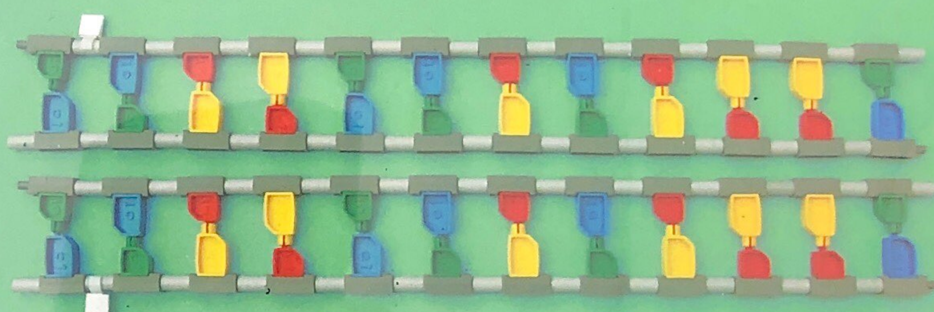
EDGERTON CENTER

DNA/RNA

Booklet 2:

Advanced DNA Topics



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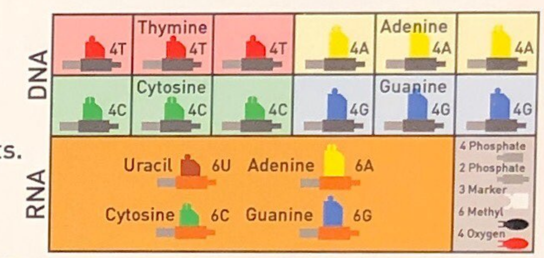
edgerton.mit.edu/dna-proteins-sets

Table of Contents

This booklet assumes you have completed DNA/RNA Booklet 1: Introduction to Structure and Function.

Using Your Booklet and Kit

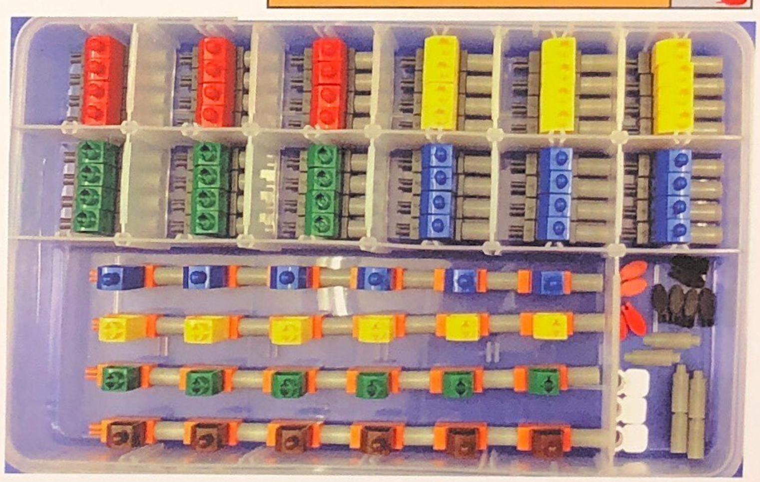
Q: = Helpful Questions (answers on Page 51)
Bold type = required actions
Underlined = new vocabulary



1. Open the kit. Count the gray DNA pieces in the small compartments. Each compartment should have 4 similar DNA pieces. Check that the colors are in the correct places. There are:
- 12 red (T)
- 12 yellow (A)
- 12 green (C)
- 12 blue (G)

2. Count the orange RNA pieces in the large compartment. Similar RNA pieces should be joined together in groups of six. There are:
- 6 brown (U)
- 6 yellow (A)
- 6 green (C)
- 6 blue (G)

3. Identify and count the pieces in the last compartment. There are:
- 6 gray cylinders (phosphates)
    - *4 with single pin*
    - *2 with double pin*
- 3 white markers
- 6 black clips (methyl)
- 4 red clips (oxygen)



PART I: NUCLEOTIDE CHEMISTRY

You will simulate cell processes in greater detail in this booklet. The chemistry of these processes is important to understand. Begin by examining the three parts of a nucleotide more closely.

