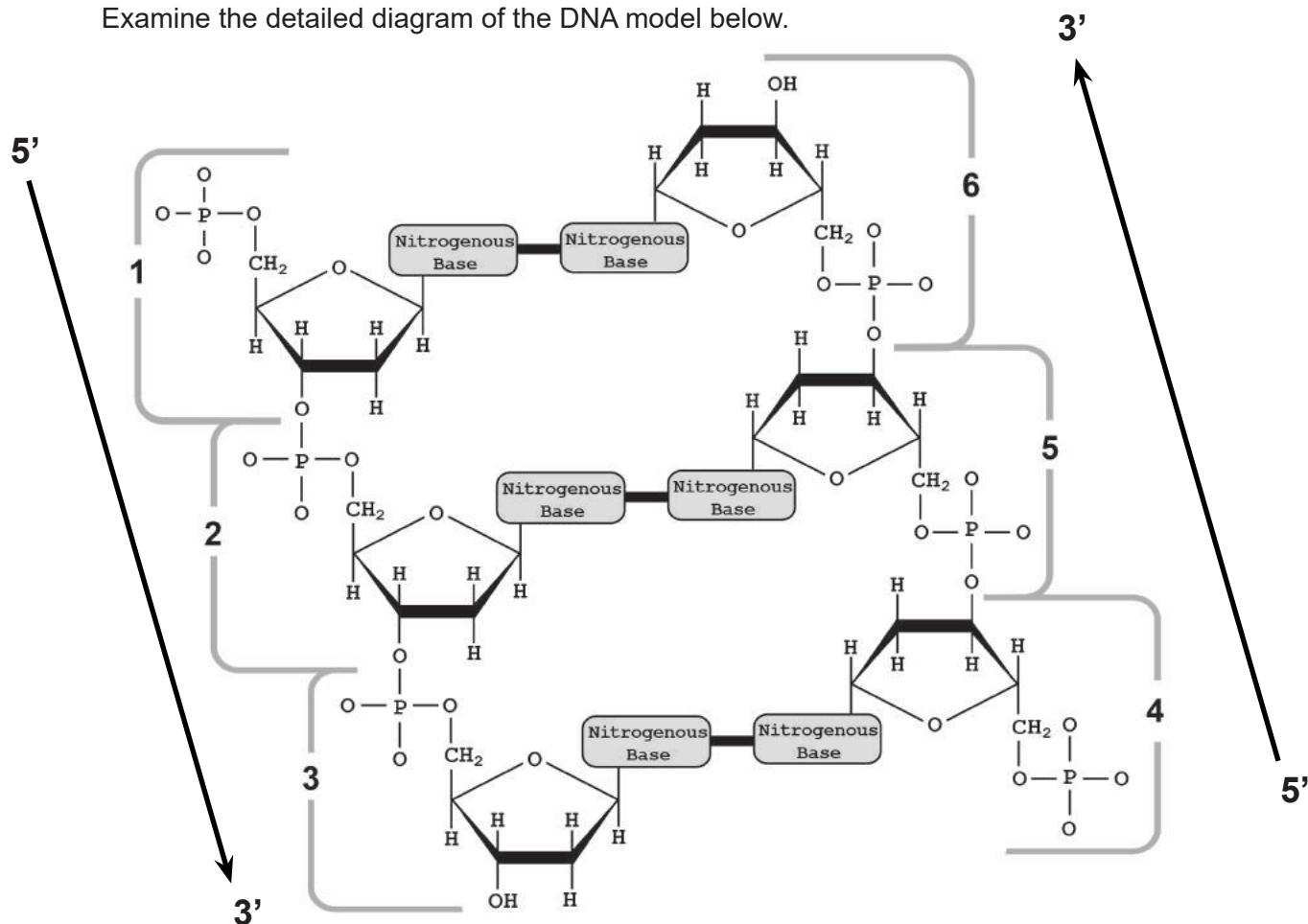


9. In the image to the right, label the locations of the 3' and 5' carbons.
10. How are the 3' and 5' carbons oriented in the strands of the DNA molecule you assembled?



DNA Replication Continued

Examine the detailed diagram of the DNA model below.

