Molecular Biology I: DNA Replication

Learning Goals:

To work with a physical model of DNA in order to help you to understand:

- rules for DNA structure
- base-pairing
- DNA replication

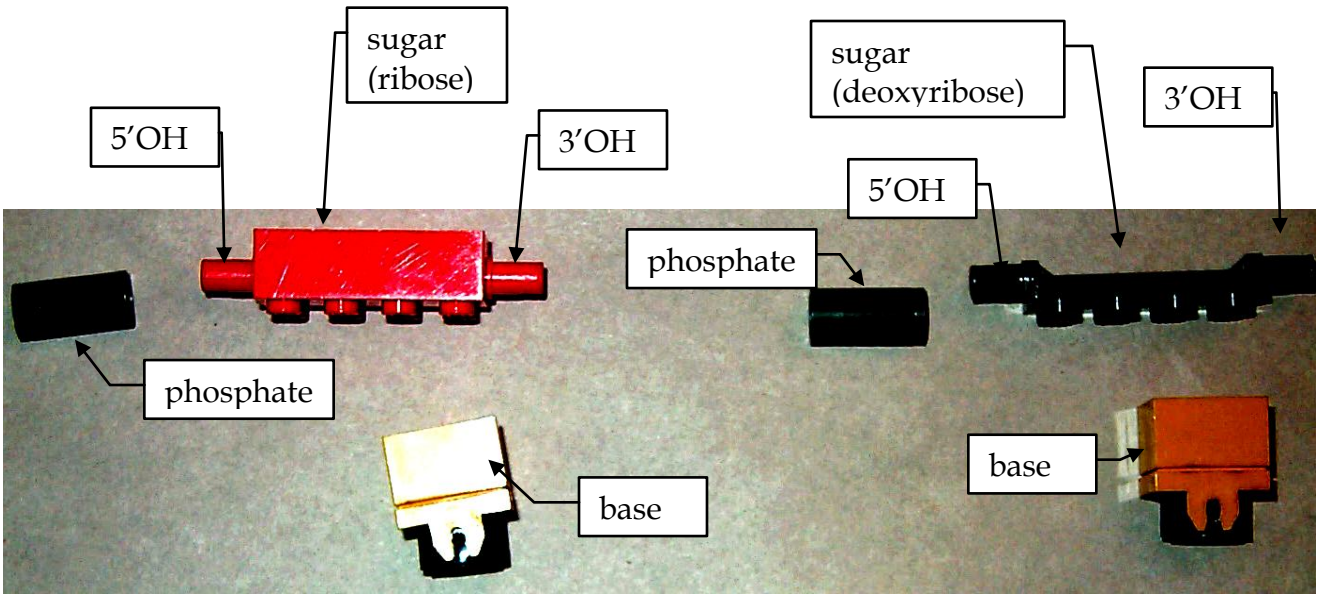
Introduction:

In order to thoroughly understand more complex molecular biology problems we will work through some simpler problems using LEGO DNA models this week and next week. This week we will only look at DNA Replication, but the kit contains both DNA and RNA, to tell the pieces apart use the diagrams below.

The LEGO models DNA and RNA as follows:

DNA and RNA are polymers of nucleotides. The LEGO models the nucleotides as follows:

RNA nucleotide DNA nucleotide



Important note: Unlike the LEGO Mitosis lab, we will not spell out all the details of what you have to do in each step of this lab. We do this on purpose - we want you to figure out some of the details with the help of your classmates and your TA. As you figure these out, the details will become clearer.

