DNA Structure and Replication

You will use simulations to demonstrate the structure and replication of DNA in this laboratory exercise. As you have studied, the genetic information for living organisms is stored in the structure of deoxyribose nucleic acid (DNA). Our DNA molecules carry the genetic code for inherited characteristics in the triplet-coded sequences of its nitrogen bases. Each time a cell divides, its DNA is precisely duplicated (DNA replication) so that the DNA of the cells formed is identical to that of the original cell.

A DNA molecule consists of thousands of nucleotides bonded together in an interconnected chain, the double helix. Each nucleotide has three components: a sugar molecule, a phosphate group, and a nitrogen base. In DNA the sugar is the five-carbon deoxyribose. The phosphate group is attached to the number five carbon end, or five-prime (5') position of the sugar molecule, and the nitrogen base attaches to the number one carbon (1') position) of the sugar molecule forming a complete nucleotide.

The three-prime (3') end of the sugar of one nucleotide bonds to the phosphate molecule of the next nucleotide forming a very long chain of alternating sugar-phosphate molecules. The DNA molecule is composed of two antiparallel sugar-phosphate chains. (This means the chains run in opposite directions to each other.) The two sugar-phosphate chains are connected to each other by hydrogen bonds between the nitrogen bases of the nucleotides. The DNA molecule looks a bit like a twisted ladder whose rungs are formed by the hydrogen-bonded nitrogen bases. By the way, this may sound more complicated than it really is!

There are four different nitrogen bases in DNA nucleotides: adenine (A) and guanine (G) are classified as purines, which have a double interlocking ring structure; cytosine (C) and thymine (T) are pyrimidines, which have a single ring structure. Because of their molecular structure, the nitrogen bases bond very specifically: adenine bonds only with thymine, and cytosine only with guanine. It is this strict base pairing between strands that dictates the double helix structure of DNA, and provides DNA with the ability to function as it does.

Pop-beads are used to represent the nitrogen bases, sugars and phosphate molecules in this set of laboratory exercises.

White beads represent Deoxyribose sugar
Red beads represent the Phosphate group
Orange beads represent Adenine (A)
Green beads represent Guanine (G)
Blue beads represent Cytosine (C)
Yellow beads which represent Thymine (T)
Clear connectors represent Hydrogen bonds

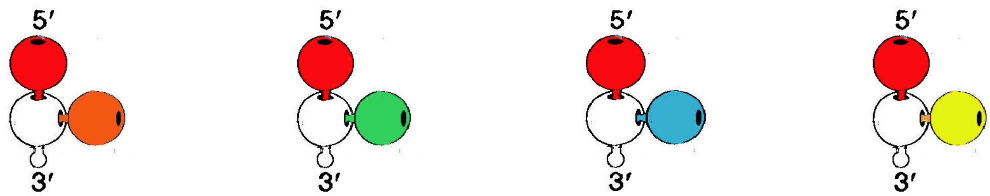
Exercise I: The Structure of DNA

Materials needed per lab group

- 10 Adenine (A) deoxyribose nucleotides (each comprised of one orange adenine bead, one white deoxyribose bead and one red phosphate bead)
- 10 Guanine (G) deoxyribose nucleotides (each comprised of one green guanine bead, one white deoxyribose bead and one red phosphate bead)
- 10 Cytosine (C) deoxyribose nucleotides (each comprised of one blue cytosine bead, one white deoxyribose bead and one red phosphate bead)
- 10 Thymine (T) deoxyribose nucleotides (each comprised of one yellow thymine bead, one white deoxyribose bead and one red phosphate bead)
- 20 clear connectors for Hydrogen bonds

Procedure

1. Be sure that each nucleotide is assembled correctly: the phosphate group (red bead) attaches to the 5' position of the deoxyribose sugar (white bead), leaving the 3' "peg" of the sugar molecule free to bond, and the nitrogen base (adenine, guanine, cytosine, or thymine) attaches to the 1' position of the same sugar.