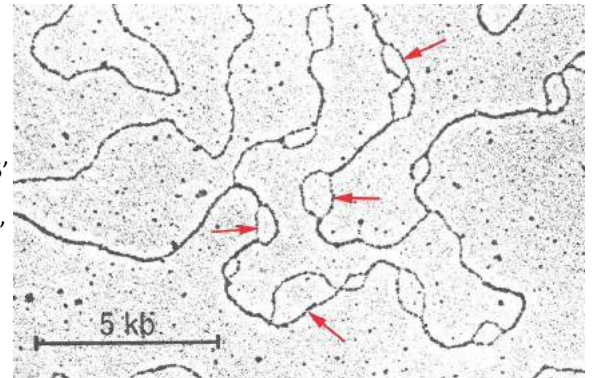
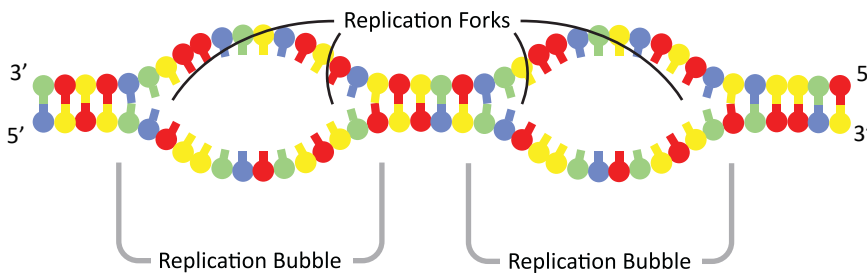


Double-stranded DNA is composed of **two anti-parallel strands**! Each DNA strand has **directionality**. The two sugar-phosphate backbones run in opposite 5' → 3' directions from each other. It is important to keep this directionality in mind as you model the process of DNA replication.

11. Find, circle and label the 5' carbon of nucleotide 1 and nucleotide 4 in the diagram above.
12. Find, circle and label the 3' carbon of nucleotide 3 and nucleotide 6 in the diagram above.
13. What group is attached to the 3' carbon? What group is attached to the 5' carbon?

DNA Replication Continued

DNA replication begins at specific sites called **origins of replication**. A eukaryotic chromosome may have hundreds or even a few thousand replication origins. Proteins that start DNA replication attach to the DNA and separate the two strands, creating a replication **bubble**. At each end of the **replication bubble** is a Y-shaped region where the parental strands of DNA are being unwound. This region is referred to as the **replication fork**.



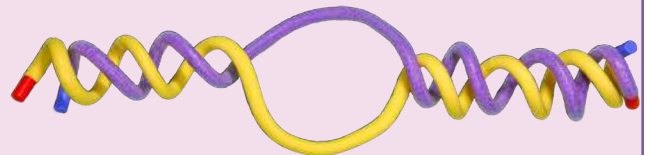
→ Replication bubble

Watch your teacher create a model of a DNA replication bubble using two mini toober like the ones shown here.



14. Identify and label the **replication bubble** and **replication forks** in the model to the right.

15. Looking at the mini toober model, what do you think might be the first step of replication?

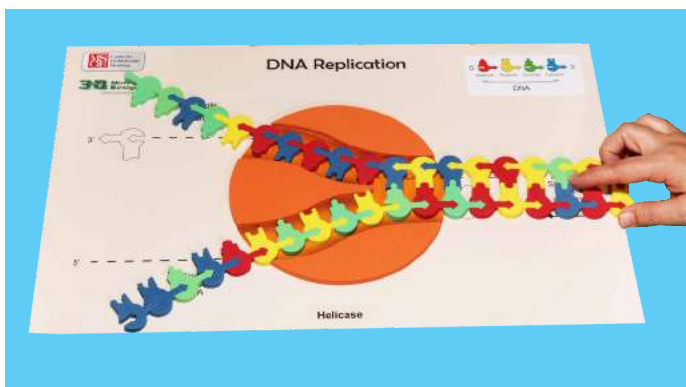


DNA Replication Continued

16. Nucleotides are added at an approximate rate of 50 nucleotides per second in eukaryotic cells. The human genome contains 6.4 billion nucleotides (3.2 billion base pairs), which must be copied. Calculate the length of time in days that it would take to copy the human genome. Show all calculations including units.