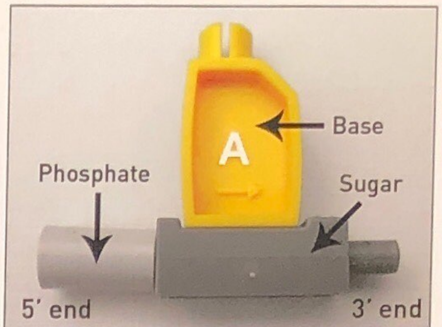
Sugar Chemistry

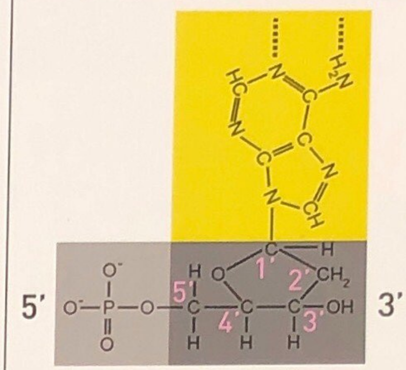
1. Pick up an adenine (A) DNA nucleotide. Identify the gray sugar component.

2. Look at the diagram to visualize the sugar's atoms. Find the carbon atoms in the sugar that are numbered 1' through 5' in pink. (The mark next to the number is called "prime.")

3. Locate the 3' carbon and the 5' carbon in the diagram. They are positioned on opposite ends of the sugar. These carbons establish the two ends of the nucleotide.

DNA Nucleotide

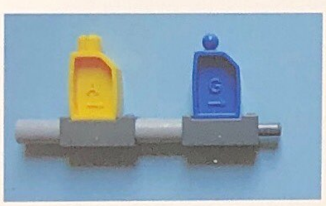




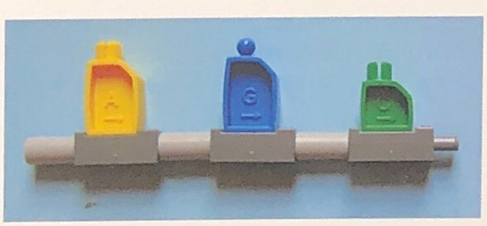
Adenine (A)

The 5' end of the sugar is always attached to a phosphate group. The 3' end is free. The enzyme DNA polymerase can only lengthen a DNA strand by adding nucleotides to the free 3' end. Thus elongation can only occur from one end of a strand.

4. Find the 3' mark on the A in your hand. Add a G onto this 3' end.



5. Add a C onto the 3' end of the G. Check your DNA strand with the photo below.



Q: Which number carbon is free (can be added onto)? Which number carbon is attached to the phosphate group? *(HINT: Refer to the diagram on page 3)*

Phosphate Chemistry

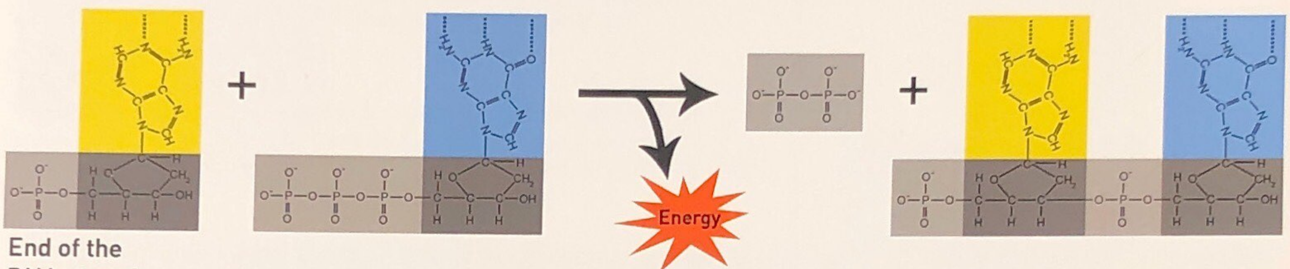
When a DNA polymerase is adding nucleotides to the 3' end of a DNA strand, each available nucleotide actually has *three* phosphate groups attached to it. So we need to revise our model of the elongation process. This process is also called DNA polymerization.