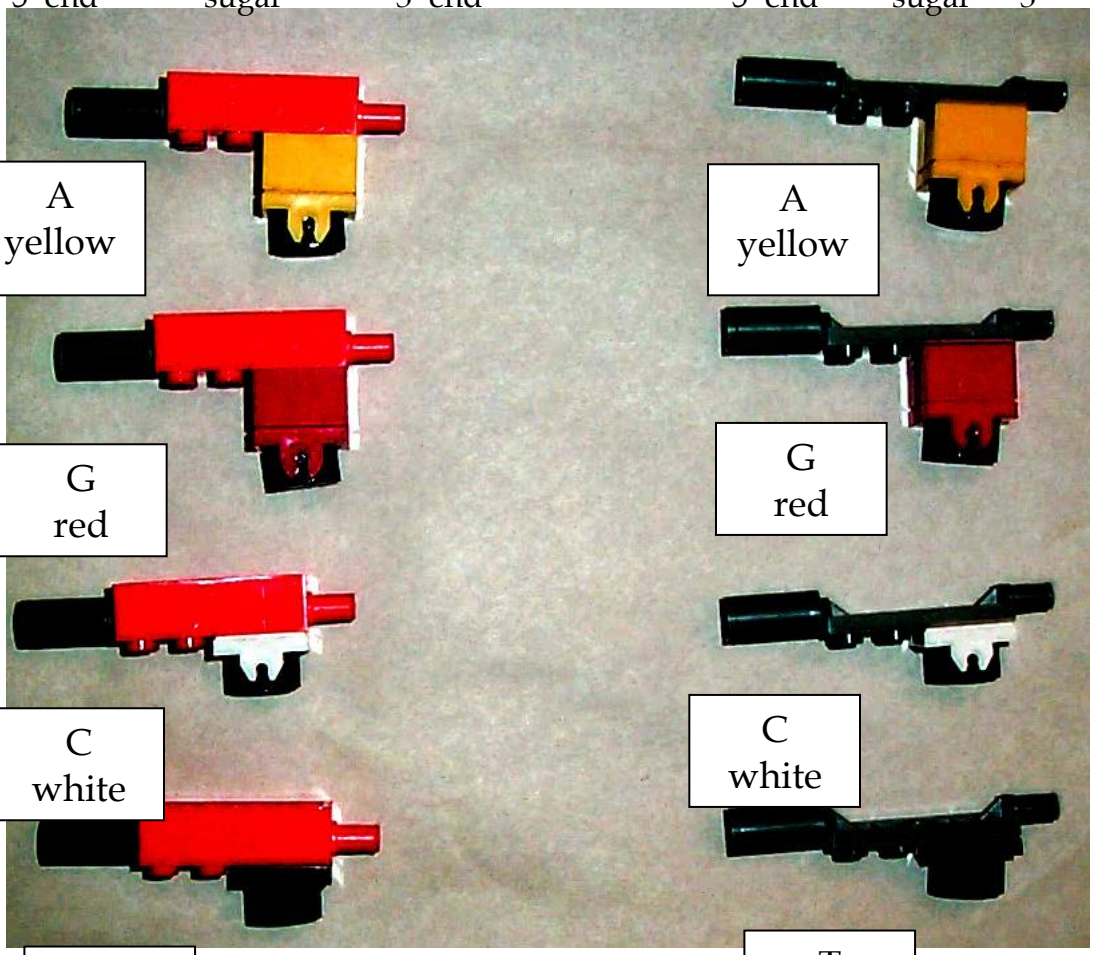
When nucleotides are assembled, they look like this:

RNA nucleotides

DNA nucleotides

5′ end sugar 3′ end

5′ end sugar 3′ end



1. The correct bases pair via hydrogen bonds simulated by the black magnets on each base.
2. The backbone is connected by covalent bonds simulated by the plug on the 3′ end and the socket on the phosphate on the 5′ end.

Procedure: 1) Check your kit. You should have the following pieces :

- 12 DNA A's (yellow base)
- 12 DNA G's (red base)
- 12DNA C's (white base)
- 12 DNA T's (black base)
- 6 RNA A's (yellow base)
- 6 RNA G's (red base)
- 6 RNA C's (white base)
- 6 RNA U's (gray base)

They should be set up as in the pictures above. That is, the phosphate should be on the 5′ end - the end farthest from the base.

