

## Lab Eight: Central Dogma/DNA Analysis

Pre-Lab: none; course text REQUIRED during lab.

Introduction: We added this brand new lab to improve practice with central dogma concepts, and to provide you with the opportunity to isolate and analyze your own genetic material using agarose gel electrophoresis - the latter valuable for the final lab. Some good resources for reviewing how gels work include: lab manual p. 242-246, 248-251; textbook: Figure 20.8.

Procedures: *This lab will require multi-tasking; carry out as recommended below.*  
Unless otherwise stated, use a new sterile plastic pipette for each new solution addition below.  
To avoid confusion, throw each away in trash immediately after use.

### ***Buccal Cell Isolation Substitution Part I: Cell Lysis - Work Individually***

Underlined text is something you should look up/understand for next week's quiz!

Carefully label the side of a 15 ml conical tube with your name using a lab pen.

Vigorously swish Gatorade (provided in paper cup) in your mouth for 30 seconds, gently rubbing/scraping your teeth/tongue against your cheeks while swishing so you get as many cells as possible. Spit Gatorade back into your cup and then pour 10 ml into the conical tube. Gatorade is isotonic with respect to your cells - why do you think this is important?

With help from your instructor, spin class tubes on highest setting (about 4000 rpm) for 3-5 minutes in the class centrifuge. Read about centrifugation in your text (Fig. 6.5, 16.4).

Centrifuges typically separate mixtures into a pellet (at the bottom) and the supernatant (at the top). Given that this first centrifugation step is supposed to concentrate your live cells, which do you want - pellet or supernatant? Which should you throw away?

Add 2.0 mL of Lysis Buffer to the cells. Flick the tube to break up the cell mass and mix. Given that Lysis Buffer contains SDS which - like most detergents - is nonpolar, what part of the cell do you think will be dissolved (because "like dissolves like")?

Add 2 DROPS Proteinase K (found in meat tenderizers). Flick the tube to fully mix - make sure there are NO CHUNKS! Incubate the cells at 65-70°C in provided water bath for 30 minutes. Given the name of the last reagent is "Proteinase," what do you think this is and does?

### ***WHILE YOU WAIT - Complete Central Dogma Exercises - Work In Pairs***

#### ***Buccal Cell Isolation Substitution Part II: DNA Purification***

Add 1.0 mL 5M NaCl and 4 mL cold isopropanol (rubbing alcohol) to the liquid in the new tube. Mix by rocking the tube gently back and forth until the DNA becomes visible. It should look like fine white fibers or lint. What is the chemistry of NaCl and isopropanol? How do you think they interact with nucleic acids to "precipitate" it from solution - think about rock candy?

With help from your instructor, spin class tubes on highest setting (about 4000 rpm) for 15 minutes in the class centrifuge. When this spin is complete, your instructor will fully dry the pellet in a fume hood and call you when they are finished. What is now in the pellet? Why?

### ***WHILE YOU WAIT - Complete Central Dogma Exercises - Work In Pairs***



## Lab Eight: Central Dogma/DNA Analysis - Pair Worksheet (6 pts - all or nothing)

All Terms in **BOLD** Below Need Answering.

Pair Names:

### Central Dogma Part One - Replication

1. Using your book, define each of the following terms related to replication.

**REPLICATION**

**ORIGIN OF REPLICATION**

**HELICASE**

**DNA POLYMERASE (2 FUNCTIONS)**

**PRIMASE**

2. In the lab, each pair will be given a long piece of genetic material (too large to fit on this paper). Complete the following exercises using this piece of genetic material.

a) **What kind of macromolecule is this - general and specific name? How do you know?**

b) Complete this molecule into its DOUBLE HELIX form by replicating the provided strand - carefully writing product below template in chalk (make letters same size as provided print). Given the complexities of real replication, we will not be modeling unwinding, leading/lagging strand, etc. today. Have your instructor check your work before proceeding.

### Central Dogma Part Two - Transcription and Translation

1. Using your book, define each of the following terms related to transcription.

**TRANSCRIPTION**

**PROMOTER**

**RNA POLYMERASE (2 FUNCTIONS)**

**TERMINATOR**

2. Using your book, define each of the following terms related to translation.

**TRANSLATION**

**RIBOSOME**

**tRNA (INCLUDE ANTICODON)**

**CODON**

**START and STOP CODON**

3. In the lab, continue to use the long piece of genetic material that you replicated earlier. Complete the following exercises using this piece of genetic material.

a) **What kind of macromolecule is this - general and specific name? How do you know?**

b) **Find promoter - what enzyme would look for and bind here to begin transcription?**

c) Transcribe this gene - carefully writing product above template in chalk (make letters same size as provided print). Have your instructor check your work before proceeding.

d) Now you are going to simulate translation by building and aligning tRNA/amino acids where they would base pair with the mRNA transcript. We are leaving the ribosome out. In this exercise, large post-it note = tRNA; small post-it = amino acid carried by a given tRNA.

e) Just like the ribosome would, find the first AUG and place a large post-it above. Write the complementary anticodon where it belongs, making sure the anticodon complements the codon. Now, using Figure 17.5, determine which amino acid should be attached to this tRNA. Attach a small post-it and label with the corresponding amino acid.

f) Continue down the line. Have your instructor check your work before proceeding.

h) The ribosome joins amino acids together in the mRNA-specified order, detaching them from their tRNAs. Once (g) is approved, hook together your amino acids as such.

**Write this information here:**

**What kind of molecule is this? How do you know?**

**What is the specific name of the joining bonds made by the ribosome?**

*Hold onto this information until the final lab. Everything you have generated today is extremely valuable for the exam!!!! Use it!!!!*

### Sample Images From Lab Eight

Top: Central Dogma Manipulatives (hand-made by Boomer/Shipley)  
Bottom Left: gel of student DNA; Bottom Right: student preparing DNA