1. Look at the chemical reaction in the diagram below.

At the start, the nucleotide to be added has three phosphate groups. Each time a polymerase attaches an available nucleotide, two phosphates are released. Releasing these two phosphates provides the energy for the reaction. In this way, DNA polymerase can synthesize DNA very quickly and efficiently.

2. Find the nucleotide with three phosphate groups in the diagram below. Find the two released phosphates in the diagram below.

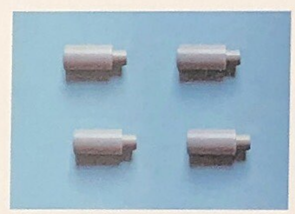
DNA Polymerization



End of the DNA strand

Let's prepare DNA nucleotides for polymerization.

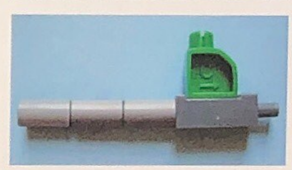
3. Take apart the DNA strand from the last activity.



4. Find four phosphates with a single pin in your kit. Check that the four phosphates match the exact shape in the photo.

5. Attach the phosphates together in groups of two.

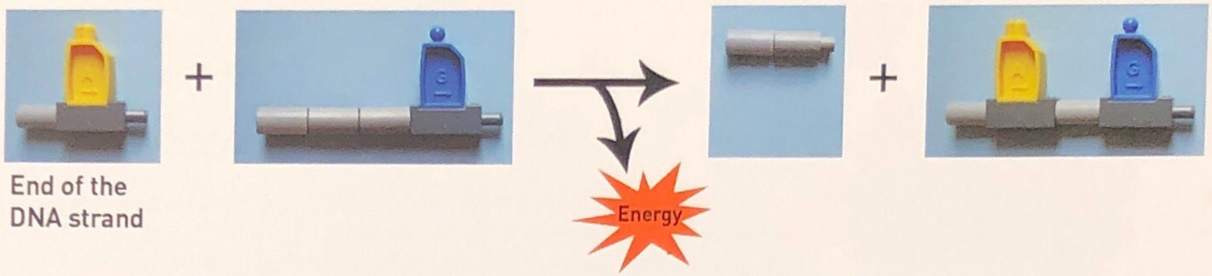
6. Attach two phosphates to the 5' end of the G.



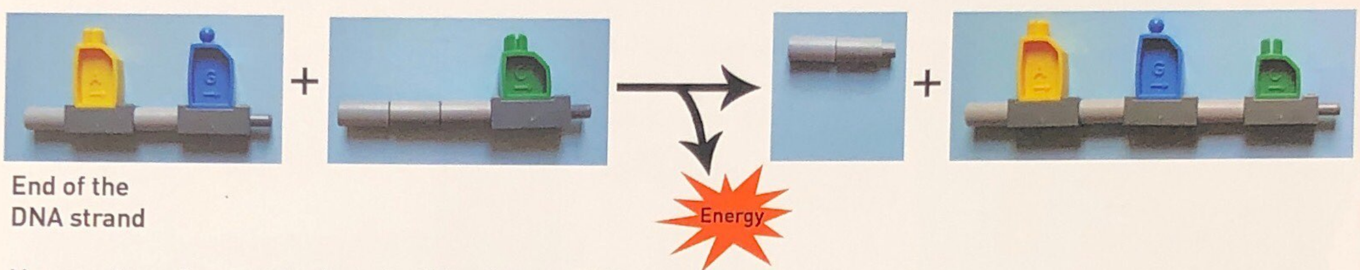
7. Attach two phosphates to the 5' end of the C.

Now let's attach these two nucleotides to the end of a DNA strand. This process takes place in the nucleus.

