Classroom Activity 1: Simple 8 Base Pair Duplex

Learning Objectives:

By the end of this activity students will:

- Be able to distinguish nucleobases from nucleotides.
- Recognize that single-stranded ssDNA has 5’ and 3’ ends.
- Be able to build a double helical duplex of B-form DNA from two antiparallel strands of ssDNA.

What You Will Need:

1. 16 DNA backbone pieces
2. 8 Adenine (red) and 8 Thymine (blue) nucleobases

Steps to Build and Investigate a Simple Duplex:

1. Assemble all 16 nucleotides, which each are built from connecting the duckbill connector of each nucleobase to its corresponding backbone (see Figure 1).

2. Build two 8-base-long single-stranded complementary DNA (ssDNA) oligomers. The simplest way to start is to build an 8 nucleotide polyA strand and an 8-nucleotide poly T strand. Arrange these strands antiparallel to each other as shown in Figure 2.

3. Hybridize these two strands by connecting each Adenine (red) with Thymine (blue) to create a simple duplex as shown in Figure 3.

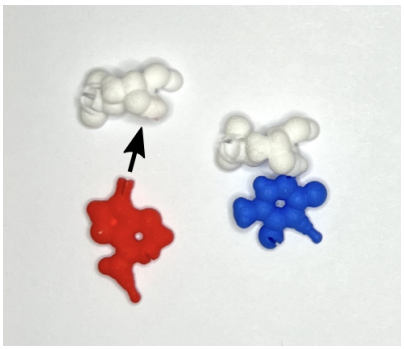

Figure 1. Nucleotides are built by snapping together nucleobases and backbone segments using the duckbill connectors.

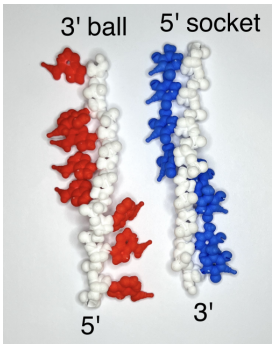

Figure 2. Flexible 8 nt ssDNA can be arranged antiparallel to each other before hybridization.

Figure 3. B-form duplex DNA takes on a helical conformation center in which the nucleobases are largely parallel to each other, and the beginnings of a major and minor groove are visible.

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Classroom Activity 2: DNA Polymerase Action

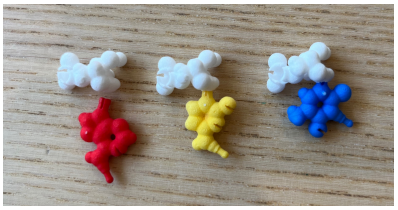

Figure 1. In Step 1 nucleotides are built by snapping together the nucleobases and backbone segments using the duckbill connectors.

Learning Objectives:

By the end of this activity students will:

- Be able to distinguish nucleobases from nucleotides.
- Be aware that DNA polymerase adds nucleotides in the 5' to 3' direction.
- Understand how polymerases can build complements and complete duplexes.

What You Will Need:

1. 64 DNA backbone pieces
2. 16 Adenine (red), 16 Thymine (blue), 16 Cytosine (yellow) and 16 Guanine (green) nucleobases

Steps to Simulate the Action of DNA Polymerase:

1. Assemble all 64 nucleotides, which each are built from connecting the duckbill connector of each nucleobase to its corresponding backbone (see Figure 1).

2. Build a 32 nt long "template" single-stranded DNA (ssDNA). For practical reasons aim for 50% GC content to avoid running out of nucleotides in later steps (see Figure 2A).

3. Build a 15 nt long "primer" sequence that is complementary to the 15 bases at the 3' end of your template strand. Once you have built your primer, hybridize it to your template strand at the 3' end of the template (see Figure 2A and B).

4. To simulate the action of polymerase, extend the primer in the 5'-to-3' direction with complementary nucleotides. Remember Adenine (red) binds to Thymine (blue) and Cytosine (yellow) binds to Guanine (green). At each step connect your backbone to the extended primer using the ball and socket connections and hybridize the next nucleobase to its complement using the pin connectors (see Figure 2C).

5. When you are done you have a complex duplex (Figure 2D). If you have enough pieces to double the activity, a fun extension of this process is to simulate multiple steps of PCR. For example, separate the strands and then apply primers to both strands and simulate polymerase action on each. This will result in 2 identical duplexes.