Adenine Nucleotide   Guanine Nucleotide   Cytosine Nucleotide   Thymine Nucleotide

2. Construct a single-stranded DNA chain by attaching the 3' peg of the sugar (white bead) of one nucleotide to the phosphate group (red bead) of another nucleotide. Use five nucleotides with adenine, five with guanine, five with cytosine, and five with thymine. Attach the nucleotides in any order you choose, but always attach the 3' peg of the sugar to the phosphate group of the next nucleotide.

3. To form the double-stranded DNA molecule, you must now construct a complementary, antiparallel chain of DNA nucleotides that will hydrogen bond with the DNA chain you have just constructed. The remaining 20 nucleotides must be linked together in the following manner:
   A. The 3' pegs of the new strand should be aligned in the opposite direction of your first strand.

   B. The nucleotides should be attached so that cytosine nucleotides on the initial strand "bond" with guanine nucleotides on the new strand, original strand guanine bonds with cytosine nucleotides, adenine with thymine, and thymine on the initial strand with new adenine nucleotides. (Figure 2).

   C. The nitrogen base pairs should now be connected with hydrogen bonds (clear connectors) to form a double-stranded DNA molecule (Figure 2). The order of the nitrogen bases in the DNA molecule codes for specific hereditary information. If the sequence of base pairs in the chain is rearranged, the meaning of the genetic code will change.

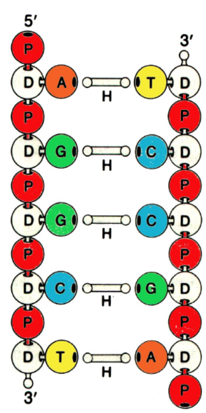


Figure 2. A simulated portion of double-stranded DNA

Take your DNA molecule apart and rearrange the nucleotide order to change the DNA code. Remember to align your two new strands of nucleotides in opposite directions. Check for proper base pairing: adenine with thymine and cytosine with guanine. Save your completed DNA molecule for Exercise II.

Exercise II: DNA Replication

Materials needed per lab group

- DNA molecule from Exercise I
- 10 additional adenine (A) deoxyribose nucleotides (each comprised of one orange adenine bead, one white deoxyribose bead and one red phosphate bead)
- 10 additional guanine (G) deoxyribose nucleotides (each comprised of one green guanine bead, one white deoxyribose bead and one red phosphate bead)
- 10 additional cytosine (C) deoxyribose nucleotides (each comprised of one blue cytosine bead, one white deoxyribose bead and one red phosphate bead)
- 10 additional thymine (T) deoxyribose nucleotides (each comprised of one yellow thymine bead, one white deoxyribose bead and one red phosphate bead)
- 20 additional clear connectors for Hydrogen bonds

Procedure

1. If you have taken your DNA molecule apart, reassemble your double-stranded DNA molecule.

2. Place your DNA molecule on your desktop so that the 5' end of the upper strand and the 3' end of the lower strand are on the left. The 3' end of the upper strand and the 5' end of the lower strand should be on the right. (Figure 3.)

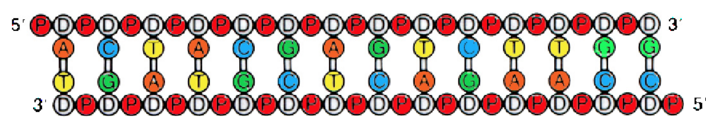


Figure 3. DNA Molecule

3. Start at the right side and unsnap the hydrogen bonds between the first 8 pairs of nitrogen bases, separating the single strands of DNA. DNA replication always occurs in the 5' to 3' direction from the 3' to 5' template (Figure 4). This sounds stranger than it really is. The upper right 3' end of your original DNA molecule is called the leading strand because replication starts at that point. Since the chains of DNA are antiparallel, or opposite, the new nucleotides you attach will be in a 5' to 3' direction.

