Lab Week 1 – DNA Extraction
Given: Friday January 22, 2016
Due: Friday January 29 by 2:30pm

Objectives:

• Students will perform an extraction to isolate DNA from their own cheek cells
• Students will review basic cell structure and properties of DNA

Reading Assignment:

• Read this laboratory packet prior to coming to lab on Friday, January 22nd

Requirements:

• None

Required Deliverables:

• Lab comprehension exercise to be distributed in lab

General Guidelines for Labs

• Biology labs in Steffee B212:
  o Food and drink are not permitted in the laboratory
  o Gloves should be worn at all times while working in the lab
  o Students should wash their hands with soap and water before and after this exercise

• Computer Science labs:
  o Work on the Alden Hall computers. If you want to work on a different machine, be sure to transfer your programs to the Alden machines and re-run them before submitting.
  o Keep all of your files! Don't delete your programs and reports after you hand them in---you might need them again later.
  o Back up your files regularly. Use a flash drive or Google Drive or whatever your favorite backup method is.

• Review the Honor Code policy on the syllabus. Remember that you may discuss experiments and programs with others, but copying answers or programs is a violation of the Honor Code
DNA Extraction
Background and Fundamentals

Deoxyribonucleic acid (DNA) is a molecule present in all living things, including bacteria, plants, and animals, and in almost all cell types. DNA is the carrier of genetic information and is responsible for determining a person’s hair, skin, and eye color, facial features, complexion, height, blood type, and just about everything else that makes an individual unique. It also carries information required for cells to perform all of the functions that are common to all members of a species, or to all living things, and thus it is sometimes referred to as a biological “blueprint”. Your personal blueprint is a combination of half of your mother’s DNA (from her egg) and half of your father’s DNA (from his sperm) during conception. All of your cells contain this complete set of instructions.

All DNA looks the same when it is extracted from cells, but it is exciting to look at your own DNA, knowing that this is really what makes you unique and alive. In this laboratory activity, you will extract your own DNA — a substance that holds your very own “blueprint” — from your cheek cells. You will use a quick and easy procedure that scientists routinely use to extract DNA from different organisms.

Every day scientists are making new discoveries as they study the information encoded in our DNA. Understanding DNA holds the possibility of curing diseases, the hope for millions who suffer from various genetic disorders and syndromes, making better products from biological sources, and even perhaps the key to longer life. We are beginning to understand who we are and why by studying our genetic material.

DNA Structure
At the molecular level, DNA looks like a twisted ladder or a spiral staircase. Two long molecules are aligned with each other, and the rungs are formed from pairs of chemical units called bases. This structure is referred to as a double helix because of the spiral, or helical form made by two strands. The bases function like letters in a code, so they are known as A, G, T, and C (abbreviations for their full names, adenine, guanine, thymine, and cytosine, respectively). Each base is connected to a sugar and a phosphate group, and the sugar and phosphate groups form the “backbones” of the ladder-like structure. (A nucleotide is one unit consisting of a base, sugar, and phosphate.) Scientists have found that A always pairs with T, and G always pairs with C in double-stranded DNA.

A schematic representation of DNA (deoxyribonucleic acid). DNA is a long chainlike molecule that stores genetic information.
The 4 chemical letters of DNA are organized to make messages that can be understood by cells, called genes. These genes contain the information to make proteins, which are the basis for almost all of your body’s structures and functions. Each of your cells contains several billion letters of DNA “text”.

A DNA sequence is the particular arrangement or order of the bases along the DNA molecule. Human DNA sequences are 99.9% identical among each other. It is the <0.1% sequence variation that makes each of us unique. In other words, what makes you different from your classmate is an occasional difference in the sequence of bases in your genes.

The Genome, Chromosomes, Genes, DNA, RNA, and Proteins…What Is the Connection?
DNA is found within the nucleus of every cell in the human body, with the exception of mature red blood cells. The DNA is organized into structures called chromosomes, in which the long thin strands of DNA are tightly coiled around proteins. Every time a cell divides — for growth, repair, or reproduction — the chromosomes replicate in a highly organized process called mitosis. The 46 human chromosomes found in human cells are analogous to 46 volumes of an encyclopedia, which collectively contain all the information in your genome.

A gene is a section of DNA that contains the information to make a protein; it is like a written recipe that specifies the composition and order of assembly of a protein molecule. The human genome contains approximately 20,000 genes. The genome is analogous to a (gigantic) collection of cookbooks (remember, there are 46 “volumes” in the entire collection); not all of the recipes in a cookbook are prepared at once to make one meal, nor are all of the genes within the genome used in every cell. This selective gene expression according to cell type generates the characteristics of different cell types within your body. Basically, all of your cells contain the same books (chromosomes), but different cells read different recipes (genes) from the books.

