Gene Expression I

Purpose:
To work with several different representations of DNA, RNA and protein in order to help you to understand:

- rules for DNA & RNA structure
- base-pairing
- DNA replication
- transcription including promoters & terminators
- translation including start & stop codons

First: Notebook Review
To be sure that you have all the information you need for this lab, with your lab partners, go over the notes you took while doing the SPOC and from lecture and be sure you have the information listed below. You should fill in any gaps in your notes so everyone in your group has all they need. You can check these items off as you go.

- The rules for DNA and RNA from lecture
- How transcription is controlled and how to figure out which mRNA will be made from a given DNA molecule
- How mRNA splicing is controlled and the difference between introns and exons
- How translation is controlled and how to figure out which protein will be made from a given mRNA molecule

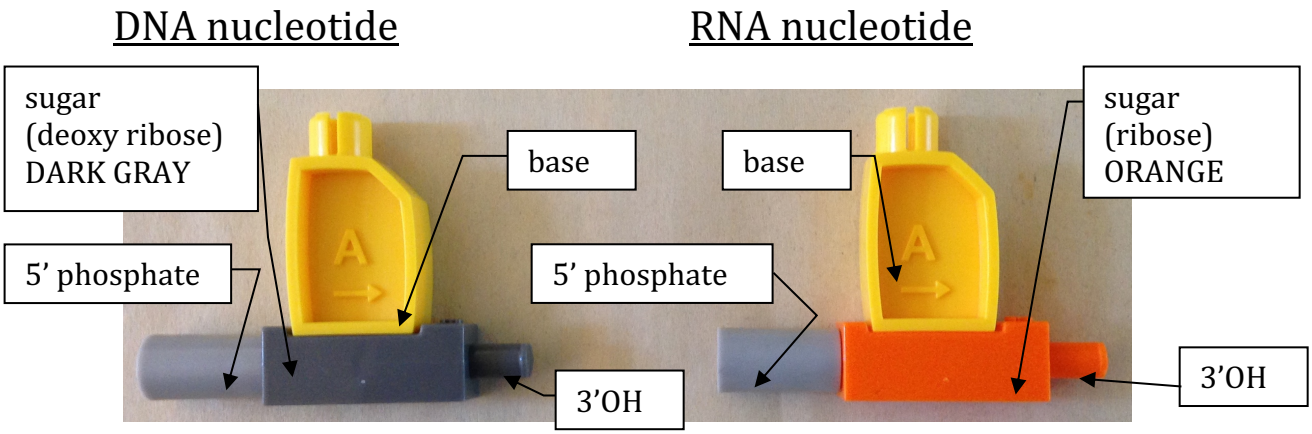
You will then discuss these with your TA as a class to clarify any issues that remain.

Part I: Giant DNA

Today, you will be working with some very scaled-up MIT-copyrighted DNA and RNA molecules developed by Karthy Vandiver and MIT's Edgerton Center (http://edgerton.mit.edu/dna-proteins-sets).

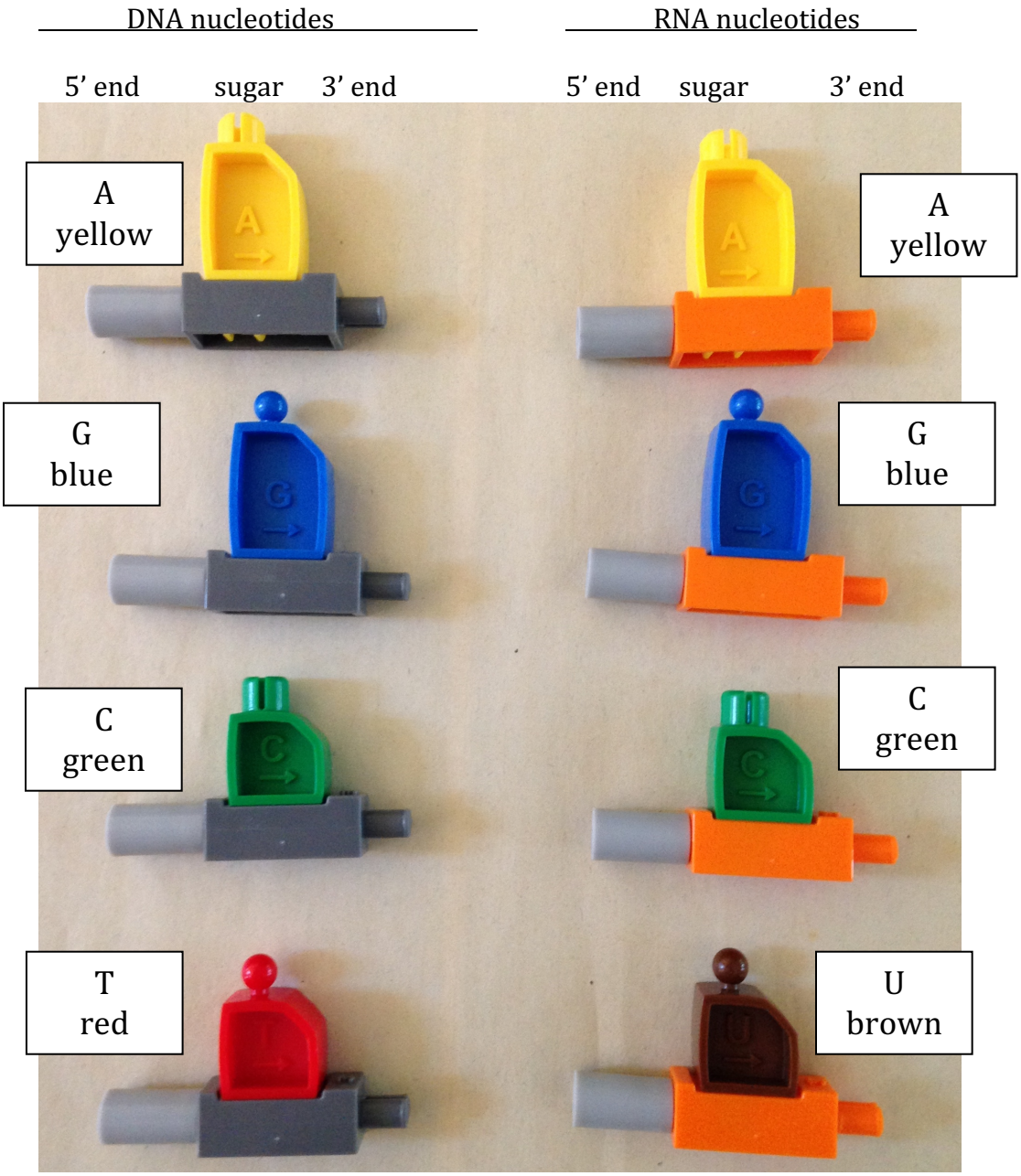
To give you a sense of scale, in these kits, each base is about 1.625 inches long (scaled up about 40 million times larger than a real DNA base). In class, we'll talk about the □-globin gene, which is about 2000bp (base pairs) long; if you built a model of the □-globin gene with these parts, it would be about 270 feet long. The smallest chromosome in humans is Chromosome #22 which is 50Mbp (million base pairs) long; in these models, that would be 1300 miles long – the distance from Boston to Topeka, Kansas. If you strung all the DNA in a single human cell – 6.4×10^9 bp – in a line, it would be 164,000 miles long with these parts – most of the way to the moon. What you'll be doing today is a very small part of a large picture…