17. Why do you think multiple replication bubbles form during the process of DNA replication?

Begin the process of DNA replication by feeding the strands of the constructed DNA into the **helicase** enzyme on the replication mat. Be sure to position the 5' and 3' ends of the DNA appropriately as you place the DNA on the mat. Continue feeding the DNA through the enzyme until you have 11 bases emerging from the helicase. Notice that helicase moves **into** the replication fork, not away from it.



18. What does the helicase appear to be doing?

19. Identify which type of bond is broken. _____

20. Why is the helicase able to break these bonds?

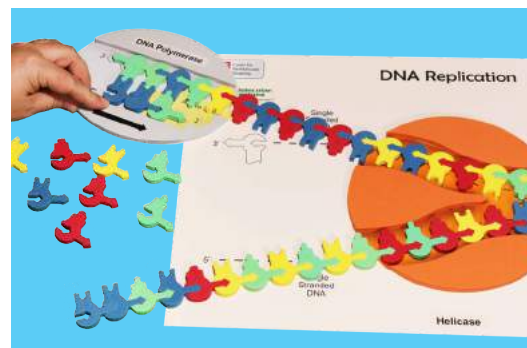
DNA Replication Continued

Note: Replication occurs on both sides of the replication fork simultaneously. For simplicity and clarification you will simulate replication on one side of the fork at a time.

Continuous Replication

The **DNA polymerase** enzyme catalyzes the synthesis of new DNA by adding nucleotides to a preexisting chain. **New DNA can elongate only in the 5'→3' direction.** The DNA strand that is made continuously is referred to as the **leading strand**.

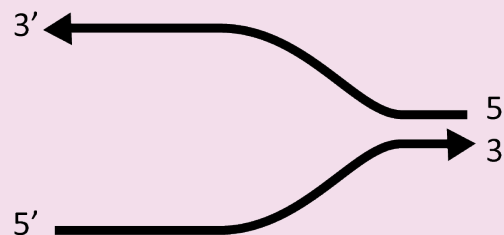
Simulate replication in the **leading strand** by placing one DNA polymerase at the **point of origin** and adding nucleotides in the active site to the parent strand. Continue adding nucleotides as you move the DNA polymerase until you reach the fork.



21. As a new nucleotide is added to the growing DNA strand, which part of the new nucleotide forms a bond with the 3' OH group? _____

22. Sketch a helicase on the diagram to the right and indicate the directionality of the newly replicated **leading strand** of DNA.

Note: The 3' OH group is essential for adding a new nucleotide to the growing DNA strand. If this group is not present – for example, if there is a 3' H instead of a 3' OH – then DNA synthesis cannot continue. This is the basis for the development of the Sanger sequencing method, which is used in determining the sequence of nucleotides.



23. Will you be able to synthesize the other strand of DNA in a continuous manner when using the model? Explain why or why not.

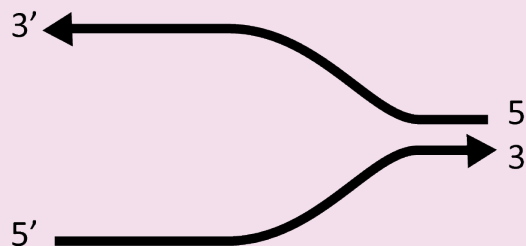
DNA Replication Continued

Discontinuous Replication

Place the second DNA polymerase at some point on the other strand of DNA. Notice that the DNA polymerase must move away from the fork instead of toward the fork, as it did in the leading strand. In order to accommodate the $5' \rightarrow 3'$ synthesis of DNA, short fragments are made on the second strand, which is referred to as the **lagging strand**. Continue adding nucleotides in the active site as you move the DNA polymerase away from the fork until you reach the end.