8. Follow the steps as shown below. Remove the two phosphates from the G. Attach the G to the 3′ end of the A.



9. Follow the steps as shown below. Remove the two phosphates from the C. Attach the C to the 3′ end of the G.



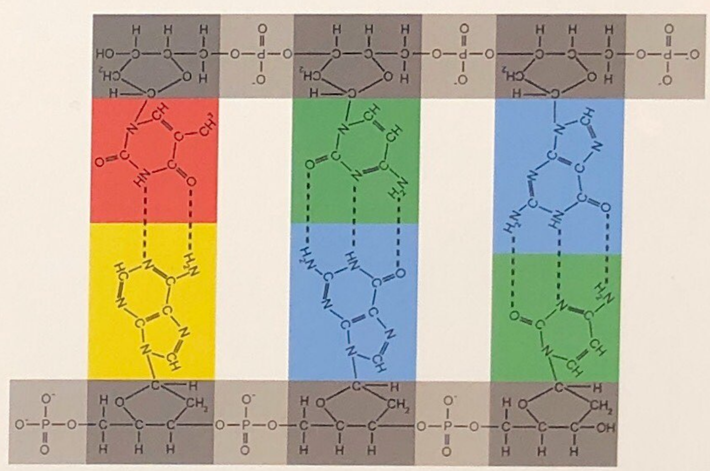
Now you know how to attach nucleotides the way DNA polymerase does!

10. Place the phosphates back in your kit.

Base Chemistry

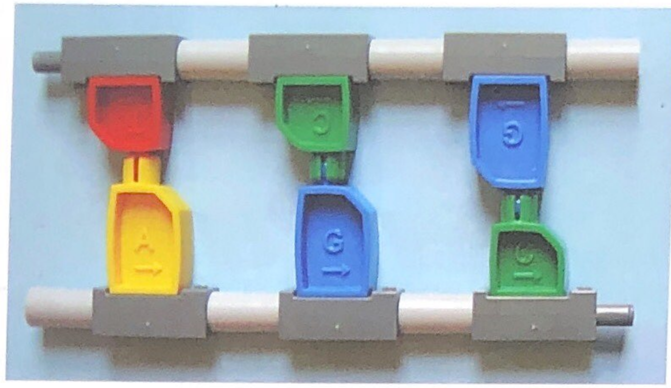
Bases in DNA are held together in pairs (A-T and G-C) by hydrogen bonding. Hydrogen bonds are weak compared to the sugar-phosphate bonds. Hydrogen bonds are easily made and easily broken, allowing the two DNA strands to separate for many important processes, such as replication. The hydrogen bonds are indicated by dotted lines in the diagram.

1. Find the hydrogen bonds in the diagram.

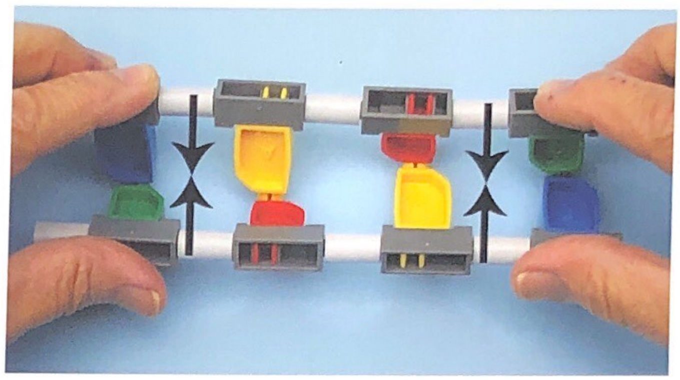


Q: Which base pair (A-T or G-C) has fewer hydrogen bonds and will separate more easily in nature?

2. Add the correct base pairs to your DNA strand. Keep the strands antiparallel as shown in the photo.



3. Pinch and pull up on the sides of the DNA strands with both hands to demonstrate the hydrogen bonds breaking. The bonds will open quickly, releasing the DNA into 2 separate strands.



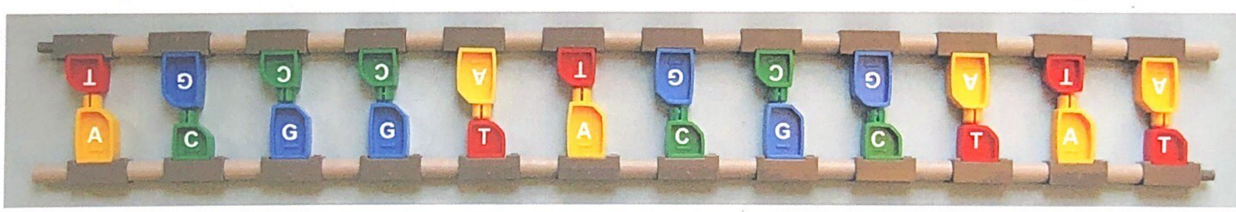
4. Join and break the hydrogen bonds two more times. Always separate the strands using this technique.