Although genes specify the proteins that are made by cells, DNA is not the direct template for protein synthesis. The templates for protein synthesis are RNA (ribonucleic acid) molecules called messenger RNA (mRNA). Each mRNA molecule is simply a copy of the DNA sequence from one gene. mRNAs are the intermediates that carry the information from the DNA within the nucleus to the ribosomes, or protein manufacturers, within the cytoplasm. The ribosomes decode the genetic information and link together the appropriate amino acids to make the protein that is encoded by the gene. All the proteins made within a cell function to give the cell its traits.
Cheek Cell DNA Extraction: Laboratory Protocol

**Workstation Checklist**

<table>
<thead>
<tr>
<th>Students’ Workstations</th>
<th>Number (4)</th>
<th>Number (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 ml tube containing 3 ml water</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Mouthwash cups</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Microcentrifuge tube labeled ‘prot’</td>
<td>1 (1.25 ml)</td>
<td>1 (1.6 ml)</td>
</tr>
<tr>
<td>15 ml tube labeled ‘lysis’</td>
<td>1 (10 ml)</td>
<td>1 (12 ml)</td>
</tr>
<tr>
<td>15 ml tube labeled ‘95% ethanol’</td>
<td>4 (10 ml)</td>
<td>5 (10 ml)</td>
</tr>
<tr>
<td>Ice bucket with ice</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Disposable plastic transfer pipettes</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td>Permanent marker</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Waste buckets</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

**Shared Equipment**

<table>
<thead>
<tr>
<th>Number</th>
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<tbody>
<tr>
<td>Water bath at 50°C</td>
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**Procedure for DNA Extraction and Precipitation**

**Steps 1 and 2: Collection and Breaking Open Cells**

To collect as many cheek cells as possible, you will gently chew the insides of your mouth for 30 seconds and then rinse your mouth with a small amount of water. Ample cell collection is critical for the success of this laboratory. For best results, make sure you spend the recommend amount of time collecting the cells.

1. Obtain a 15 ml tube containing 3 ml of water and label it with your initials.

2. Obtain a mouthwash cup. Pour the 3 ml of water from the tube into your mouthwash cup. Take your cup into the hallway. *Eating and drinking are not permitted during biology labs.*

3. Gently chew the insides of your mouth for 30 seconds.

4. Take the 3ml of water from your cup into your mouth and swish vigorously for 30 seconds. Do not swallow the water!!

5. Carefully expel all of your water mouthwash back into your cup.

6. Return to the lab and carefully pour your mouthwash back into your labeled 15 ml tube.
7. Locate the 15 ml tube at your workstation labeled ‘lysis’. Using a fresh disposable plastic transfer pipet, add 2 ml of lysis buffer to your tube.

![Image of a pipet with measurement markings]

8. Place the cap back on your tube. Gently invert your tube 5 times to lyse your cells. DO NOT SHAKE THE TUBE.

**Step 3: Removing proteins**

1. Locate the pink microcentrifuge tube labeled ‘prot’. Using a fresh disposable plastic transfer pipet, and add 5 drops of protease + salt solution to the 15 ml tube containing your cell extract. Cap the cell extract tube and gently invert 5 times to mix.

2. Place your cell extract tube in the 50°C water bath for 10 minutes. This is the optimal temperature for the protease to degrade the proteins bound to your DNA.

**Steps 4 and 5: Making the DNA visible**

1. Locate a 15 ml tube labeled ‘95% ethanol’ in the ice bucket on your bench.

2. Using a fresh disposable plastic transfer pipet, add 10 ml of alcohol to your tube as follows: hold your tube at a 45° angle and add the alcohol by slowly dispensing it down the inside wall of the tube. It will take repeated additions to add 10 mls. The alcohol and cell extract will form two separate layers. Screw the cap back onto your tube.

![Image of a pipet with alcohol and cell extract]

*Slowly add alcohol (pink) while holding the tube at a 45°*
3. Place your 15 ml tube upright in the tube rack and **leave it undisturbed** at room temperature for 5 minutes.

4. After 5 minutes, look at the contents of your tube, especially the area where the alcohol and cell extract layers meet. What do you see?

5. With the cap of your tube tightly sealed, mix the contents of your tube by slowly inverting the tube 5 times. Look for anything stringy, white or clear material. **This is your DNA!**

6. Using a fresh disposable plastic transfer pipet, gently withdraw your precipitated DNA along with about 1 ml of alcohol solution and transfer it to a microcentrifuge tube.
Below are a set of questions designed to assess your understanding of the DNA Extraction lab. While you are permitted to discuss the questions with your classmates, this is an individual assignment and all answers should be your own.

You should begin by reviewing the introductory material on pages 2-3 of this handout.

1. Imagine you are trying to explain the difference between chromosomes, genes, and DNA to your younger brother or sister who is middle school. Write down your explanation in simple words that they could understand.

2. If you wanted to isolate a copy of the gene that codes for a protein found in the stomach, could that gene be located in cheek cells? Explain your reasoning.

3. Match the outcomes on the left with the laboratory steps on the right:

   _____ Harvest the cells
   _____ Dissolve cell membranes
   _____Precipitate the DNA
   _____Break down proteins
   _____Make DNA less soluble in water

   A. Gently chew the insides of your mouth and then rinse vigorously with water
   B. Add protease, incubate at 50°C
   C. Mix in a detergent solution
   D. Layer cold alcohol over cell extract
   E. Add salt