2) Build a single-strand of DNA with the following sequence:

5′-ACGGTACGCTAT-3′

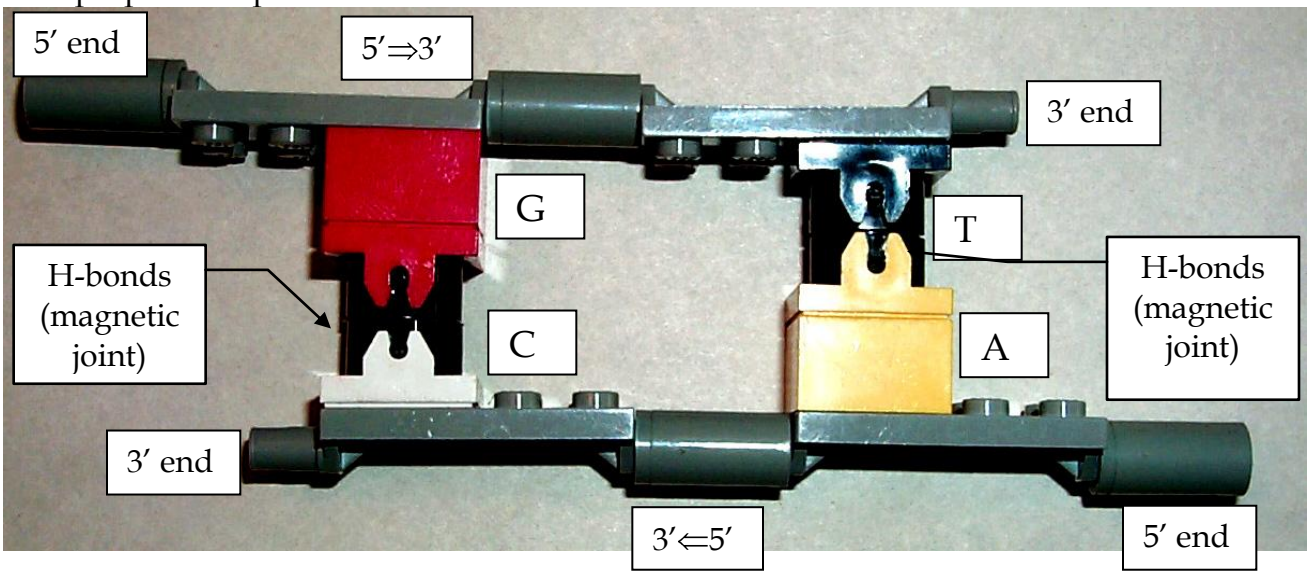
Notice that all the sugars run in the same direction.

3) Build another DNA strand properly base-paired to the one you made in step (1).

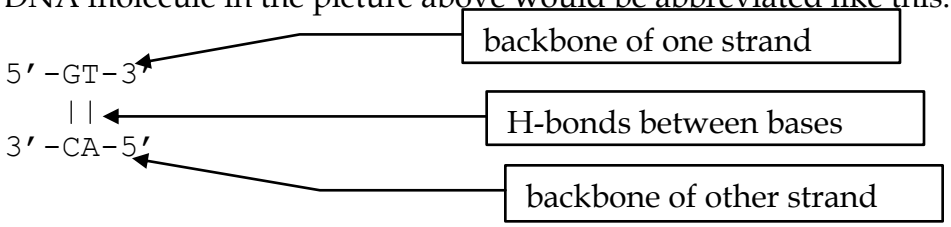
Note:

- the strands must be anti-parallel (run 5′ ⇒ 3′ in opposite directions)
- A pairs with T (yellow with black) the magnets won't let you pair it any other way
- G pairs with C (red with white) the magnets won't let you pair it any other way

