Pilot Study Version
DNA Starter Kit
1-Group Set

3-D Molecular Designs
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DNA Replication and Transcription Activity

The purpose of this exercise is to explore the steps involved in DNA replication and transcription, simulating these activities using foam models of the nucleotides. At the end of this activity, you should be able to describe each process and identify the enzymes involved.

Open the bag labeled “DNA Starter Kit” and separate the parts. Each kit should contain:

- 12 red adenine
- 12 blue cytosine
- 12 green guanine
- 12 yellow thymine
- 6 white uracil
- 4 long gray DNA backbone pieces – note the arrows on the backbone – point from 5’ phosphate end to 3’ OH end
- 24 gray sugar-phosphate backbone pieces (representing deoxyribose linked to a phosphate for DNA)
- 12 purple sugar phosphate backbone pieces (representing ribose linked to a phosphate for RNA)
- 56 orange phosphate pieces
- 48 orange phosphate linkers

If something is missing from your kit or you have too many pieces, notify your instructor.

Construct Double-Stranded DNA

1. Look at the long gray backbone piece. Note the arrows on one side of these pieces:

   ![Arrow pointing from 5' to 3']

   ![Arrow pointing from 3' to 5']

   5’ phosphate end of the backbone  3’ OH (sugar) end of the backbone

This model is a schematic model – since the sugar and phosphate structures are not clearly evident. The holes in the backbone are the attachment sites for the nucleotides, so they represent the 1’ carbon of the sugar molecules.

The arrows point from the 5’ phosphate end of the backbone towards the 3’ OH (sugar) end of the backbone. The smaller individual sugar-phosphate pieces also have an arrow that points from the 5’ end to the 3’ end of the piece:
In the smaller pieces, the shape of the sugar (pentagon) is more visible, and the phosphate is represented by the circle. You’ll also see a stamp that shows $P \rightarrow S$ on this model as well.

Below the images of the schematic phosphate-sugar backbone pieces is a more accurate representation of the backbone, based on the X-ray crystal structure, displayed in Jmol.

The Jmol image is color-coded, such that:

- Gray is carbon
- Red is oxygen
- Orange is phosphorus

In each depiction, the phosphate group is circled in red, and the sugar group is circled in blue.

Each of these models, though different, represents the same structure.

All nucleic acids (both DNA and RNA) are synthesized in the 5’ to 3’ direction – by polymerases. DNA polymerases make DNA, and RNA polymerases make RNA.

2. Using 3 each of adenine, cytosine, guanine and thymine and a long DNA backbone piece, construct a segment of single stranded DNA. Place the nucleotides in any order on the chain. (See figure at right.)
3. Next, build the second strand of DNA **complementary** to the first, using 3 of each of the nucleotides A, G, C and T, and a second long DNA backbone. Be sure the backbone is running **antiparallel** (in the opposite direction) to the first backbone. Add the diphosphate to the 5’ end of this DNA strand, and join the base pairs.

4. Twist your DNA into a right handed helix by holding the two ends in your hands and twisting toward you with your right hand and away from you with your left hand. Verify that this is a right-handed helix. (Hint: Imagine walking up the DNA as a spiral staircase. Which hand is on the OUTSIDE railing?)

5. **Complete Part A of the DNA Replication and Transcription Worksheet before proceeding.**

**DNA Replication**

1. DNA is synthesized by DNA polymerase using nucleotide triphosphates as substrates. Build nucleotide triphosphates with the remaining A, G, C and T bases. Each of these will have a base (A, G, C or T) joined to a gray sugar (the pentagon side of the gray sugar phosphate). See illustration at right. The base is attached to the 1’ carbon and the phosphate group is attached to the 5’ carbon of the sugar molecule, but these individual atoms are not identifiable in this model of DNA. Two phosphate groups will be attached to the phosphate using the orange phosphate connectors.

2. Create replication bubble – pulling the middle 6 bases apart. The end bases will remain intact for the time being.
3. New DNA strands are synthesized in the 5’ to 3’ direction – so opposite directions on each strand.

4. Energy for each nucleotide addition comes from splitting off the two phosphates. Just as ATP is an energy carrier molecule, so are GTP, CTP and TTP.

5. Initiation of DNA replication begins with a short RNA primer. Since you are working with such a short strand of DNA, we’re not going to simulate the RNA primer in this activity.

6. Add a nucleotide that is complementary to the nucleotide at the 3’ end of one of the template strands within the replication bubble. Be sure you orient the nucleotide so the arrows on the sugar-phosphate piece are running in the opposite direction from the arrows on the backbone of the template strand. Similarly, add a nucleotide complimentary to the nucleotide at the 3’ end of the other template strand. Make sure that the arrows on the backbone pieces on the two strands point in opposite directions. Note that replication on the two strands does not start in the same place.