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



Note that the 3' end of each nucleotide is indicated by a little 3' marked on the sugar.

The four DNA and RNA bases are shown below:

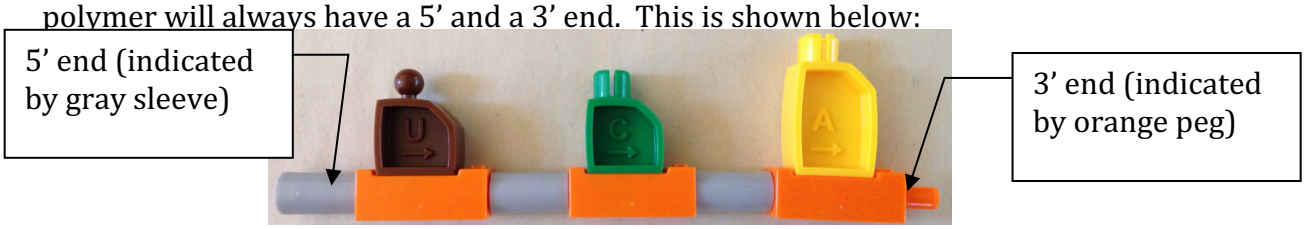


The letter for each base is molded into the side of the base.

Important note: In this lab, we will not spell out all the details of what you have to do in each step. 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.

- The correct bases pair via hydrogen bonds simulated by the ball and socket joint on each base.
- 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.

- Since each nucleotide has a 5' and a 3' end, if they are connected 5' to 3' in a long polymer, this polymer will always have a 5' and a 3' end. This is shown below:

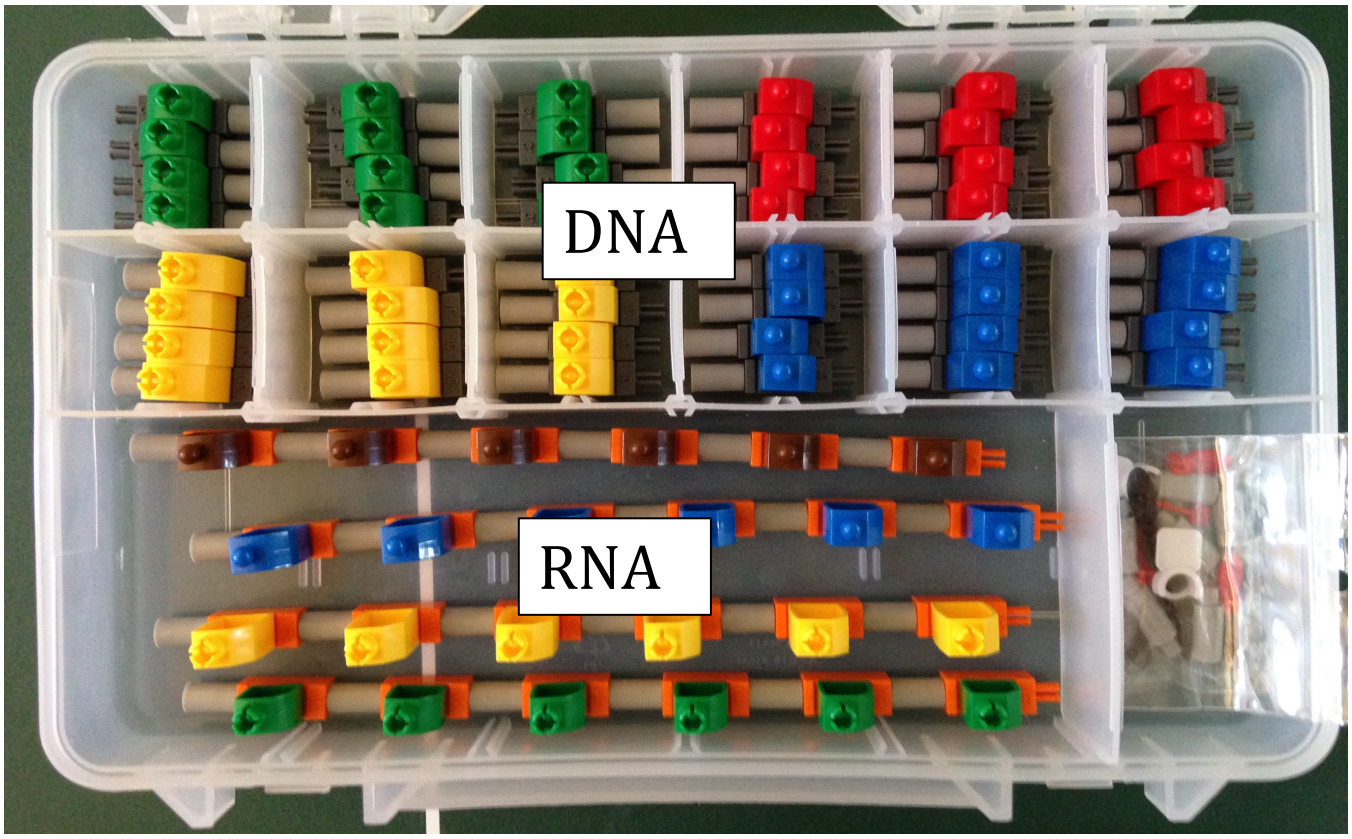


The sequence of this small RNA molecule would be: 5'-UCA-3'

