Student Guide: DNA Replication DNA Simulation BioKit®

Deoxyribonucleic acid (DNA) carries inherited genetic information in the coded sequence of its nitrogenous bases. It is essential that DNA be exactly duplicated from one cell division to the next to maintain the code of heredity. This duplicative process is termed DNA replication. For replication to begin, the DNA double helix must be unwound and separated into two single-stranded patterns of nucleotides called templates. Complementary DNA nucleotides are brought to each template, where hydrogen bonds form to link the nucleotides of the new strand to the template. The new, antiparallel strands grow in a 5'-to-3' direction, as opposed to the 3'-to-5' template. DNA replication produces two double-stranded molecules from one initial molecule.

Name	
Date _	

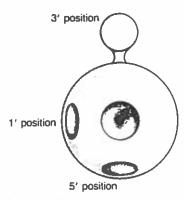


Figure 1 Carbon positions on the deoxyribose sugar (white) bead.

ASSEMBLY

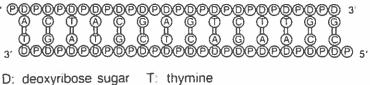
Each team needs the materials listed below. Note the designation of each component at this time.

Quantity per team	Kit component	Component designation Deoxyribose sugar	
60	White beads		
60	Red beads	Phosphate group	
₁₁₁ 15	Orange beads	Phosphate group Adenine (A)	
15	Green beads	Guanine (G)	
15	Blue beads	Cytosine (C)	
15	Yellow beads	Thymine (T)	
30	Clear connectors	Hydrogen bonds	

Assemble 60 nucleotides by attaching a phosphate group (red beads) to the 5' position of the deoxyribose sugar (white beads) and by attaching any one of the four nitrogenous bases (orange, green, blue, or yellow beads) to the 1' position of the same sugar (Fig. 1).

By attaching the phosphate group of one nucleotide to the 3' peg of the deoxyribose sugar of another nucleotide, construct the DNA molecule shown in Fig. 2. Join the two antiparallel strands with hydrogen bonds between the

nitrogenous bases. Remember that adenine (A) always pairs with thymine (T) and cytosine (C) with guanine (G). Grasp the completed DNA molecule by the base pair at each end of the double strands. Gently twist the molecule into the form of a spiraling rope ladder, being careful not to separate the weak hydrogen bonds between base pairs. This represents the double-helical structure of the DNA molecule.



D: deoxyribose sugar T: thymine P: phosphate group A: adenine C: cytosine G: guanine

Figure 2 A simulated DNA segment.

REPLICATION

Unwind the DNA molecule and position the double strands on your desk as shown in Figure 2. Place the 5' end of the upper strand and the 3' end of the lower strand on the left. The 3' end of the upper strand and the 5' end of the lower strand should be to the right.

Beginning on your right, unsnap the hydrogen bonds between the first eight pairs of nitrogenous bases and separate the two single strands (Fig. 3). Replication occurs in a 5'-to-3' direction off a 3'-to-5' template. Therefore new complementary nucleotides should be brought to the 3' end of the upper template and positioned antiparallel to the nucleotides on the template strand. Place hydrogen bonds between the nitrogenous base pairs and attach the 5' phosphate group of each new nucleotide to the 3' peg of the last sugar on the growing strand. In the case of the first new nucleotide to be brought to the template, connect only the nitrogenous bases via a hydrogen bond.

Growth of the new strand proceeds one nucleotide at a time from the open 3' end of the upper strand toward the separation point of the two original single-stranded templates. Continue adding nucleotides until the template separation point is reached. This replicating strand proceeding toward the separation point of the two templates is called the leading strand. As the templates separate, the leading strand replicates continuously.

On the lower template with the terminating 5' phosphate group, replication moves away from the separation point of the two templates but still moves in a 5'-to-3' direction. The lower, lagging strand must replicate in short, discontinuous segments to keep pace with the separating template strands.

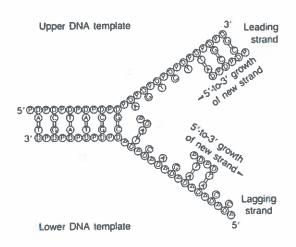


Figure 3 Separation of the two DNA strands and initiation of replication.

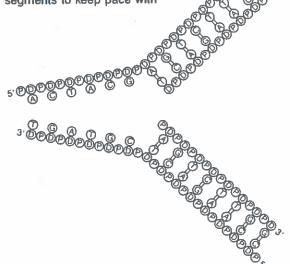


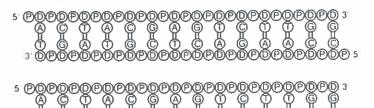
Figure 4 Separation of the six remaining base pairs.

Bring the first complementary nucleotide to be attached to the lower strand to the fourth nucleotide from the 5' end. The fourth nitrogenous base on the template strand is adenine; therefore the new nucleotide to be hydrogen-bonded must have thymine as its base. Replicate a short nucleotide segment by bonding the next three nucleotides to the right in an antiparallel manner. Move up to the eighth nucleotide on the template (thymine), bond an adenine-based nucleotide to the template thymine base, and continue pairing the next three bases to the right until the first fragment of new DNA nucleotides is reached.

Separate the remaining six nitrogenous base pairs of the template strands by unsnapping the hydrogen bonds between them (Fig. 4). Continuously replicate the upper leading strand by adding the 5' phosphate group of a new complementary nucleotide to the 3' peg of a nucleotide already on the new strand. Move up three nucleotides on the bottom strand and add a complementary nucleotide. Move to the right and hydrogen-bond two more nucleotides to the template to form another short fragment on the lagging strand. Finally, move to the last nitrogenous base (thymine) at the 3' end of the lower strand and bond a complementary adenine-based nucleotide to the template. To complete the replication, bond the last two complementary nucleotides to the right. The four short nucleotide fragments attached

to the lower template should be bonded by snapping together neighboring 3' sugars and 5' phosphate groups present at fragment end points.

Two new, antiparallel DNA double strands of 14 nucleotide pairs should be on your desk (Fig. 5). Twist each into a double helix. Inspect both DNA molecules. Are the double strands of each molecule antiparallel? Are all the nitrogenous bases paired correctly (adenine with thymine and cytosine with guanine)? Are both DNA molecules identical? Analyze and correct any errors that are present. Repeat the exercise until you are familiar with DNA replication.



D: deoxyribose sugar T: thymine P: phosphate group A: adenine G: guanine

Figure 5 A completed pair of double-stranded DNA hybrids.

Carolina Biological Supply Company

Burlington, North Carolina 27215