7. Bring in the next nucleotide on each strand – being sure to ‘grow’ your new chain in the 5’ to 3’ direction. Remove 2 phosphate groups as you add each new nucleotide to the sugar of the preceding nucleotide. Note that the first nucleotide retains its phosphate groups. Recall that ATP is considered the “energy currency” of the cell, and that removing one or more phosphate groups from ATP releases energy to drive chemical reactions. Similarly, CTP, GTP and TTP also have stored energy in the phosphate bonds. Energy released from cleaving the phosphates from each of these molecules is used to form the phosphodiester bond in the DNA backbone.

8. Build your two DNA strands, 5’ to 3’, opening the bubble and adding bases complimentary to the template strand.

9. When you are done, you should have two double stranded DNA sequences that lack two nucleotides on the 5’ end. Because the replication bubble proceeds in BOTH directions, and DNA synthesis can only occur from 5’ to 3’, for each replication fork, there is a leading strand (synthesized 5’ to 3’) and a lagging strand (running 3’ to 5’). In order to fill in the gaps on the lagging strand, synthesis is delayed, then build 5’ to 3’ in
segments of approximately 200 nucleotides. These short segments were discovered by Okazaki and coworkers, they are aptly named “Okazaki fragments”.

10. Simulate the filling in of the Okazaki fragments by adding bases at the 5’ end of your DNA chains. Be sure to add the bases 5’ to 3’. Remember that DNA polymerase can add bases one at a time in the 5’ to 3’ direction. But DNA polymerase cannot join the backbone between two nucleotides that have already been added to the chain.

11. A separate enzyme, DNA ligase, joins the backbone between the Okazaki fragments. Simulate DNA ligase to create intact sugar-phosphate backbones on both of your DNA strands.

12. At this point you should have two nearly identical DNA double strands. What is the difference between the two strands? [Hint: Observe the solid vs. the pieced backbone pieces. Which strand is pieced on the two models?] The solid strand represents the ORIGINAL DNA, and the pieced strand represents the newly synthesized DNA. Back in the 1950’s scientists didn’t know exactly HOW DNA replication occurred – whether the old sequence remained intact and a completely new DNA was synthesized (termed “conservative replication”) or whether each strand served as a template for a new strand, so that the daughter DNA contained one old strand and one new strand (termed “semi-conservative replication” since one half of the DNA is conserved in the new strand). Through a series of experiments using two isotopes of phosphorous, Meselson and Stahl were able to demonstrate that DNA replication is semi-conservative and that each strand serves as a template to build a new complementary strand of DNA. Your model demonstrated semi-conservative replication.

6. Complete Part B of the DNA Replication and Transcription Worksheet before proceeding.

Transcription into mRNA

1. Human DNA contains sequences coding for genes, sometimes thousands of bases long, as well as regulatory sequences that are involved in turning genes on and off. Sometimes regulators function more like dimmer switches, fine tuning the expression of genes. Your segment of DNA is too short to model regulatory sequences. – Instead, you’ll focus on the basics of transcription into RNA.

2. When DNA is transcribed into mRNA, only one strand of DNA is used. This is termed the template strand. Select one of your double-stranded DNA molecules to use. Take apart the other model of DNA. (You’ll be using pieces of it for the next step.) Next, create ribonucleotide triphosphates by attaching the lavender ribose phosphate, then two additional orange phosphate groups, to each of three A (red), C (blue), G (green) and U (white) nucleotides.
You will be using the newly synthesized strand of DNA (the one with the pieced backbone) as the template strand. The RNA polymerase binds to the DNA and unzips the DNA, forming a bubble similar to replication. In this simulation, you should form a bubble of 6 bases beginning two bases in from the 3’ end of the template strand. Since RNA polymerase builds the RNA from 5’ to 3’, it must read the DNA from 3’ to 5’. Using the template strand, build a complementary mRNA. As the transcription bubble moves along the DNA, each time you unzip one DNA base pair to add a ribonucleotide to the growing mRNA chain, you should remove one ribonucleotide from the start of the mRNA molecule and rezip the DNA. In this simulation, only 4 base pairs of DNA should be unzipped at once. (In reality, about 10 base pairs are unzipped and bound to mRNA at any given time.) You will have a double stranded DNA model with a few bases unzipped. One strand of the unzipped DNA will be bound to mRNA, which will have a single stranded “tail” that grows as RNA polymerase moves down the DNA.

3. Once you reach the end of the DNA, the mRNA will continue detaching from the DNA and the DNA will rezip. In the end, you should have a single-stranded mRNA molecule complementary to the DNA template strand, and a double-stranded DNA molecule.
4. **Complete Part C of the DNA Replication and Transcription Worksheet before proceeding.**

5. Disassemble your DNA and mRNA and place all the parts in the appropriate bags.