**BIOL 104 Forensic Biology**

**Lab 8 DNA Extraction**

1. **Introduction**

Deoxyribonucleic Acid (DNA) consists of a double helix of nucleotides, but what does DNA actually look like? We will first precipitate DNA using alcohol. Then we will extract our own DNA as a first step toward DNA fingerprinting. We will also examine epithelial cells from our buccal swabs using the compound light microscope.

1. **Materials & Methods**

**Wipe down your lab bench and wash your hands. Be sure to wear your gloves and safety glasses.**

1. **DNA Precipitation**
2. You have a small beaker filled with DNA dissolved in 3 ml of a buffer.
3. Transfer 6 ml of cold 70% isopropyl alcohol (rubbing alcohol) using a

disposable pipette. Slowly drip the alcohol along the side of the beaker so that it forms a layer on top of the DNA. Do not mix the two liquids!

1. DNA is soluble in the buffer and insoluble in alcohol, so a precipitate will form where the two liquids meet.
2. Insert a glass rod just below the line separating the two liquids and swirl the glass rod in a circular motion to collect the DNA.
3. Swirl the glass rod in the opposite direction in a conical tube filled with more 70% isopropyl alcohol to collect the DNA.
4. You may also use the disposable pipette to transfer more DNA into the conical tube.
5. Record your observations about the appearance of DNA in the Results section.
6. **DNA Extraction**
7. Unwrap a new sterile swab and gently scrape the inside of your

cheek (buccal) area to collect the epithelial cells on the surface.

1. Place the swab into your microcentrifuge tube containing 1 ml of

Phosphate Buffered Saline (PBS) and agitate to dislodge the cells. Dispose the swab in the Biohazard Waste.

 3. Close your tube and label the top with your lab number.

 4. Use a vortex to mix the cells.

 5. Place your tube into a microcentrifuge which will spin the tubes

10,000 times per minute for 5 minutes in order to collect the cells at the bottom of the tube.

6. When the microcentrifuge has finished spinning, find your tube. Can

you see a white pellet of cells at the bottom? Record your observations in the Results section.

7. Without disturbing your pellet, pour the liquid on top, or supernatant,

into the sink being careful not to lose your pellet of white cells.

8. Vortex the 10% Chelex and then transfer 0.5 ml of it to your tube

using a disposable pipette. (Check with your instructor if you cannot see the 0.5 ml marked line.)

9. Resuspend your cells in the Chelex by pipetting up and down

several times. The Chelex will help to separate the

cellular components and protect the DNA from degradation by DNAses, enzymes that degrade DNA.

10. Place your tube in a boiling water bath for 10 minutes. This will

lyse, or break open, your cells, releasing the DNA.

1. Allow your tube to cool for 2 minutes and then use a vortex to mix

it for 10 seconds.

1. Place your tube into a microcentrifuge for 5 more minutes to

collect the other parts of your cells at the bottom. The DNA will be dissolved in the liquid.

1. Your instructor will help you to transfer 200 μl of your DNA solution to a new microcentrifuge tube.
2. Label your tube with your lab number and “DNA.” The tubes will

be stored in a freezer at -20°C until next week.

 **C. Observing Epithelial Cells Under the Microscope**

 1. Obtain a glass slide and add one drop of 0.025% methylene blue to

the center.

2. Unwrap a new sterile swab and gently scrape the inside of your

cheek (buccal) area to collect the epithelial cells on the surface.

1. Roll the swab through the drop of methylene blue. Dispose

the swab in the Biohazard Waste.

1. Place a cover slip on top of the drop of methylene blue containing

your cells.

5. Use your compound light microscope to view your cells under high

power. Draw a cell and label the cell membrane, cytoplasm and nucleus.

Name\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Score:

Date\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

1. **Results**
2. **DNA Precipitation**
3. How does the DNA appear in the isopropyl alcohol?
4. **DNA Extraction**

2. Could you see a pellet of white cells after the first microcentrifugation?

**C. Observing Epithelial Cells Under the Microscope**

Cheek epithelial cells Total Magnification\_\_\_\_\_

1. **Conclusions**
2. Why couldn’t we see the DNA in the buffer? What allowed us to see the DNA in the isopropyl alcohol?
3. What types of cells are collected with a buccal swab?
4. Why is it important to correctly label tubes at each step in the procedure?
5. What does a vortex do?
6. What does a microcentrifuge do?
7. What does the term supernatant mean?
8. What is the purpose of using Chelex?
9. What does boiling do to the cells?

9. Where is DNA located in your cheek epithelial cells?