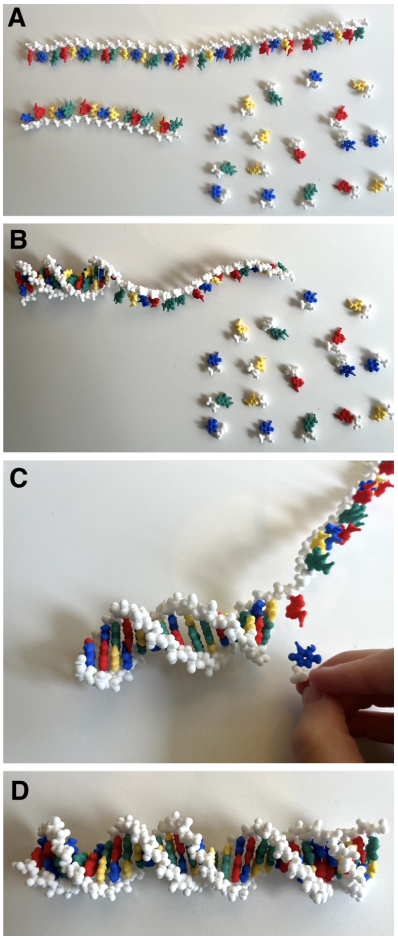

Figure 2. To simulate the action of a polymerase, build your template strand (A) and primer (B) as described in steps 2 and 3. Hybridize the primer to the 3' end of the template. Simulate polymerase action by extending the primer in the 5'-to-3' direction with complementary nucleotides, hybridizing into the duplex at each step (C) until complete (D).

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Classroom Activity 3: Molecular Tweezers

Learning Objectives:

By the end of this activity students will:

- Be able to describe how strand displacement reactions enable the dynamic actuation of DNA-based nanosystems
- Have experience building simple molecular tweezers

What You Will Need:

1. 236 DNA backbone pieces (15 bundles of 16 backbone pieces required)
2. 59 Adenine (red), 60 Thymine (blue), 59 Cytosine (yellow) and 58 Guanine (green) nucleobases
3. We recommend students read the article in which this nanosystem was introduced, "A DNA-fueled molecular machine made of DNA" by Yurke et al. *Nature*. 406(6796):605-8 (2000).

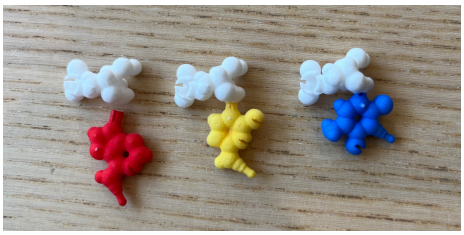

Figure 1. In Step 1 nucleotides are built by snapping together the nucleobases and backbone segments using the duckbill connectors.

Steps to Build and Investigate Molecular Tweezers:

1. This activity is best done in groups of 3-5, because each student can put together one of the 5 required sequences below. Groups of 3 can build the first three sequences and assemble an open tweezers. Groups of 4 can build the first 4 sequences and assemble the locked tweezers, and Groups of 5 can assemble all 5 sequences and perform strand displacement to unlock the tweezers and return them to their open configuration.

2. Start by connecting the backbone and nucleobase pieces using the duckbill connectors as shown in Figure 1. Make sure to note that the 5' end of the backbone is the socket end.

A	5′ TGCCTTGTAAGAGCGACCATCAACCTGGAATGCTTCGGAT 3′
B	5′ GGTCGCTCTTACAAGGCACTGGTAACAATCACGGTCTATGCG 3′
C	5′ GGAGTCCTACTGTCTGAACTAACGATCCGAAGCATTCCAGGT 3′
F	5′ CGCATAGACCGTGATTGTTACCAGCGTTAGTTCAGACAGTAGGACTCCTGCTACGA 3′
F′	5′ TCGTAGCAGGAGTCCTACTGTCTGAACTAACGCTGGTAACAATCACGGTCTATGCG 3′

Table 1. Sequences for the molecular tweezers (Yurke et al. *Nature* (2000).

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3. Students should work together to hybridize A to B as shown in Figure 2A. Note these bases are highlighted in yellow in the table above, and as a reminder, the oligonucleotide backbones must be antiparallel during hybridization, which means that one is oriented 5'-->3' while the other is oriented 3'-->5' during hybridization.

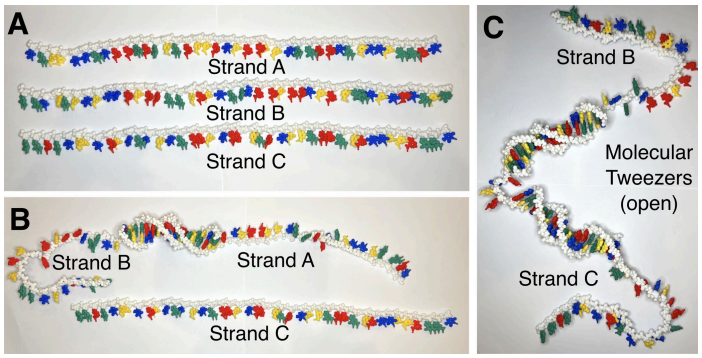

Figure 2. (A) Build Strands A, B, and C, and then follow steps 2 and 3 as shown in (B) and (C) to construct the open tweezers.

4. Next, students should work together to hybridize A to C as shown in Figure 2C. Note these complementary bases are highlighted in gray in the table above. Now you have molecular tweezers in the open configuration!

5. To begin closing the tweezers hybridize the first section of sequence F to the single stranded region of sequence B (see Figure 3A and 3B). These complementary bases are written in blue above.