Procedure:

1) Check your kit. You should have:

- 12 DNA A's (yellow base)
- 12 DNA G's (blue base)
- 12 DNA C's (green base)
- 12 DNA T's (red base)
- 6 RNA A's (yellow base)
- 6 RNA G's (blue base)
- 6 RNA C's (green base)
- 6 RNA U's (brown base)



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

5′–ACGGTACGCTAT–3′

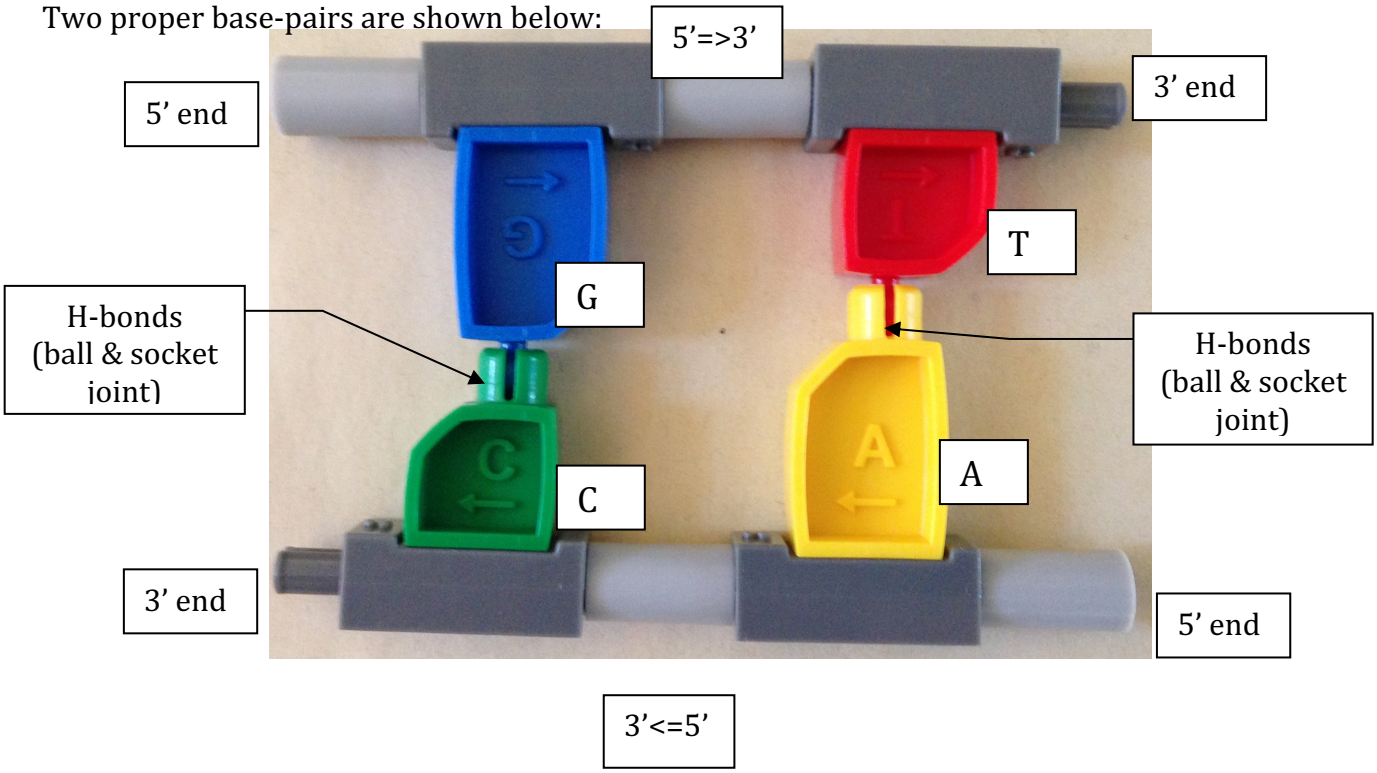
Notice that all the sugars run in the same direction.

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

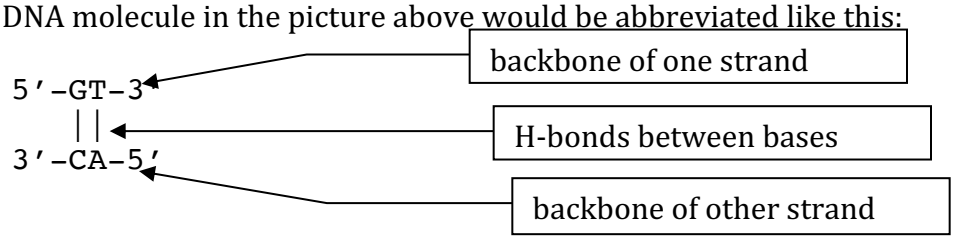
Note:

- the strands must be anti-parallel (run 5' => 3' in opposite directions)
- large bases (A and G - purines) pair with small bases (C and T - pyrimidines); NEVER pair a large with a large or a small with a small (the ball & socket joints might let you do this, but it is biologically impossible).
- A pairs with T - (yellow with red) the joints won't let you pair it any other way
- G pairs with C - (blue with green) the joints 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:

Try GENTLY twisting the molecule to make a double helix.