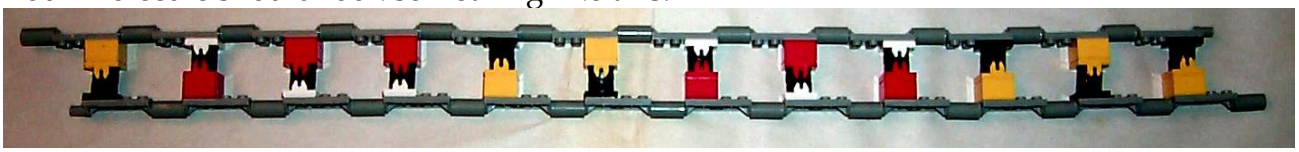
Two proper base-pairs are shown below:



The sequence of the DNA molecule in the picture above would be abbreviated like this:



Your molecule should look something like this:



What is the sequence of the DNA strand you just built?

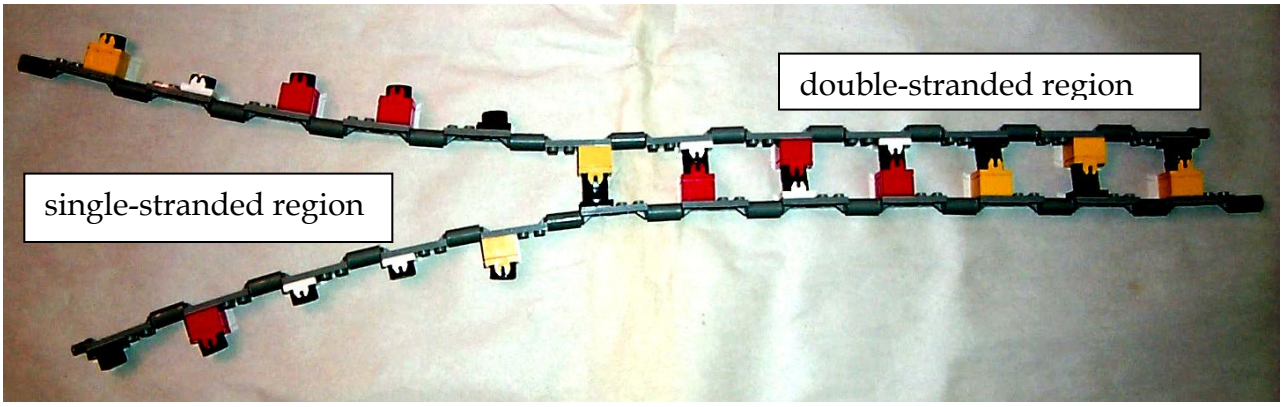
5′-_____-3′

What is the structure of the double-stranded DNA molecule you now have?

DNA Replication

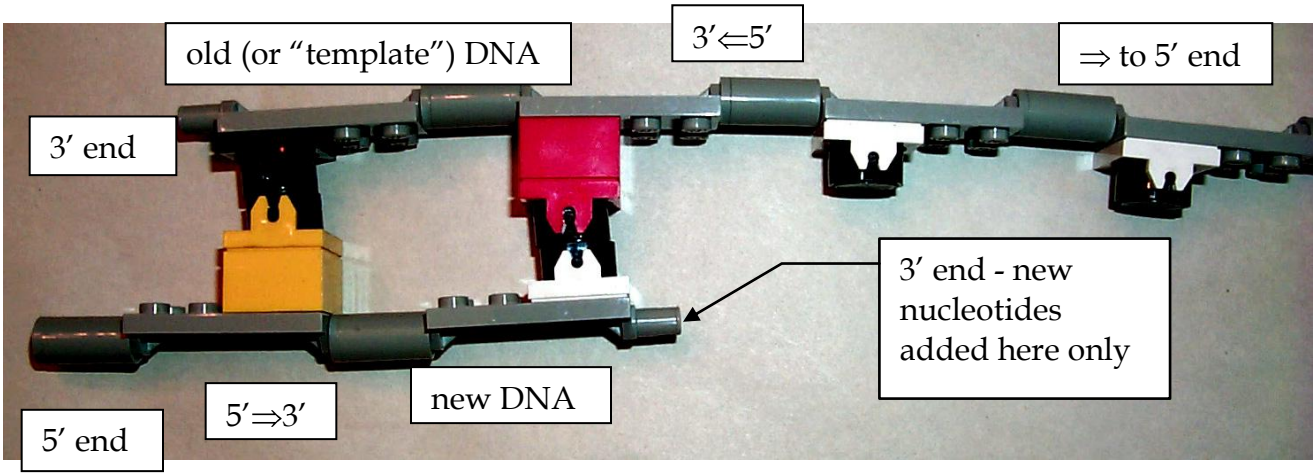
You will now simulate the replication of this DNA molecule.