PART II: REPLICATION, DAMAGE, AND REPAIR

Advanced DNA Replication

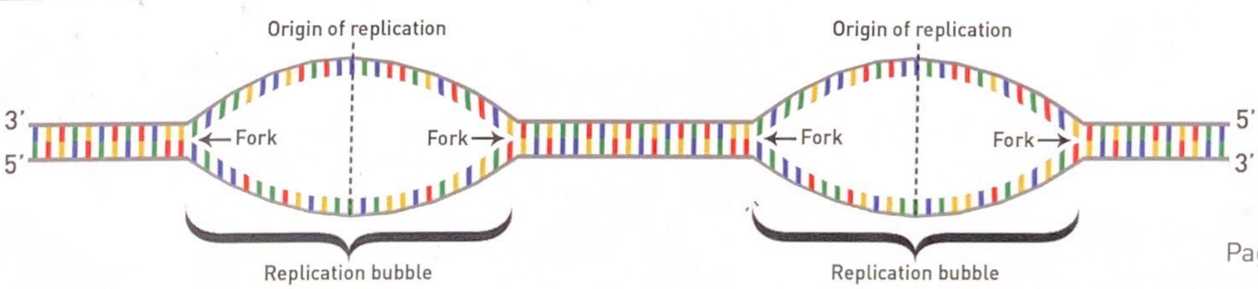
1. Build the molecule of DNA as shown in the photo. The bottom strand is 5' to 3' and the top strand is 3' to 5'. Note these expressions (5' to 3' and 3' to 5') tell you the direction of the nucleotides from left to right.

3' 5'

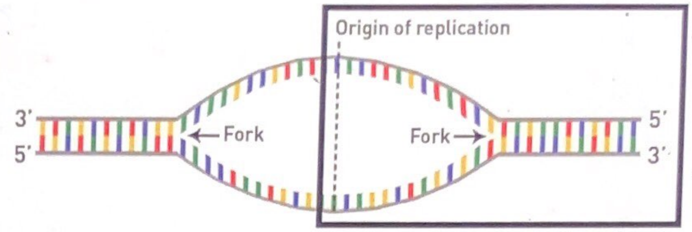


5' 3'

Origins of replication are places where DNA replication starts along the chromosome. Replication bubbles are made by proteins separating the two strands of DNA. These proteins keep unzipping the DNA, creating replication forks that continue to open in both directions.



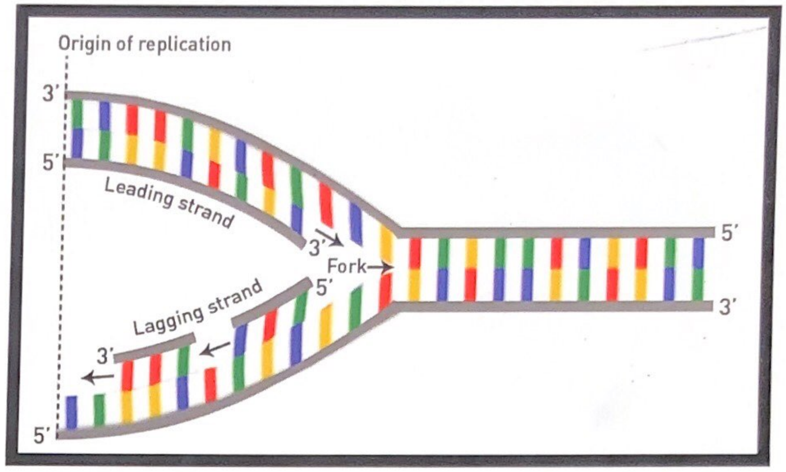
To make the replication process easier, we will only replicate one side of the replication bubble as shown in the box.



Since DNA polymerases can only add nucleotides to the 3′ end of a strand, the two new strands are replicated differently:

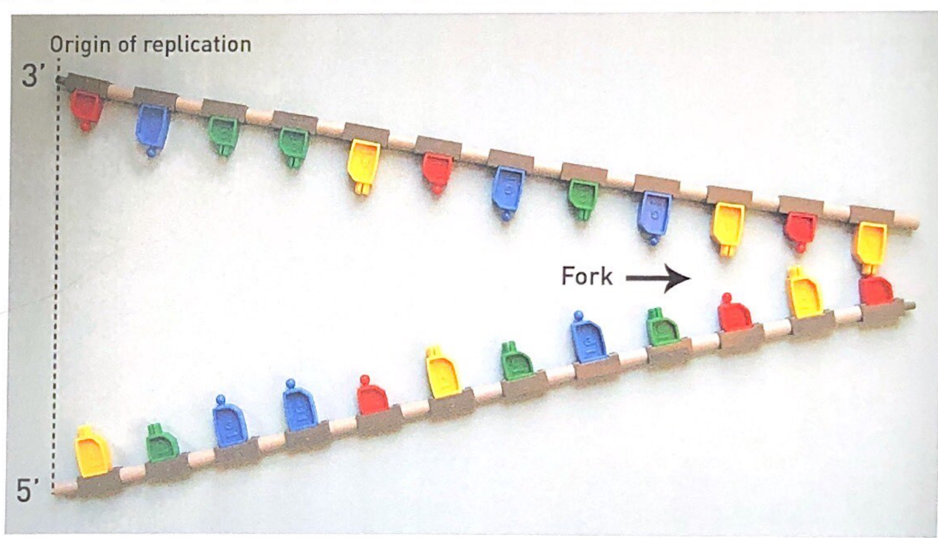
A leading strand is built in the direction of a fork. A leading strand has its 3′ end free to be added onto as the fork opens.

A lagging strand is built away from a fork. Because the fork keeps opening, a lagging strand is created in many pieces, called Okazaki fragments.



2. Pinch open the DNA from the left, leaving the last base pair attached as shown in the photo. The open left side of the DNA represents the origin of replication.

DNA polymerase will pair nucleotides to these original strands. We will start by building the leading strand first.



3. Answer this question before continuing on the next page: Which strand (top or bottom) will the DNA polymerase pair with to build the leading strand?
The leading strand is created in the direction of the replication fork. Nucleotides can only be added to a 3′ end.

Fantastic Fact!

Opening the DNA requires two proteins in cells. Helicases unwind and separate the DNA strands, and topoisomerases relieve the twisting stress on the double helix.

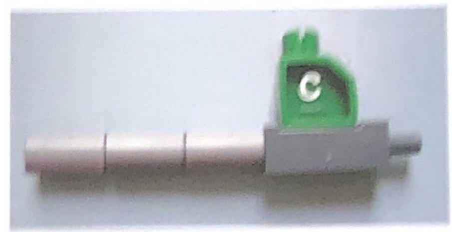
DNA polymerase will pair nucleotides with the top strand to build the leading strand. The 3' end of the new nucleotide is free in the direction of the replication fork.

4. Pair an A with the first T on the top strand to build the leading strand.

5. Check the 3' and 5' ends of the A with the photo.

Remember that nucleotides have three phosphates when they are being added to the DNA strand. The release of the two phosphates provides the energy needed for polymerization.

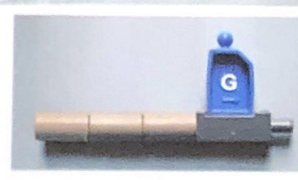
6. Add two phosphate groups to a C as shown in the photo.



7. Base pair the C to the G to build the leading strand.

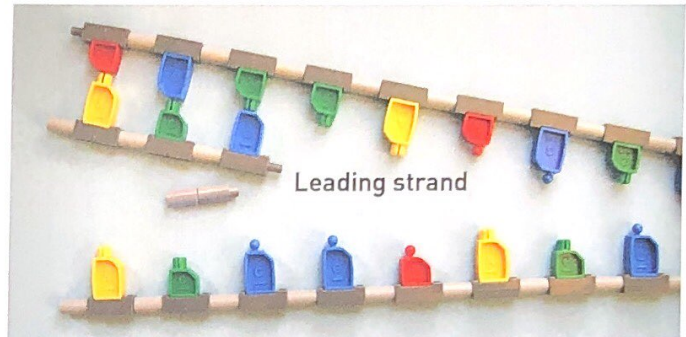
8. Remove the two phosphates to make the sugar-phosphate bond on the 3' end of the growing strand.