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

5'-_____-3'

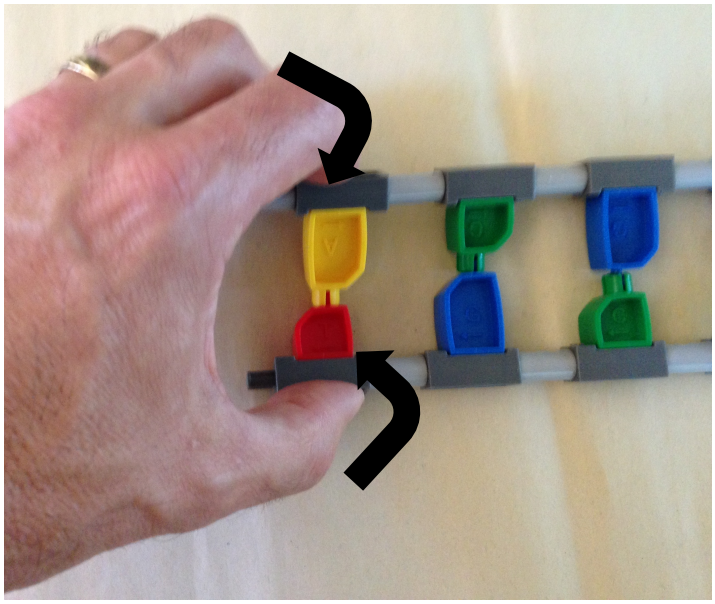
What is the sequence of the double-stranded DNA molecule you now have?

DNA Replication

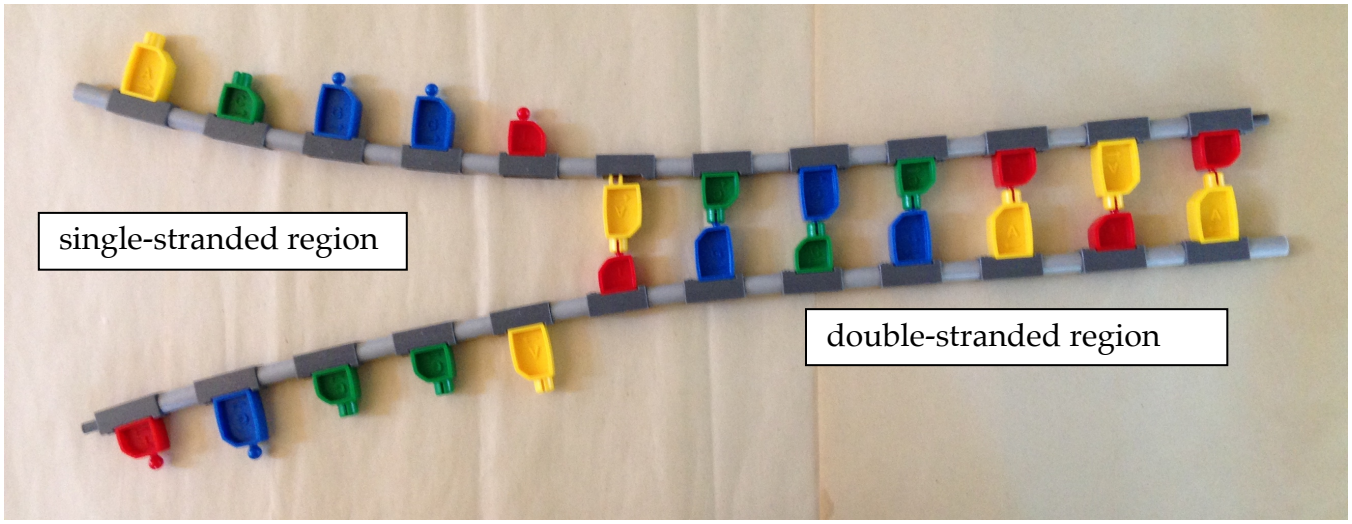
You will now simulate the replication of this DNA molecule.

VERY Important Note: Although you may be tempted to pull the bases apart to simulate breaking the hydrogen bonds – you should never do this since it will weaken the ball and socket joints.

Instead, you should gently pinch the backbone strands together as in the figure below. The ball and socket joints will open easily and not wear out – this is the way they were designed to open. Your TA will demonstrate.

