Experiment 3

The Chemical Nature of DNA

Chemically, DNA is a polymer composed of four simple repeating subunits, the nucleotides. Each nucleotide is comprised of a molecule of deoxyribose, a phosphate group, and one of four nitrogenous bases, adenine and guanine (purines) and thymine and cytosine (pyrimidines). The bases are linked to a molecule of deoxyribose, a five-carbon sugar, at the 1’ position, and the deoxyribose molecules are linked to one another by phosphodiester bonds between the 3’ and 5’ positions of adjacent nucleotides. DNA is double-stranded and the strands are held together by hydrogen bonds between the bases. Adenine on one strand always pairs with thymine on the other, and guanine always pairs with cytosine. The sequence of nucleotides determines the genetic code. Sets of three nucleotides are referred to as codons and specify the inclusion of a particular amino acid in a protein.

In this experiment we will characterize the chemical nature of DNA. The chemical nature of DNA can be analyzed by acid hydrolysis and thin-layer chromatography in order to visualize the four bases. In acid hydrolysis, DNA is treated with formic acid at very high temperature and pressure. This treatment will reduce the DNA to its component parts, leaving the free bases intact. The sample is then analyzed by thin-layer chromatography, during which each base migrates up the silica gel chromatogram at its own unique rate. Following the chromatography, the bases can be visualized with ultraviolet light. The chromatography sheet contains a fluorescent dye, but the bases will absorb UV, creating dark spots against a bright background. The intensity of the spots can be used to calculate the concentration of each base. It was through experiments on DNA using the then new technology of paper chromatography that Erwin Chargaff discovered that the ratios of the four bases vary from species to species, but the amount of adenine in DNA always equals the amount of thymine, and guanine always equals the amount of cytosine. This discovery disproved the theory that DNA was merely a repetition of the four base sequence ATGC and laid the groundwork for Watson’s and Crick’s discovery of the structure of DNA.
**Materials**

**First Day:**
- 5% trichloroacetic acid (TCA)
- 95% ethanol
- formic acid

**Third Day**
- Tubes of:
  - Adenine, Thymine, Cytosine, and Guanine
  - Chromatography Solvent

YOU MUST WEAR SAFETY GLASSES DURING ALL PARTS OF THIS EXPERIMENT

| Procedure for First Day | 1 Transfer 150 μg of *E. coli* chromosomal DNA to a microfuge tube containing 1 ml of 5% TCA. This will precipitate the DNA and leave protein and other contaminants in solution. Centrifuge for 10 minutes and then decant the TCA.
| Caution: TCA is extremely caustic |
| 2 Add 0.5 ml of ice-cold 95% ethanol to the tube. DNA is insoluble in ethanol and will remain precipitated in a pellet, but the residual TCA will be washed out. Centrifuge for 10 minutes. |
| 3 Add 200 μl of formic acid to your sample. |
| Caution: formic acid is extremely caustic |
| 4 Select a hydrolysis tube. Each tube must be weighed prior to use. If your tube weighs less than 4 gm, its walls will not be strong enough to withstand the high temperature and pressure, and will explode. Any tube 4 gm and over is acceptable. |
| 5 Carefully transfer your sample to the hydrolysis tube. |
| 6 Seal your tubes according to the following diagram. First heat the tube with a torch near the top until the glass becomes soft. Pull the glass out to a thin neck, but DO NOT SEAL THE TUBE YET. Sealing the tube at the same time as making the constriction may cause the tube to explode during heating. After the glass has cooled, return return the tube to the flame and seal the tube by heating at the top of the constriction. |
| Remember to keep your tube slanted so that the sample does not run out! |
Acid Hydrolysis of DNA

Procedure for Second Day

1. Break the seal on your tubes. The tube is under considerable pressure that was built up during hydrolysis, which must first be relieved. To do this, freeze the sample in a dry ice/ethanol bath to contract the gases.

2. Place the tube into a holder and insert the sealed end into the flame of a torch. There will be a (possibly) dramatic but harmless pop and recoil as the tube ruptures.

3. Next, briefly heat the widest part of the tube in the flame for a few seconds, allow a few seconds to cool, and then drip some water on the glass. This will cause the tube to crack.

4. The end may be knocked off with a pair of forceps. Your sample will still be mostly frozen.

   **Be careful of the sharp edges!**

5. This is all we will do today. By next lab, the instructor will place the samples in a heating block to evaporate off the formic acid.

Procedure for Third Day

1. Add 30 µl of water to your samples and vortex to mix.

   **Be careful of the sharp edges!**

   Transfer the 30 µl of dissolved hydrolysate to two microfuge tubes, 10 µl in one, and 20 µl in the other. This should give you two samples containing 50 and 100 µg of DNA, respectively.

2. Obtain a thin-layer chromatography sheet. This is an acetate sheet coated with a fine powder of silica gel which, if your are

7. Leave tubes in rack. Stop at this point. Before the next lab, the instructor will heat tubes for 40 minutes at 175º and return them for use in lab next time.
not careful, will rub off easily. They come as 20 x 20 cm sheets and you will have to cut them in half (10 x 20 cm). With a blunt pencil, draw a line 2 cm from one edge and a second line 10 cm farther. Along the first line, draw six evenly spaced spots.

3 You will spot your samples on the center spots, and spot samples of adenine, thymine, cytosine, and guanine on the flanking spots. If you spot your entire sample at once, the gel will be unable to absorb it quickly and the liquid will spread out too far. You need to make a small, tight sample spots. Carefully pipet 2 µl on each spot and allow the spots to dry. When dry, spot an additional 2 µl on each. Repeat this until the entire DNA samples have been spotted.

The standards are concentrated enough that you only need one 2 µl applications. As you spot, remember to try and not touch the chromatogram with the pipet. Otherwise, you will scrape off the silica gel.

4 Place your chromatogram in a tank containing chromatography solvent. Make sure your samples are above the solvent.

5 Allow the solvent to migrate up the chromatogram until it has travelled 10 cm, about 1 - 1.5 hr. Remove the chromatogram and allow it to dry.

6 When dry, observe the chromatogram in the BioDoc-IT. Place the chromatogram on the transilluminator powder-side down. Save the image to memory. To print, slide the thermal printer size button to the left, to the “SIDE” position. This will print a nearly actual size image of your chromatogram. Be sure to return the size button to the central NOR position when you are done.
7 Calculate the retention factor ($R_f$) for each spot.

\[
R_f = \frac{\text{Distance starting line - middle of spot}}{\text{Distance starting line - solvent front}} = \frac{b}{a}
\]

Mix and place in separatory funnel. Discard the bottom phase and retain the top phase.

**Chromatography Solvent**

- ethyl acetate ......................... 160ml
- n-propanol .............................. 40 ml
- water ..................................... 80 ml

Mix and place in separatory funnel. Discard the bottom phase and retain the top phase.

**100% Trichloroacetic Acid**

Add 100 ml of water to a bottle containing 500 g of TCA. Using a magnetic stirring bar, mix until the TCA is completely dissolved. If necessary you can add additional water. When the TCA is completely dissolved, add enough water to bring the final volume up to 500 ml. Store in a dark glass bottle. Dilute as needed.

**Hydrolysis Tubes**

- Corning Pyrex Rimless Culture Tube #9820
  - 10 x 75 mm
  - 401770 7740

**Thin-Layer Chromatography Sheets**

- Machery-Nagel #805023
  - POLYGRAM Thin layer chromatography polyester sheets
  - SIL G/UV$_{254}$ 20 x 20 cm