9. Add the two phosphates to a G, the next incoming nucleotide.



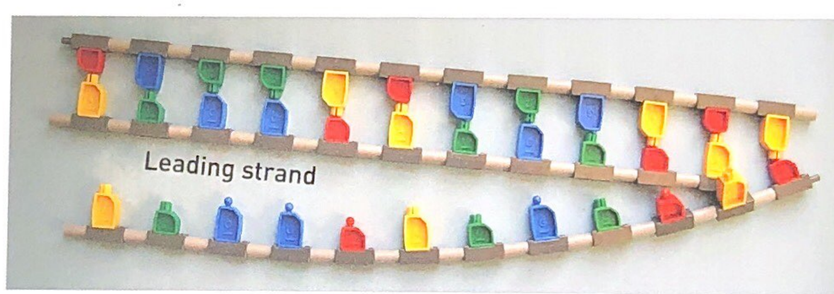
10. Base pair the G to the C to build the leading strand.

11. Release the two phosphates to make the sugar-phosphate bond.

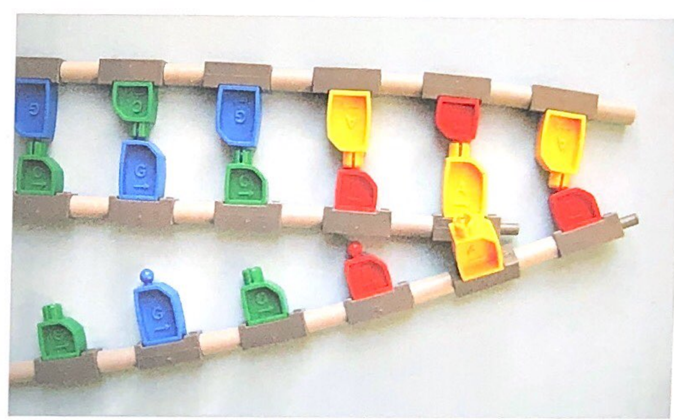


12. Place the phosphates back in your kit.
To make this easier, stop adding the two phosphates to the incoming nucleotides. Join the nucleotides directly to each other for the rest of the activity.

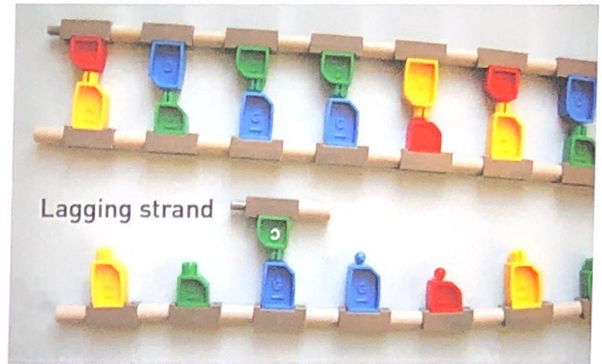
13. Add as many nucleotides as you can to the leading strand. Leave the last original base pair as shown in the photo.



14. Let the leading strand and the original bottom strand overlap as shown in the photo.



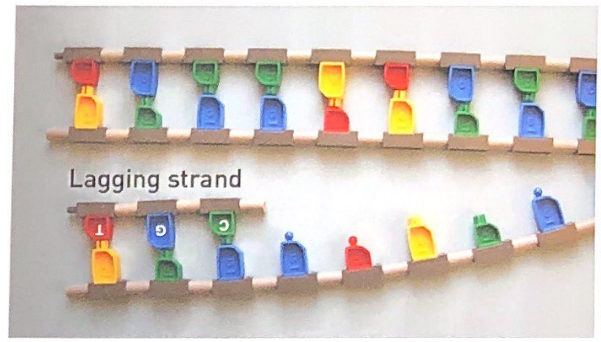
Now we'll build the lagging strand. The lagging strand is built away from the replication fork.
It is created in segments called Okazaki fragments.