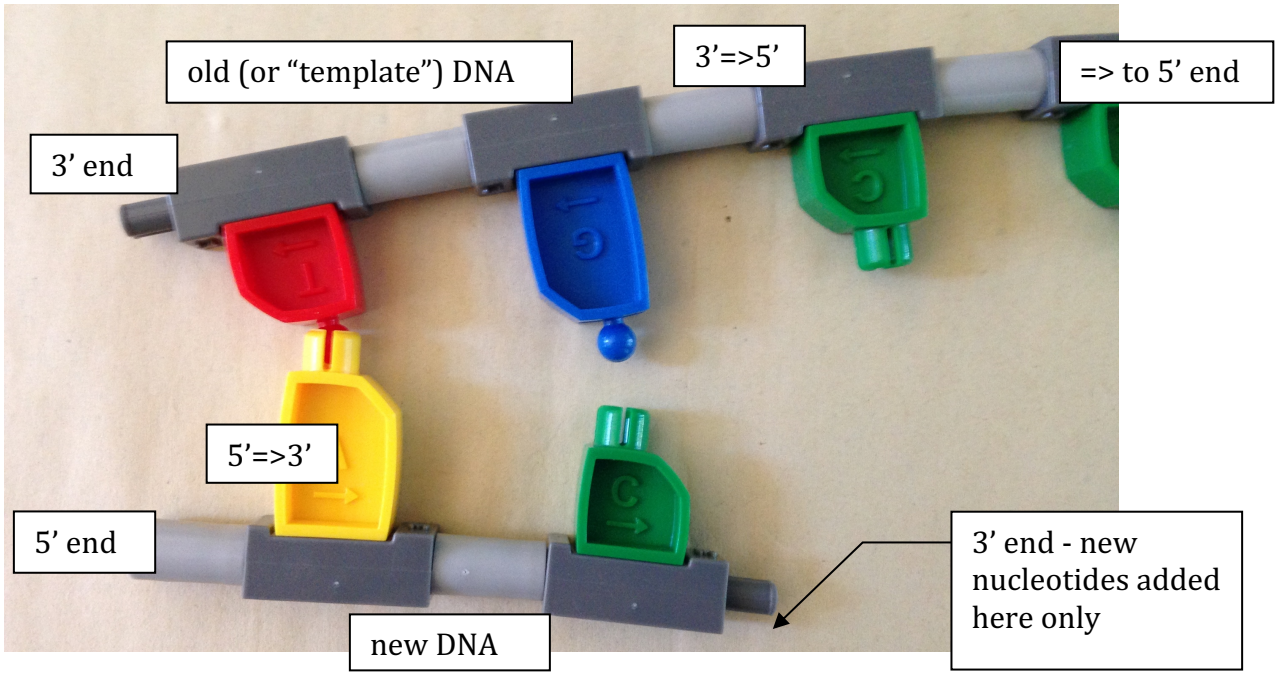


4) Prepare the left-hand end of the molecule for replication. Un-zip (break the hydrogen bonds - simulated by PINCHING the strands apart) the 5 base-pairs at the left end of your DNA molecule to make a region of single-stranded DNA. You should turn the bases to face out from the center to make them ready for the next steps. This is shown below:



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

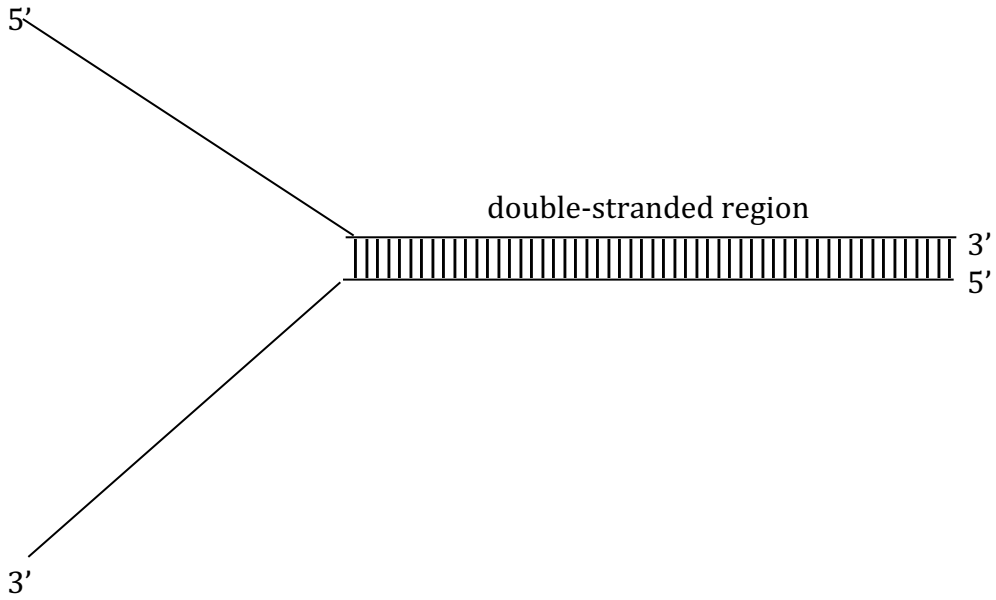
- the strands must be anti-parallel (run 5'=>3' in opposite directions)
- A pairs with T
- G pairs with C
- DNA polymerase can <u>only</u> add nucleotides to a 3' end. This is shown below:



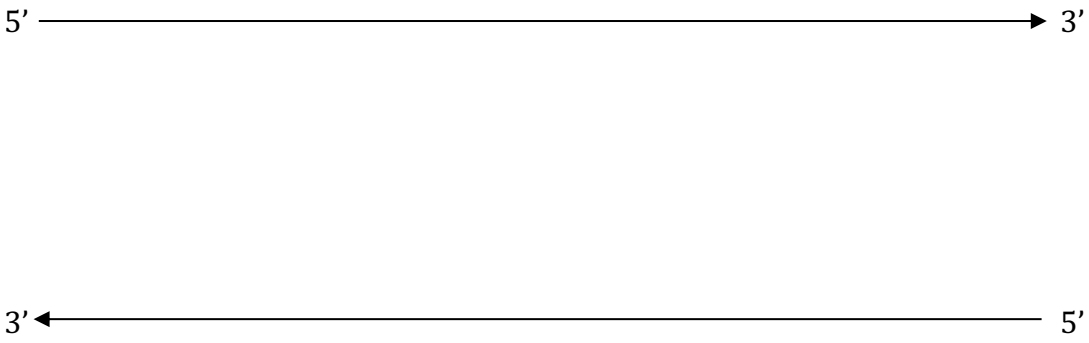
6) Continue replicating this strand until you have to stop - either because you've reached the end of the template strand or you've run into the double-stranded region.

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. On the drawing below, draw the new DNA strands; the solid lines represent the template DNA. Use a wavy line for the DNA you made in step (6) and a dotted line for the DNA you made in step (7). Be sure to indicate the 5' and 3' ends as appropriate.



List the differences between the replication on the two strands:

Leading strand:

Lagging strand:

* You will note that there are gaps in the backbone of the new DNA on the lagging strand. These are also present in the new DNA on the lagging strand in real cells. An enzyme called DNA ligase seals these breaks.

10) Disassemble the DNA molecules you made. This simulates the hydrolysis that occurs during digestion.

A Small Gene

In this part, you will build a small gene and simulate how it produces a protein.

11) Build the gene. Build a single-strand of DNA with this sequence (the spaces are to make it easier to keep your place in the sequence - they are not gaps in the backbone):

5′–CTATA AGCAT GCCCC TATGA GGGT–3′

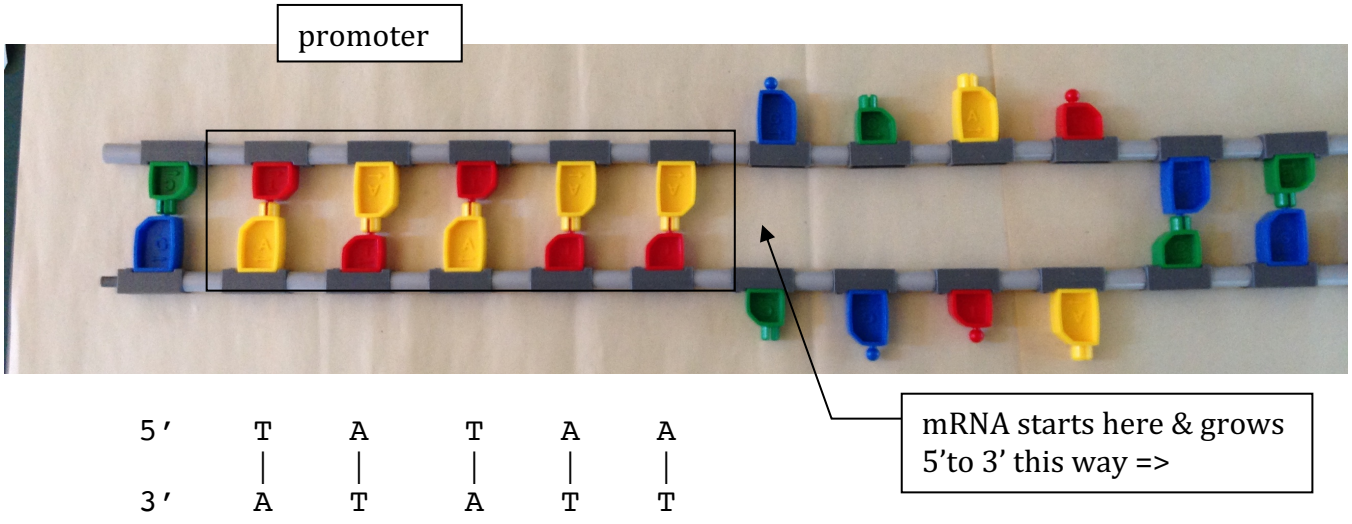
12) Build the corresponding other strand of DNA. If you have got the sequence exactly right, you will use up all of your DNA nucleotides.

Transcription

Transcription in this simulated organism starts at the first nucleotide after a promoter. In this organism, promoters have this sequence:

```
      DNA bases
5′–TATAAx.....–3′
   ||||||
3′–ATATTy.....–5′
```

The 5′ end of the mRNA starts at base pair x-y. This is shown below:




```
5′    T    A    T    A    A
      |    |    |    |    |
3′    A    T    A    T    T
```

Transcription in this organism ends at the base-pair just before the terminator. In this simulated organism, terminators have the following sequence:

```
      DNA bases
5′–xGGGT.....–3′
   |||||
3′–yCCCA.....–5′
```

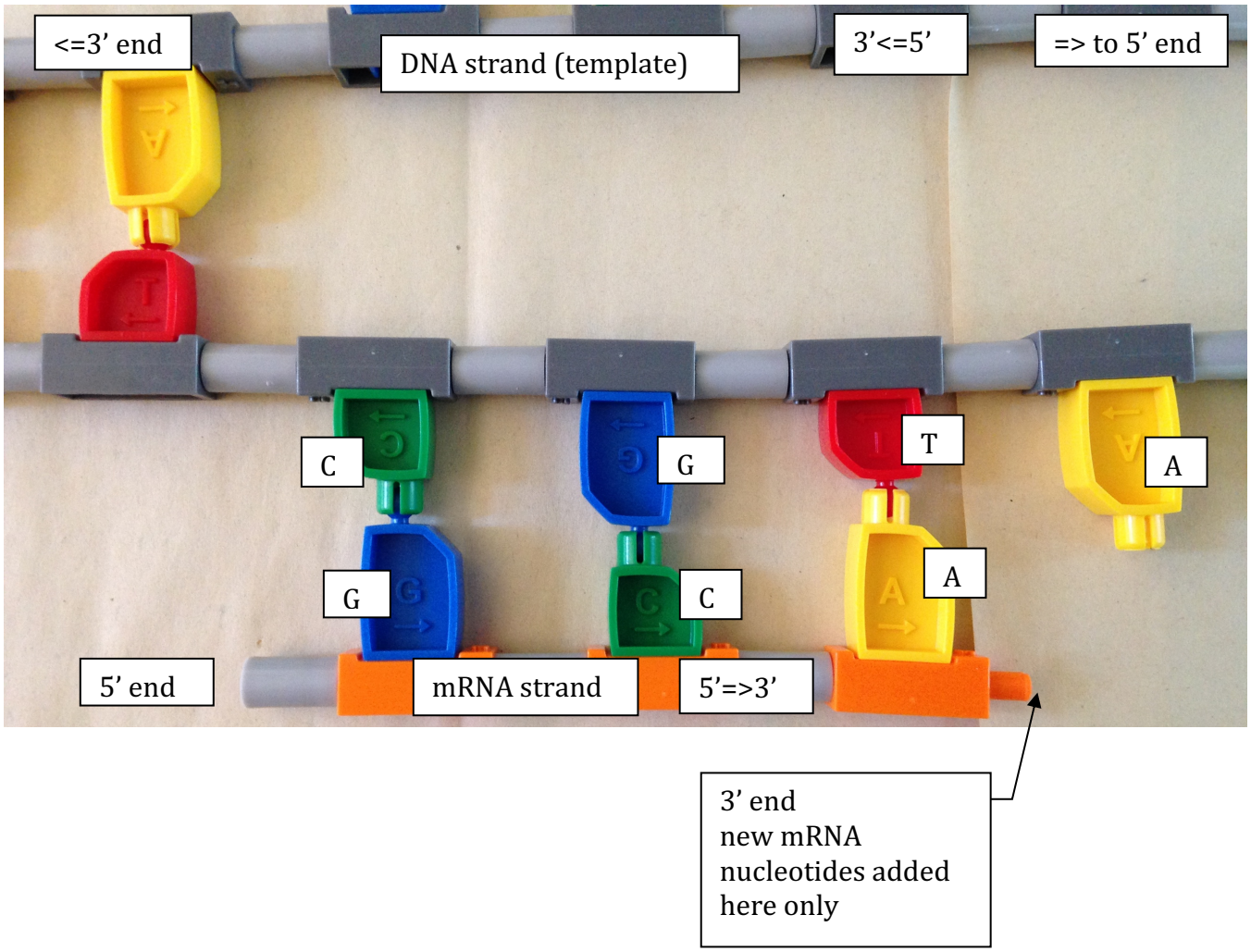
The 3′ end of the mRNA ends with base pair x-y.