24. Sketch and indicate the directionality of the fragments composing the **lagging strand** of DNA right.



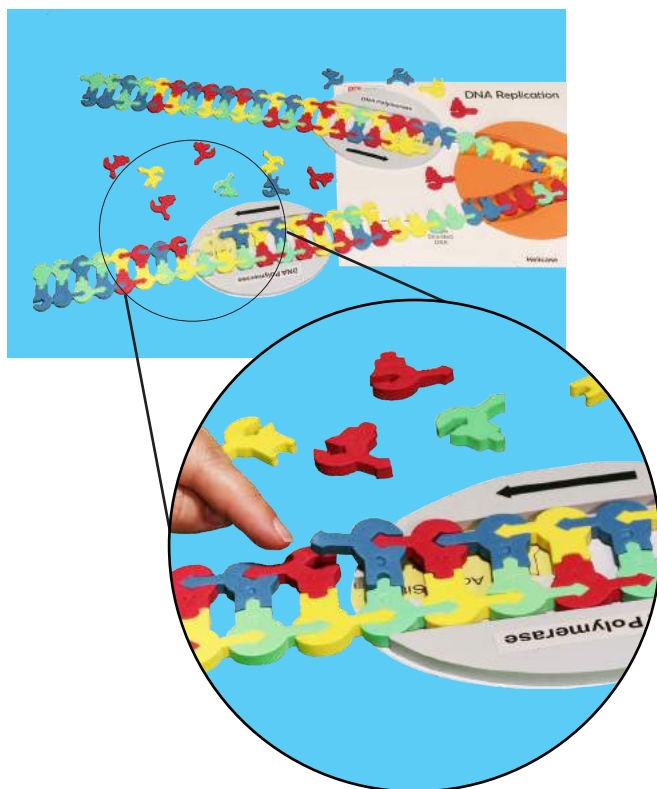
Feed the next 11 nucleotides through the helicase. Continue sliding the DNA polymerase along the **leading strand**, adding more nucleotides as you progress.

DNA Replication Continued

The **lagging strand** requires you to move the DNA polymerase! Place the DNA polymerase back at the fork junction to create the next fragment. Move the DNA polymerase so that the bases may be added from the 5' → 3' direction. You have now created a second fragment of DNA on the lagging strand. These fragments are referred to as **Okazaki fragments** and are usually 100-200 nucleotides long in eukaryotic cells.



When your polymerase bumps into the first fragment, you will need to remove the DNA polymerase and join the two fragments together with the appropriate nucleotide. The actual process of joining the Okazaki fragments together is more complex and involves several other proteins.

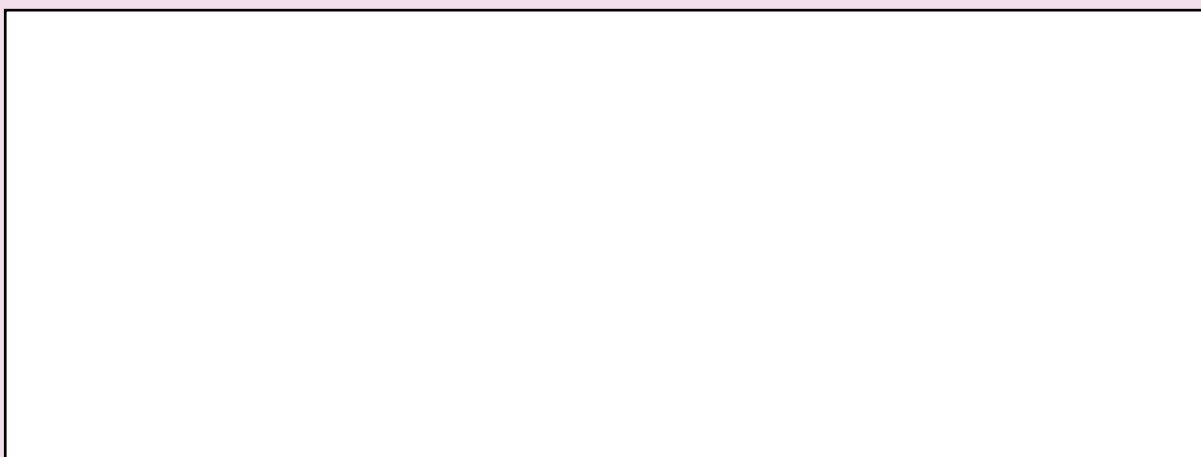


Complete the process of DNA replication with the remaining 11 nucleotides on both the leading and the lagging strands. DNA replication is considered to be a **semi-discontinuous** process.

DNA Replication Continued

25. Why is DNA replication considered to be a **semi-discontinuous** process?

26. Create a sketch which models the semi-discontinuous process of DNA replication at a replication bubble. Be sure to label the following aspects of your representation: leading and lagging strands, helicase, Okazaki fragments, parental strands, 3' ends and 5' ends.

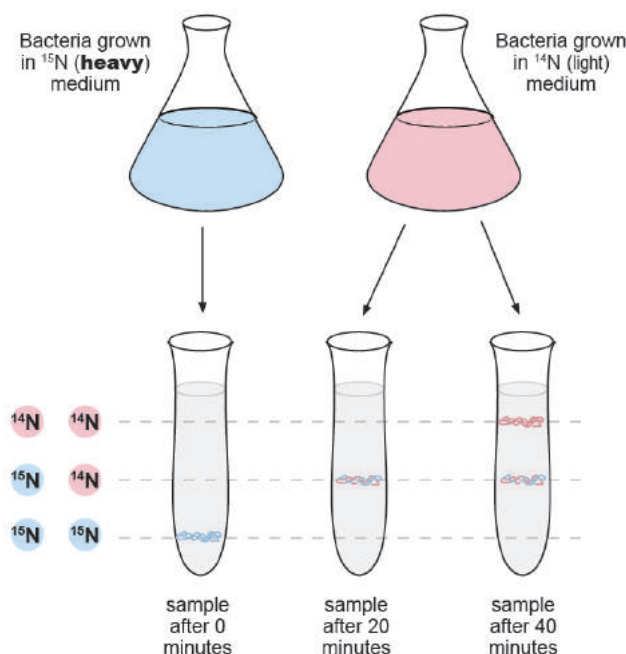


27. How do these two new strands compare to the original (parental) strand?

DNA Replication Continued

Three Models for the Process of DNA Replication

In 1958 at the California Institute of Technology, Matthew Meselson and Franklin Stahl devised an elegant series of experiments to determine which one of three models explained the mechanism of DNA replication. (You can find and read their published paper on our website at 3dmoleculardesigns.com/Teacher-Resources/Flow-of-Genetic-Information-Kit/Replication-Student-Handout-and-Key.htm.) Meselson and Stahl cultured *E. coli* in a medium containing nucleotides labeled with a heavy isotope of nitrogen, ^{15}N . They transferred the bacteria to a medium with only ^{14}N , a lighter isotope. A sample was taken after the DNA had replicated once (20 minutes). Another sample was taken after the DNA replicated again (40 minutes). The DNA was extracted from the bacteria in the samples and then centrifuged to separate the DNA of different densities. Their results are shown below.



Obtain and assemble 4 nucleotide base pairs of the colored DNA foam pieces. Find the matching gray base pair pieces but DO NOT assemble them. These colored DNA strands represent the parental strands from *E. coli* grown in a medium tagged with ^{15}N nucleotides. The gray foam pieces represent the nucleotides used to synthesize new DNA.

You will use the foam DNA models to detect which mechanisms of replication would most likely explain Meselson and Stahl's results.

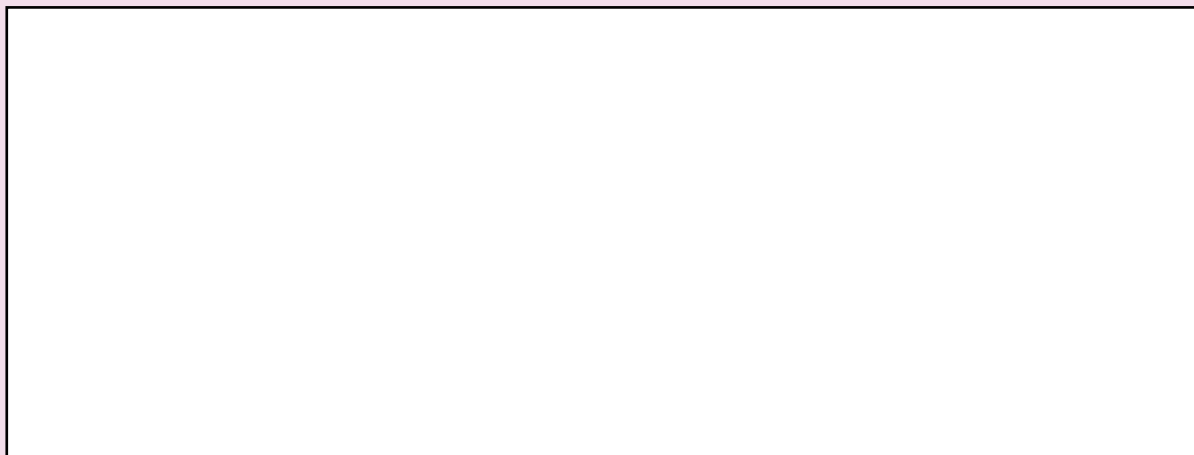
You will create a physical representation of the three models of DNA replication: (1) conservative, (2) semiconservative, and (3) dispersive. Begin with modeling the first round of replication of the DNA after the bacteria were transferred to a medium with only ^{14}N .

DNA Replication Continued

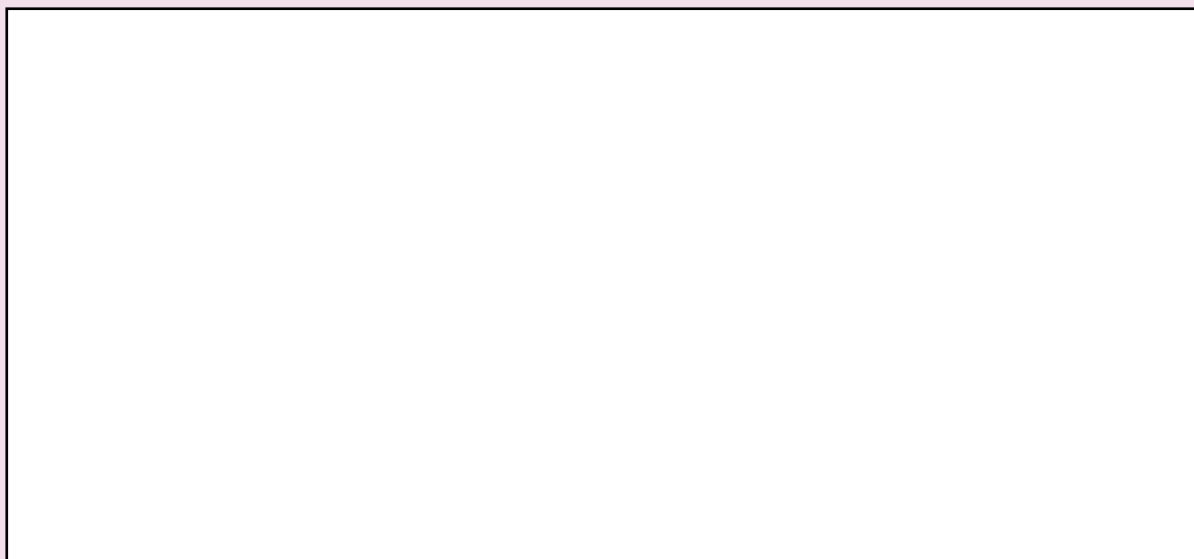
Conservative Model

In the conservative model of DNA replication, the parental strands are used as templates for the new DNA molecule and somehow come back together to *conserve* the parental molecule. Using the colored DNA parental strands you have just created and the gray nucleotides, model the end result of the conservative method of DNA replication. You should have one parental model made entirely of colored pieces and one daughter molecule with the same sequence of base pairs, but made entirely of gray foam nucleotides.

28. Sketch the new and old strands after one round of replication. It will be helpful if you have two different colored pens or pencils to create your sketches.



Sketch a test tube showing the density gradient of ^{15}N tagged DNA after one round of conservative replication.