6. Next to fully close the tweezers hybridize the second section of sequence F to the single-stranded section of C extending from the tweezers. These complementary bases are written in green above. Finishing this step fully closes the tweezers as shown in Figure 2B. Note: Due to the geometry configuration of sequence F, it may not be possible to connect a couple of the central bases of sequence F (Figure 3B).

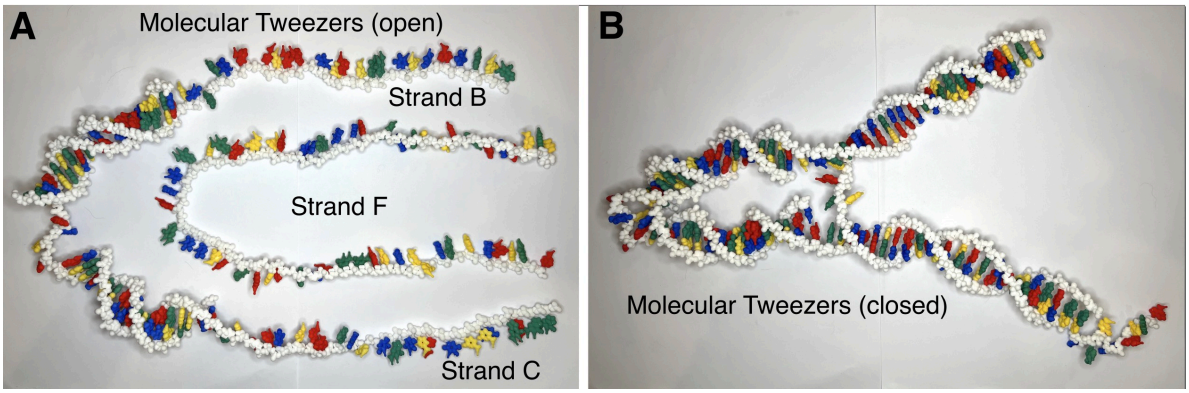

Figure 3. (A) Build Strand F and arrange the complementary sections along the regions of Strands B and C extending from the open tweezers. (B) Hybridize F to single-stranded regions of the open tweezers to close them. Note that some of the central bases of Strand F may not be able to fully bind, and also note the single-strande toehold that now extends from the closed tweezers.

7. The 8-bases of sequence F that extend single stranded from the closed tweezers serve as a toehold. To remove F from the tweezers and re-open the tweezers, bind sequence F' to the toehold and slowly displace F from C and then from B. This process is called strand displacement.

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Classroom Activity 4: Holliday Junction Investigation

Learning Objectives:
By the end of this activity students will:

- Understand the sequence complementarity in Holliday Junctions
- Be able to construct a Holliday Junctions
- Recognize that DNA is flexible and can bend substantially in nanostructures
- Appreciate that limited base unpairing occurs at the center of a Holliday Junction

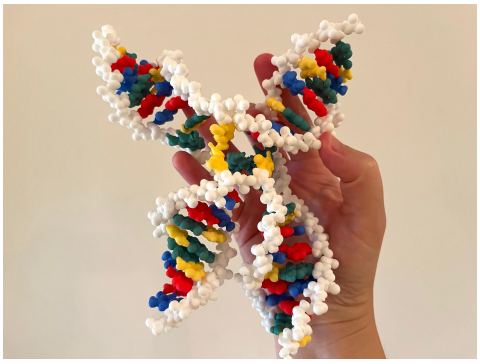

Figure 1. Holliday Junction model.

What You Will Need:

1. 64 DNA backbone pieces
2. 16 Adenine, 16 Cytosine, 16 Thymine, and 16 Guanine nucleobases

Figure 2. Nucleotides are built by snapping together the nucleobases and backbone segments using the duckbill connectors.

Steps to Build and Investigate Holliday Junctions:

1. Assemble all 64 nucleotides, which each are built from connecting the duckbill connector of each nucleobase to its corresponding backbone (see Figure 2)
2. Build two 16-base-long single-stranded DNA (ssDNA) oligomers with any sequence. To connect nucleotides, snap together the ball element from one backbone piece into the socket of the next.
3. We can describe the first strand as having domains A1 and A2, while the second strand has domains B1 and B2 (see Figure 3). To create the third and fourth strands use the schematics in Figure 3 to create a third strand whose first half is a complement to B2 and whose second half is a complement to A1. Create a fourth and final strand whose first half is a complement to A2 and whose second half is a complement to B1.
4. Assemble your strands into an X-shaped pattern and hybridize the complementary bases (see Figure 4).

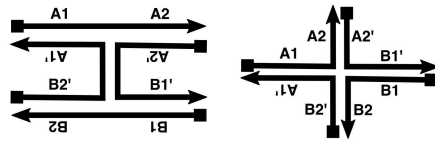

Figure 3. Holliday junctions can be represented at H-shape or X-shaped structures.

Figure 4. Arrange the strands in an X-shape and hybridize the complementary bases for each of the four domain duplexes. Note that you may not be able to connect all of the bases at the

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