13) Unzip (**PINCH**) the base-pairs from the end of the promoter to the start of the terminator - don't forget to flip the bases out so they'll be ready. This is shown in the picture on the previous page.

14) Make the mRNA using the following rules:

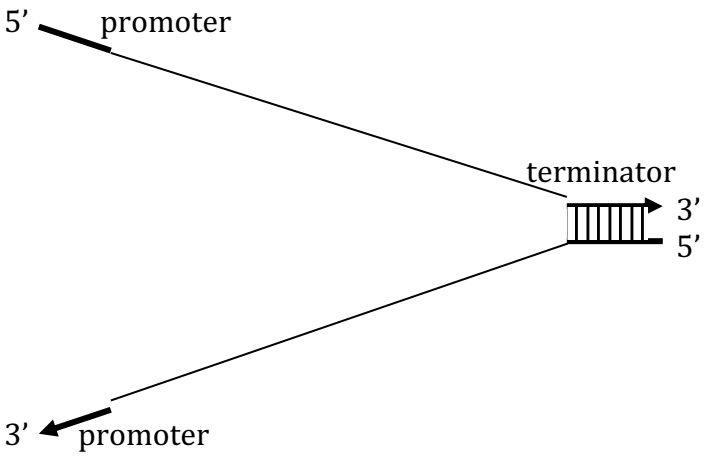
- the strands must be anti-parallel (run 5'=>3' in opposite directions)
- A pairs with U (brown)
- G pairs with C
- RNA polymerase can only add nucleotides to a 3' end.

Correct RNA-DNA base-pairs are shown below:



Notice that only one mRNA strand can be made that follows these rules.

The solid lines in the diagram below represent the DNA strands. Draw in the mRNA that you just made. Be sure to indicate the 5' and 3' ends.



Why is it not possible to make any other mRNA? Which rule(s) would this other mRNA break?

What is the sequence of this mRNA?

5'-_____-3'

Translation

15) **PINCH** to separate your mRNA from the DNA.

You will now act like a ribosome and read this mRNA 5' to 3' to produce a protein.

Ribosomes in all organisms start at the 5' end of the mRNA and look for the first start codon. This is 5'-AUG-3' and encodes the N-terminal methionine. Translation ends with a stop codon. A table of the genetic code can be found at the end of this section of the lab manual.

Acting as a ribosome, translate this mRNA.

What is the resulting protein sequence? (A table of the genetic code can be found at the end of this section of the lab manual)

N-_____-C

A mutant gene

Mutations are alterations in DNA sequence. You can simulate their effects by changing the LEGO bases at a particular place in your simulated gene.

16) The 12th base-pair in your gene is a C-G base pair. Change it to a G-C base-pair. That is, the original DNA in that region **was**:

```
5'-...TGCCC...-3'
      |||||
3'-...ACGGG...-5'
```

Change it to (the altered base-air is **bold-underlined**)

```
5'-...TGGCC...-3'
      |||||
3'-...ACCGG...-5'
```

What is the resulting mRNA sequence? 5'-_____-3'

What is the resulting protein sequence? N-_____-C

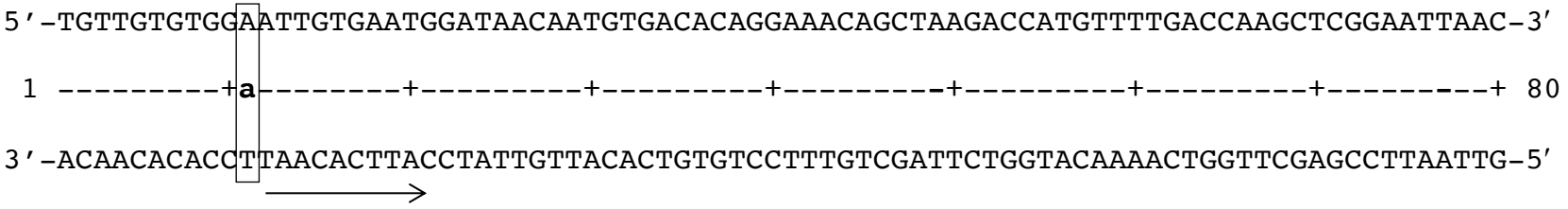
17) Disassemble your DNA and mRNA molecules and sort them into the kits. Check that your kit looks like the picture on page 5 before returning it to your TA.

Part II: Larger Genes

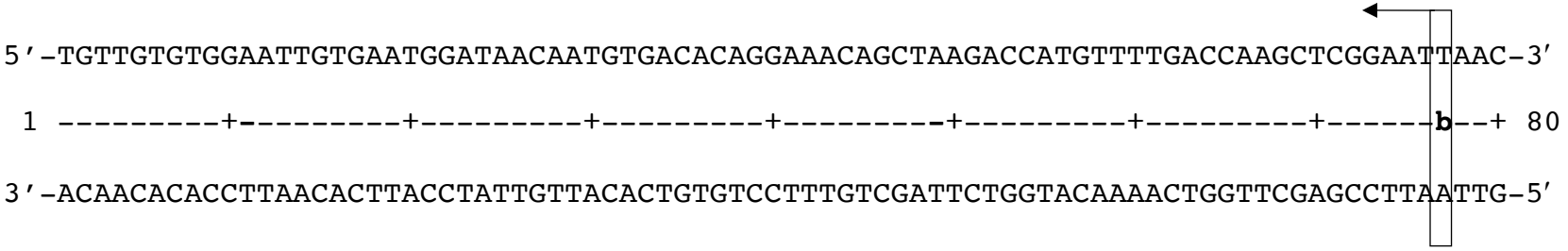
You will now apply what you have just learned to problems like those you might see on an exam. You should work through problem 4.1.2 (a) through (e) from Chapter 3 of *A Problems Approach to Introductory Biology*.