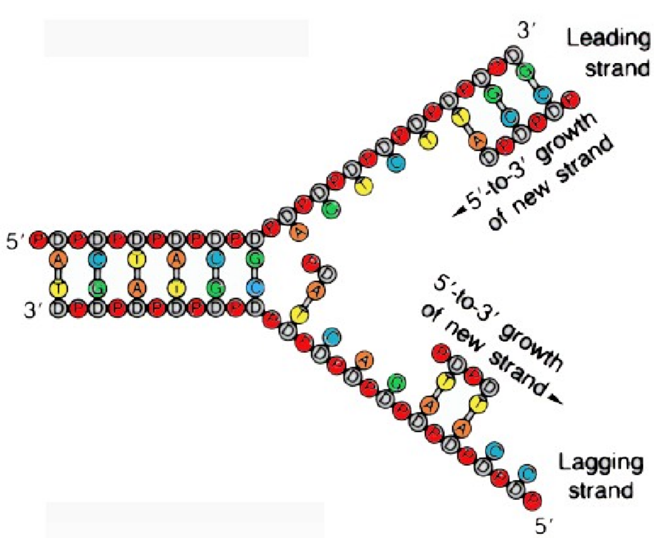
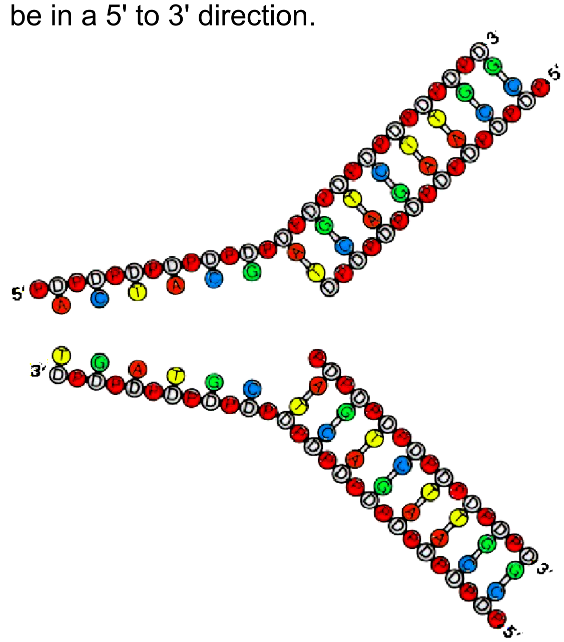


Figure 4. Separation of the two DNA strands



DNA replication

4. Attach new complementary nucleotides to the separated DNA molecule. The nucleotides you bond to the leading DNA strand will have the 3' end of the sugar molecule free. Attach the free 3' peg of the sugar to the 5' phosphate group of each new nucleotide being added to the growing strand. Make hydrogen bonds between the complementary nitrogen bases (adenine to thymine and guanine to cytosine). The DNA replication will occur one nucleotide at a time. As the replication continues, the original DNA will continue to "unwind", and the leading strand will replicate continuously.

5. The lower end of your original DNA molecule, which terminates at the 5' end, has to replicate away from the separation point, rather than toward it as the upper strand can. This lower end is called the lagging strand, and has to replicate in short, discontinuous pieces, but always in a 5' to 3' direction. You can replicate your lower strand in small segments, too.

6. After you have replicated the first 8 nucleotides, separate remaining nucleotide pairs of your original DNA molecule, and continue replication as before. When you finish you should have two DNA molecules, each identical to your original DNA strand. (Figure 5.) You may want to repeat your DNA replication until you are confident that you understand the process.

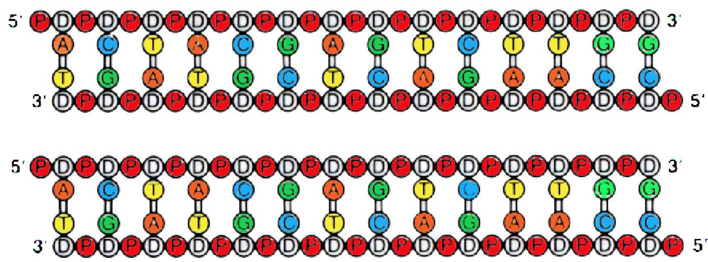


Figure 5. Replicated DNA Molecule

Thought Questions

1. What enzymes are involved in the process of DNA replication? What do each of these enzymes do during DNA replication?

2. How would you represent primase and the RNA primer in this exercise?

At the completion of the exercise, please separate the assembled pop-beads into their nucleotides and return the nucleotides and hydrogen bond connectors to the appropriate containers. Return other materials to the designated locations.

This laboratory exercise is adapted from Carolina Biological Supply's DNA Simulation Biokit # 17-1030.