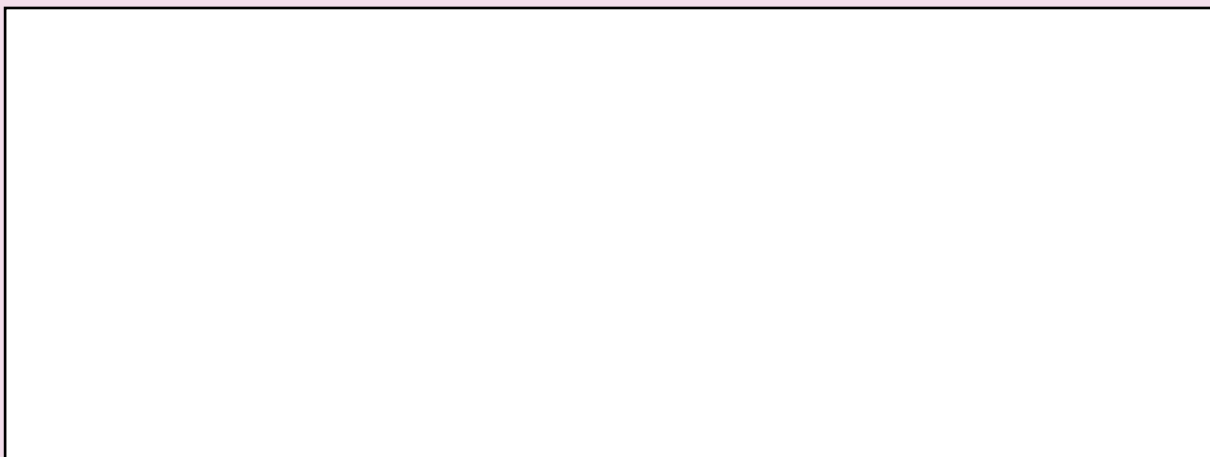
DNA Replication Continued

Semiconservative Model

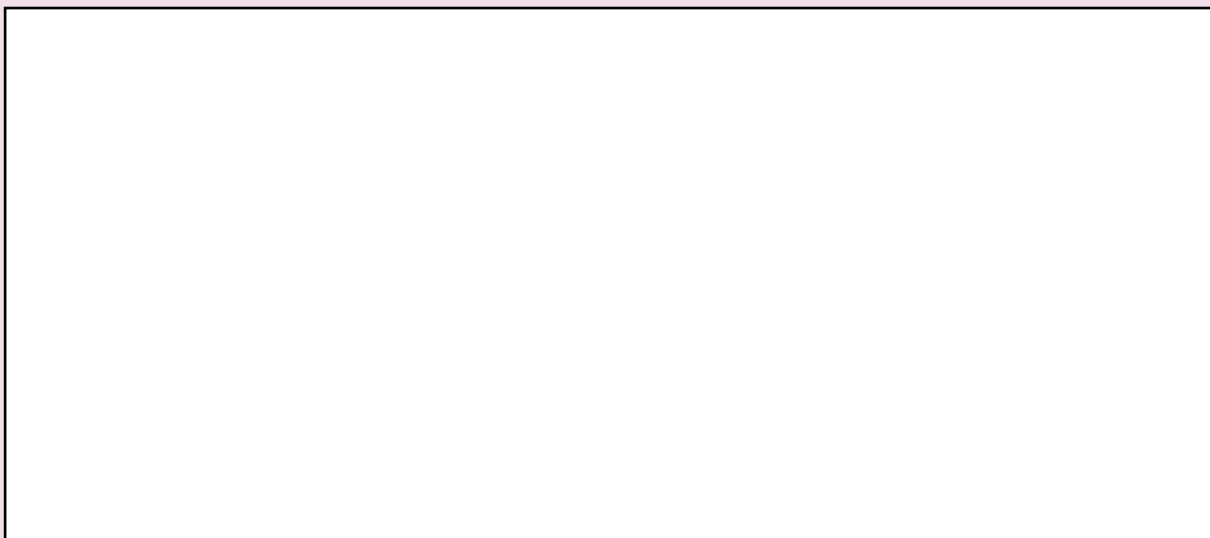
In the semiconservative model of DNA replication, each of the two daughter molecules will have one old strand from the parental molecule and one newly-made strand.

Now using the colored DNA parental strands you have created and the gray nucleotides, model the semiconservative method of DNA replication.

29. Sketch the results of one round of DNA synthesis after the semiconservative method of replication.



30. Sketch a test tube showing the density gradient of ^{15}N tagged DNA after one round of semiconservative replication. Refer to the Meselson and Stahl experiment to help you create your sketch.



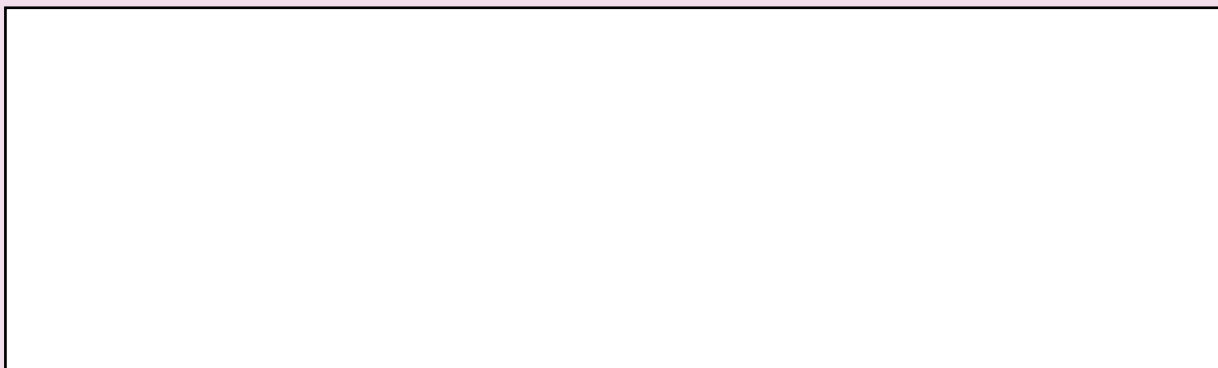
DNA Replication Continued

Dispersive Model

In the dispersive model of DNA replication, each strand of both daughter molecules contains a mixture of old and newly synthesized DNA.

Finally, using the colored DNA parental strands you have just created and the gray nucleotides, model the dispersive method of DNA replication.

31. Sketch the results of one round of DNA synthesis after the dispersive method of replication.



32. Sketch a test tube showing the density gradient of ^{15}N tagged DNA after one round of dispersive replication.



33. Which of the methods can now be eliminated based on the results that Meselson and Stahl got after one round of replication? Why?

DNA Replication Continued

Use the foam pieces to visualize what the newly synthesized strands of DNA would look like after a second round of replication in each of the methods. Sketch your results in the first column in the table below. In the second column, sketch what the DNA density gradient would look like in the test tube.

DNA Synthesized After A Second Round of Replication	DNA Density gradient
Conservative Model	
Semiconservative Model	
Dispersive Model	

DNA Replication Continued

34. Which method of DNA replication may now be eliminated after the second round of DNA replication based on the results of the Meselson and Stahl experiments? Why?

35. Based on the results of Meselson and Stahl's experiments, DNA is shown to replicate in a _____ manner.

Post-Lab Questions

36. What is the relationship of DNA replication to cell division?

37. Of the representations of DNA models (foam pieces, paper diagram, mini toobers), identify the strengths and weaknesses of each.

38. Based on what you have learned from this activity, explain why semiconservative replication is the preferred process of DNA replication as opposed to dispersive or conservative.

Big Ideas

Each time one of your cells divide, it must duplicate its DNA genome so that the new cell will have an exact replica of DNA contained in the original cell. This process of duplicating the double-stranded DNA is called replication. Because the nucleotide sequence of one strand of DNA is complementary to the sequence of the opposite strand – during DNA replication, the two strands of the original DNA are separated, and two different DNA polymerase enzymes use each single strand as a template for the synthesis of the second, complementary strand. This process is known as semiconservative DNA replication – meaning that each new cell receives a double-stranded DNA composed of one old strand of DNA and one newly-synthesized strand.