**Bio4250 – Evolutionary Genetics Dr Carr June 2022**

**Lab #1 DRAFT - (Re)familiarization with the structure of Deoxyribonucleic Acid (DNA)**

Modern evolutionary genetics centers on inferences made from the **DNA** molecule. Famously, **DNA** is a **double-stranded helix**, whose **sugar-phosphate (deoxyribose-PO4) backbone** strands run in **anti-parallel directions**, and are held together by hydrogen bonds (**H-bonds**) between four bases, **Adenine**, **Cytosine**, **Guanine**, and **Thymine** (**A**, **C**, **G**, & **T**). This much of the structure is easily appreciated in flat drawings, with the work already done. It is less obvious in three-dimensional models, where structure must be appreciated from molecular properties.

In this lab, you will construct three-dimensional models of the **DNA** molecule from solid **3D** printer models of the **sugar-phosphate backbone** and the **A**, **C**, **G**, & **T** subunits. The purpose is to reinforce your understanding of **DNA** structure as it pertains to molecular evolution of the molecule. The lab will also introduce **Single-Nucleotide Polymorphism** (**SNP**) variation as the basis of molecular evolution.

1. Familiarize yourself with the **building blocks**.
   1. Identify the **5’** and **3’** ends of the **deoxyribose-PO4** backbones. What features identify these?
   2. Identify each of the colored molecules with the correct bases. What features allow you to recognize them? [***Note***: the base colors conform to the standard convention]
   3. Identify the appropriate **base pairs**. What features allow them to pair, and how does this relate to question 1.b ?
2. Construct a **3D** model of an **ATG** **Start** triplet.
   1. Attach an **A** base to the **deoxyribose-PO4** molecule in the **correct 5’-3’ orientation**. How do you recognize the correct orientation? What is this molecule called?
   2. Add the **T** and **G** bases at the correct end of the **A**. How do you know which is the correct end?
   3. **Check your construction with the Instructor**.
3. Add the appropriate bases for the **complementary strand**, with the *proper strand orientation*, to form a double-stranded 3bp molecule. [***Note***: base pairs should join & separate easily; **deoxyribose-PO4** molecules will require a bit of twisting to the proper orientation to join each other. Don’t force a fit].
4. Construct a **multi-triplet DNA molecule** as instructed [***Note***: this may or may not start with the **ATG**].
   1. Why are the **3bp runs** called ‘***triplets’*** rather than ‘***codons’*** ?
   2. Recall that separation between adjacent base pairs is **0.34 nm**, and that one complete helical turn of **3.4 nm** comprises **10 bp**. As best as you can, adjust the backbone to achieve this.
      1. Recall that **DNA** is a **right-handed** helix: how do you arrange & recognize this ?
   3. Add the appropriate bases as in Step 3 to form a **dsDNA** molecule as instructed.
      1. Try constructing each strand *separately* (pay attention to **5’-3’** orientation) AND
      2. Try adding *successive* base pairs to the appropriate end.
   4. **\*\*\*If** you come up with a clever means of achieving a 10bp period, share it with the class.
5. **Join** the separate molecules from each working group into a **single molecule**.
   1. Identify the **coding** and **non-coding** strands, and the **5’-3’** orientation. What do you look for? [Have all groups done it right?]
   2. Identify the **amino acid polypeptide** encoded by the molecule, beginning with the start triplet. Use the **universal genetic code** [next page].
6. **SNP variation:**
   1. The gene coding for the **β-globin** chain of Hemoglobin (**Hb**, standard from **Hb-A**) is known to exist in a variety of allelic forms, some of which result in severe anemias. The most famous of these is **Hb-S**, which results in [**Sickle-Cell Anemia**](https://www.mun.ca/biology/scarr/Recessive_Co-Dominant_&_Dominant_genetic_diseases.html#HbS) in West African populations and their descendants in North America. The allele is due to a **2nd position transversion** in the **6th triplet**.
      1. **Make a model** of the first six triplets of **Hb-A**.
      2. **Alter the model** to demonstrate the **SNP** that results in **Hb-S**.
      3. **NOTE TO SELF**: Given only the amino acids, the base sequence of the **DNA** molecule is ambiguous, and either of two **SNPs** produce the same amino acid substitution. **Probably**, *give students the base sequence of the first six triplets*, AND have them discover the ambiguity of the **SNP** substitution themselves.
   2. The table below left gives the amino acid sequences of the **β-globin** molecule for seven allelic variants, including **Hb-A** and **HB-S,** at different positions in the molecule. For one of the last five allelic variants as assigned, construct an artificial **DNA** sequence based on positions **6**, **26**, **63**, & **121**.
      1. **NOTE TO SELF**: The Instructor can construct the **Standard** [“*Normal*”] sequence as a demonstration:



**Questions**

1. Amino acid residues ##6, 26, & 121 (Nos. 3, 4, & 6) involve **SNP** variants of **GLU** triplets to three alternative coding triplets. What is the **DNA** **SNP** in each case?
2. Same question for residue #5, which involves **SNP** variants to two alternatives of the **HIS** triplet.
   1. Which of these **SNP** variants might result in functional changes to the β-chain? Explain.
   2. Why are the 3-letter combinations called ‘***triplets’*** rather than ‘***codons***’ ?
   3. Why are the nucleotide variants called ‘***SNPs****’* rather than ‘***mutations****’*?
   4. **FOR FURTHER THOUGHT**:
      1. The table at right is the **Universal Genetic Code**: how does it differ from a **DNA translation table**?
      2. Why might the **GLU** triplet be especially prone to **SNP** variation? [How many amino acid substitutions are possible given a single SNP in the triplet?]

**NOTE TO SELF**: Construction of two six triplet molecules may be excessive. Given the purpose of the lab, proceed directly to second exercise for its focus on SNPs, with only middle four triplets that show SNP variation.