(4.1.2) Shown below is an 80 base pair segment of a hypothetical gene. It includes the promoter and the first codons of the gene. The sequences of both strands of the DNA duplex are shown: the top strand reads 5′ to 3′ left to right (1 to 80); the bottom, complimentary, strand reads 5′ to 3′ right to left (80 to 1).

a) Synthesis of the mRNA starts at the boxed A/T base pair indicated by the (a) below (#11) and proceeds left to right on the sequence below. Write the sequence of the first 10 nucleotides of the resulting mRNA.

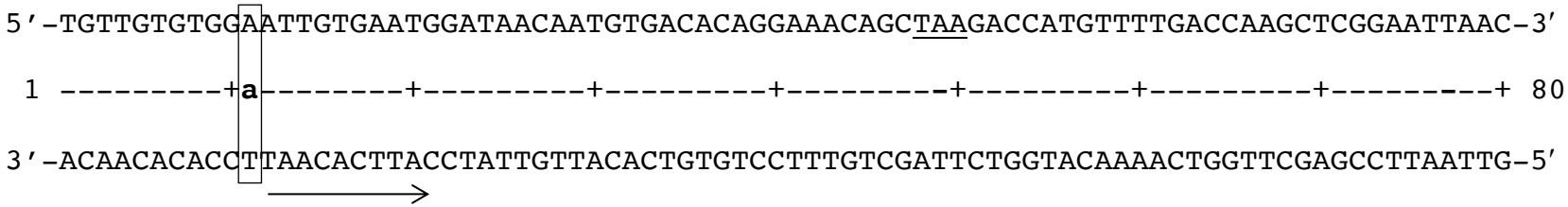


b) Suppose the synthesis of mRNA started at the boxed T/A base pair indicated by the (b) below (#77), and proceeded right to left. What would be the first five nucleotides of the mRNA?



c) The mRNA you just wrote has almost the same sequence as one of the DNA strands. Which DNA strand is this? What is the difference between it and the mRNA sequence?

This is the sequence from part (a):



d) What are the first three amino acids of the polypeptide that would result from translation of the mRNA from part (a)?

e) Does translation terminate at the UAA in the mRNA corresponding to the underlined bases at positions 48-50? Why or why not?

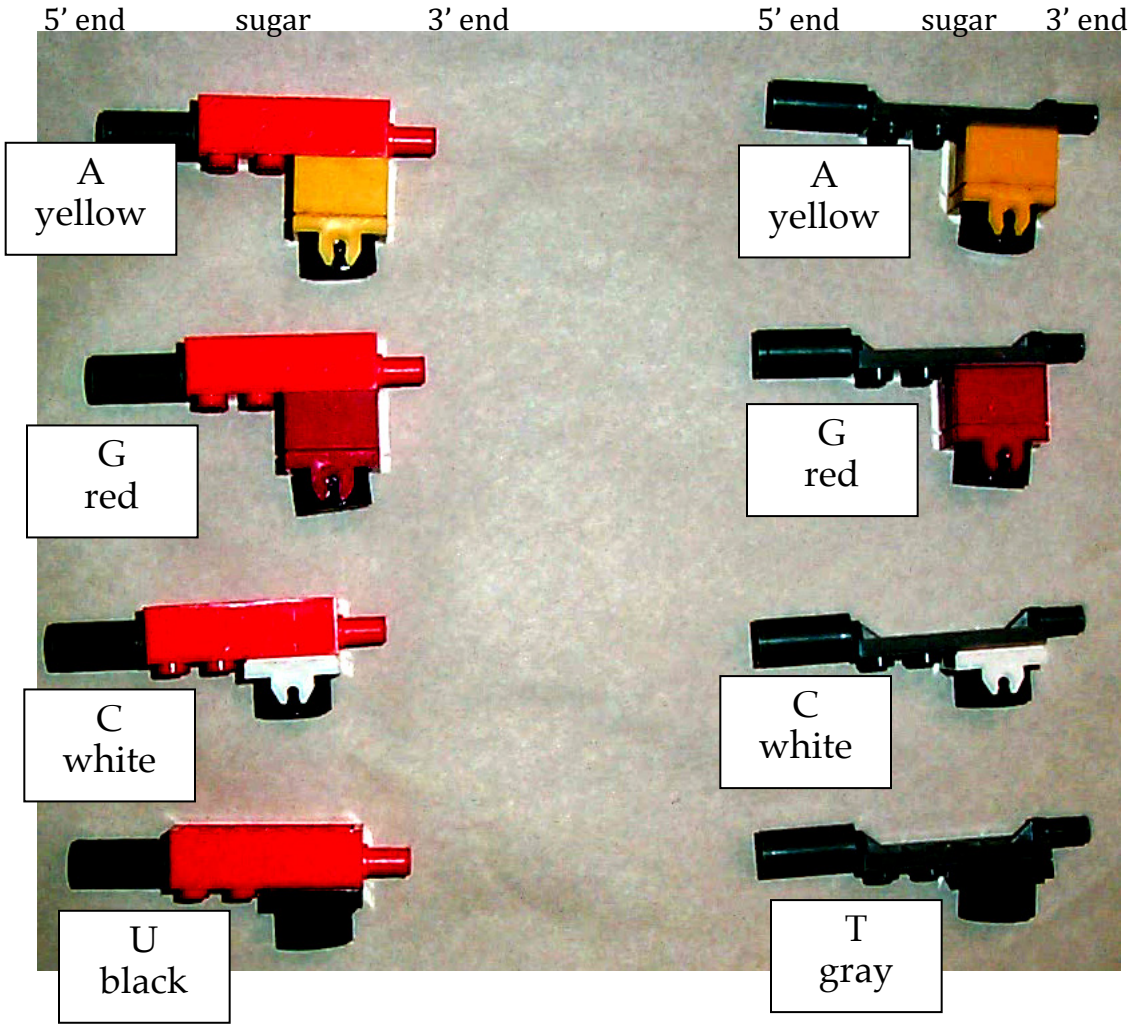
APPENDIX: LEGO DNA Models

In the past, we have used LEGO versions of these models. In a pinch, some groups may need to use the LEGO. In that case, the LEGO models look like this:

When nucleotides are assembled, they look like this:

RNA nucleotides

DNA nucleotides



- The correct bases pair via hydrogen bonds simulated by the black magnets on each base.
- 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.