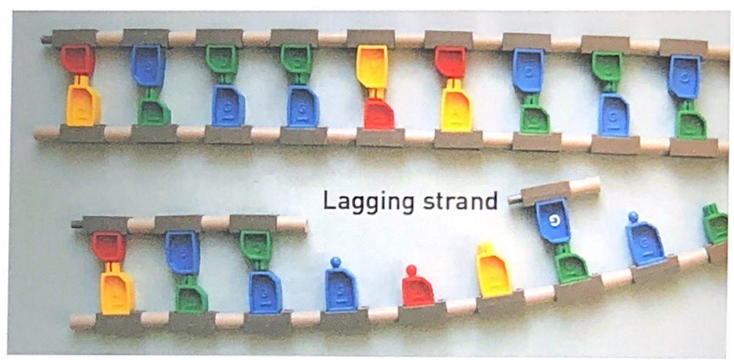
15. Add a C nucleotide to the third nucleotide (G) to build the lagging strand as shown in the photo.

16. Check the 3' and 5' ends of the C with the photo.

DNA polymerase uses nucleotides with 3 phosphates to create the lagging strand. But to make this easier, you will join the nucleotides directly to each other.

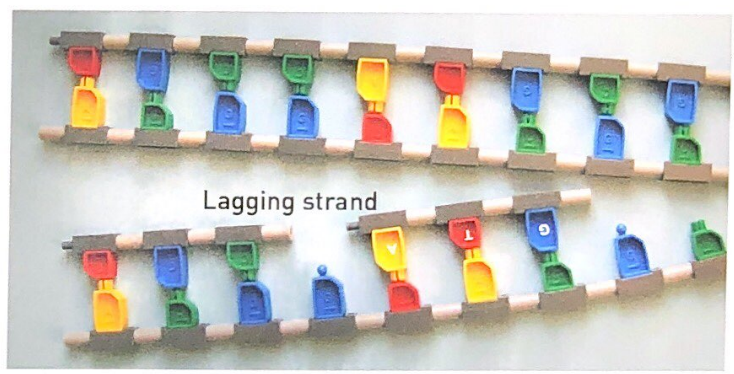


17. Add 2 more nucleotides to create one Okazaki fragment for the lagging strand as shown in the photo.



18. Add a G nucleotide to the seventh nucleotide (C) to build the lagging strand as shown in the photo.

19. Check the 3' and 5' ends of the G with the photo.

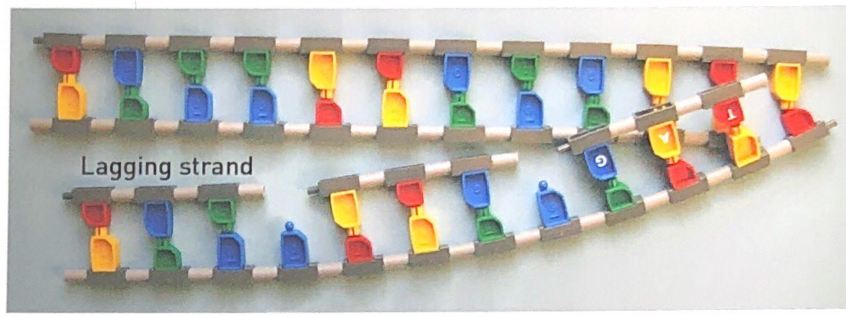


20. Add 2 more nucleotides to create the second Okazaki fragment for the lagging strand as shown in the photo.

21. Add a T nucleotide to the eleventh nucleotide (A) to build the lagging strand as shown in the photo. Allow the T to overlap the leading strand.

22. Check the 3' and 5' ends of the T with the photo.

23. Add 2 more nucleotides to create the third Okazaki fragment for the lagging strand as shown in the photo. These nucleotides should overlap the leading strand.



Let's finish replication on both the leading and lagging strands.

24. Pinch open the replication fork completely as shown in the photo.