4) Prepare the left-hand end of the molecule for replication. Un-zip (break the hydrogen bonds - simulated by separating the magnets) the 5 base-pairs at the left end of your DNA molecule to make a region of single-stranded DNA. You will have to turn the bases to face out from the center or they will stick back together. This is shown below:



5) Start replicating DNA on one of the single-stranded regions of your DNA molecule. Remember to follow the rules:

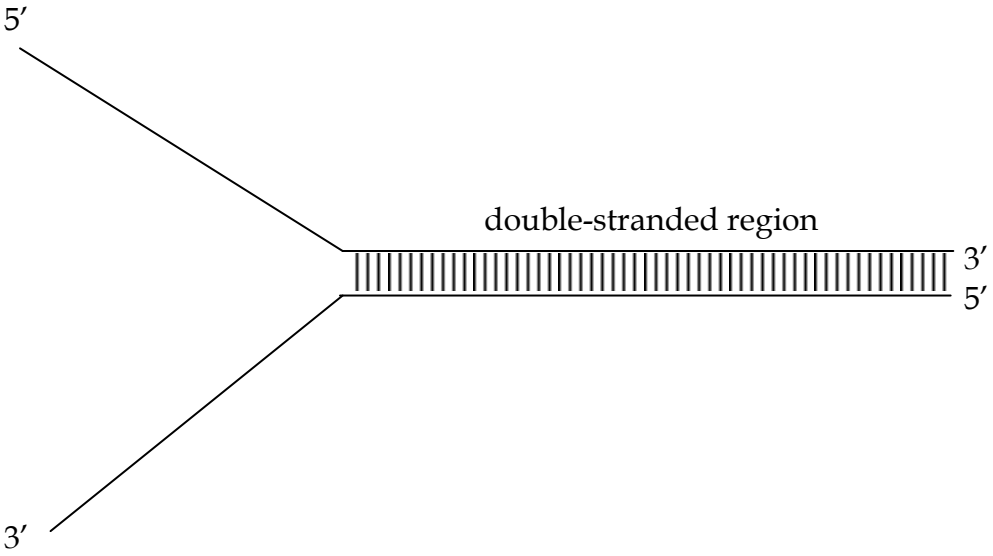
- the strands must be anti-parallel (run 5′ ⇒ 3′ in opposite directions)
- A pairs with T (yellow& black) the magnets won't let you pair it any other way
- G pairs with C (red & white) the magnets won't let you pair it any other way
- DNA polymerase can <u>only</u> add nucleotides to a 3′ end. This is shown below:



6) Continue replicating this strand until you have to stop-because you've reached the end of the template strand or you've run into the double-stranded region.

7) Replicate the other strand in the single-stranded region. Keep in mind the rules from step (5). You will notice an important difference between the two strands.

8) The lines in the diagram below represent the template DNA strands. On the diagram below, draw the two new DNA strands you made. Be sure to indicate their 5′ and 3′ ends. Put an arrowhead on the 3′ end to indicate that this is where the strand can grow. (5′ ⇒ 3′)



9) Unzip the remaining base-pairs in the double-stranded region and finish replicating the DNA strands. List the differences between the replication on the two strands:

Leading strand:

Lagging strand:

10) Disassemble the DNA molecules you made. Do this carefully so that the phosphates stay on the 5′ ends of the nucleotides (the end farthest from the base). This simulates the hydrolysis that occurs during digestion. This is shown below: