Familial Hypercholesterolemia: A DNA Electrophoresis Lab

Introduction

Cholesterol is a complex lipid (fat) found in every cell of the body. Cholesterol is vital in the formation and stability of cell membranes. It is needed for the synthesis of Vitamin D, bile salts, and hormones, such as testosterone, estrogen, cortisol, and progesterone. Cholesterol is made by the liver and is absorbed in the intestine from ingested food. It circulates in body fluids in spherical bodies known as lipoprotein particles. Lipoproteins are classified according to their densities determined by centrifugation. Cholesterol made in the liver is packaged into particles known as very low density lipoproteins, or VLDL. These VLDL's then form LDL, or low density lipoproteins. Lipoproteins are simply proteins that attach to a fat or lipid. **Low-density** lipoproteins (LDL) are known as "lousy" cholesterol. LDL transports cholesterol from the liver into the bloodstream where it can accumulate and form a plaque in the arteries. Amassing of plaque can eventually lead to occlusions that restrict blood flow to the heart or brain, resulting in heart attack or stroke. **High-density** lipoproteins (HDL) remove cholesterol from LDL and peripheral tissues then transport it back to the liver for repackaging or excretion. Hence, HDL is termed as the "good" cholesterol because it removes the "bad" lipoproteins.

Correlation between cardiovascular diseases and elevated blood cholesterol and serum cholesterol levels are now routinely tested. Levels of another potentially harmful lipid known as triglycerides, are also tested along with serum cholesterol. Elevated levels indicate the need for cholesterol reduction by diet, lifestyle changes, or cholesterol-reducing drugs known as statins.

Familial Hypercholesterolemia

As mentioned, most circulating cholesterol if found in the LDL particles. Cells take up the LDL from the circulation by a specific receptor. Familial Hypercholesterolemia, FH, is a condition where an individual may possess a mutation in the LDLR gene that encodes for the LDL receptor protein. This gene is responsible for removing the LDL cholesterol from circulation. Patients who have one copy, or are heterozygous for the mutation, still have one functional gene and possess 50% the normal level of receptors of unaffected individuals. Patients who are homozygous for the mutation, however, completely lack the LDL receptor and therefore possess extremely elevated levels of serum cholesterol, often greater than 600 mg per 100 ml of serum (150-200 mg/100ml is considered normal). Left untreated, patients usually die in childhood of coronary artery disease.

FH results from any of hundreds of different mutations in the FH gene. In some ethnic groups, however, the disease may be passed on as a single mutation. The mutation may be detected by a combination of genetic-based diagnostics, such as Polymerase Chain Reaction along with Restriction Fragment Length Polymorphism analysis. PCR targets the specific gene or site near the disease-causing gene and amplifies or makes millions of copies. PCR separates the strands of DNA and with the addition of oligonucleotides, or primers, synthesizes the copies. The RFLP process makes use of restriction enzymes to digest the target region of the gene that has been amplified. RFLP analysis relies on the fact that there are variations in the number of restriction sites in the specific DNA region in a diseased vs. a healthy individual. The digested DNA is then run on an agarose gel in the electrophoresis chamber. The process sorts and separates the DNA. The gel is stained to reveal bands on the gel that indicate the mutation.

In this experiment, simulated DNA samples from 3 patients will be analyzed for the FH mutation. Each sample was amplified and digested with a restriction enzyme and is ready to be separated by the process of electrophoresis. Once stained, gels can reveal whether the patients are normal, heterozygous, or homozygous

for the mutation. Homozygous individuals for the mutation will possess two mutated alleles. Electrophoresis is a process that consists of using a gel made by mixing agarose powder (derived from seaweed) and a buffer solution. The mixture is cooked in a microwave, cooled, then poured into a frame and allowed to cool and set. The gel is loaded with samples of DNA. The gel is placed in an electrophoresis chamber along with the buffer solution. The chamber is wired with a negative end and a positive end. The wires are connected to a power supply unit. Electricity travels from the negative (anode) to the positive (cathode). As the electric current travels through the box, it pulls the negatively charged DNA fragments through the gel toward the positive end. The gel is made of millions of tiny pores. The DNA fragments travel through the pores based upon their sizes. Large fragments cannot travel as far. Small fragments get pulled further down the gel. Once the gel is stained with a methylene blue dye, dark blue bands show up on the gel. The gel is analyzed by comparing their location to a control sample or to a DNA ladder band.

Safety:

- No food, drink, or gum in the lab
- Wear safety goggles
- Use hot gloves when preparing agarose
- Wear latex or nitrile gloves when staining gels

Equipment/Supplies

Electrophoresis Chambers
1X Buffer
Electronic balances
Gel beds, dams,
6 comb templates
Microwave
Small and large massing pans
50X buffer
Agarose
Instastain squares
Micropipettors & tips
Erlenmeyer flask
Hot gloves

Procedure

Part I. Gel Preparation

1. Assemble the rubber dams on each end of the gel bed frame. Place the rubber dam on the table with the groove facing up, push the end of the gel bed frame into the groove starting at the corner of the groove. Insert the blue comb template into the first set of notches on the end of the gel frame.
2. Place a small massing pan on the balance and tare the balance. Add 0.23g of agarose powder using a spoon or spatula.
3. Pour the powder into the Erlenmeyer flask.
4. Measure 30.0 ml of 1X buffer using the graduated cylinder.
5. Pour the buffer into the flask containing the agarose. Gently swirl the mixture.
6. Place the flask into the microwave. Cook the solution for one minute. The mixture will come to a boil and be extremely hot.
7. Use protective gloves and remove the flask. Place the flask on top of the microwave and check the solution for shiny crystals or flecks. If the solution is not clear, microwave again in short bursts of 20 seconds. Continue to check until the solution is clear like water. Do not overcook or the solution will evaporate.
8. Using the gloves, take the flask to the lab station and allow it to cool. The cooling process should take about 10 minutes or until you can comfortably touch the flask. It should be warm, not hot. Do not cool too long as the solution will start to thicken and set up in the flask.
9. Pour the solution into the assembled gel bed. Place the gel bed on the table where it will not be bumped or disturbed. The solution will begin to set up and become a gel.

PART II. Practice Pipetting Techniques

1. Instructor will demonstrate the proper technique for using a pipettor.
2. Practice using the pipettor using the practice gel and loading solution at the lab station.

STEPS FOR PIPET USE:

A. Dial pipet to designated amount of microliters (not needed with gray/purple pipets)
B. Obtain a new tip from the tip box
C. Open sample tube
D. Depress pipet plunger to FIRST STOP and HOLD
E. Insert tip end of pipet into the bottom of the sample tube through the foil top
F. SLOWLY release the pressure on the plunger
G. Remove pipet from tube; sample should fill the tip with no air bubbles
H. Position pipet tip above the first well (work from left to right)
I. Do not place tip INTO the well as this will tear or place a hole in the well
J. Place thumb on plunger and slowly push the plunger without hesitation to the second stop. Continue to HOLD the thumb down and lift the pipet up and out of the chamber
K. Release plunger and eject the used tip into the waste cup

PART III. Prepare solidified gel for loading of DNA

1. When the gel is translucent and firm (about 20 minutes after pouring), remove the blue toothed template by lifting it straight up out of the gel. Use both thumbs and index fingers to grasp the comb. It may help to have a lab partner hold the gel bed firmly on the table. Do not wiggle or jiggle the comb; simply pull straight up.
2. Use a stainless steel spatula and carefully run it along the inside of the rubber dam on one side of the gel. This will "break" the seal between the gel and the rubber dam. Do not cut into the gel; press the spatula close to the rubber dam.
3. Repeat on the other side. Slowly pull the rubber dams away from the plastic bed.
4. Place the gel bed into the electrophoresis chamber. The chamber has notches on the left and right sides that match the notches on the gel bed frame. This firmly holds the bed in place. NOTE: Each chamber can hold 2 gel beds.

PART IV. Preparing for Electrophoresis and DNA loading

1. Pour 1X buffer solution into the electrophoresis chamber until it just covers the gel. The gel wells must be submerged in the buffer. The buffer may already be in the chamber; if so, gently place the gel bed into the chamber. If any air bubbles are on the gel, gently lift the plastic frame and place it back in.
2. Check the DNA samples in the test tube rack. There are six samples A-F that contain the following:
   - A contains the DNA marker ladder of standards
   - B Normal DNA
   - C FH Control
   - D Patient # 1 DNA sample
   - E Patient # 2 DNA sample
   - F Patient # 3 DNA sample
3. Begin with the first well on the left looking down into the chamber and pipet Sample A.
4. Use a **NEW** tip with each sample. Eject the used tip into the waste cup.
5. Be sure to take the correct sample tube as you load from A to F.
6. Place a Ziploc bag under the chamber below the gel you loaded. Write your name (s) on the bag with a sharpie marker.

PART V. Running the Electrophoresis Chamber

1. Once the gel or gels are loaded, place the chamber lid on the chamber. Match the negative (black wire, anode) on the lid with the black wire on the chamber.
2. Plug the black and red wires into the corresponding plugs on the power supply unit.
3. Depress the switch for 125 V. Turn the power on.
4. Check the buffer in the chamber for evidence that the unit is working properly. Bubbles should be visible on the bare wires inside the box. This indicates that electricity is flowing through the unit.
5. The gel will run 30-45 minutes. A tracking dye is added to the DNA to monitor the progress of the movement down the gel. Once the dye is about 2 cm from the end of the gel, turn off the power, otherwise the DNA will run off of the gel.

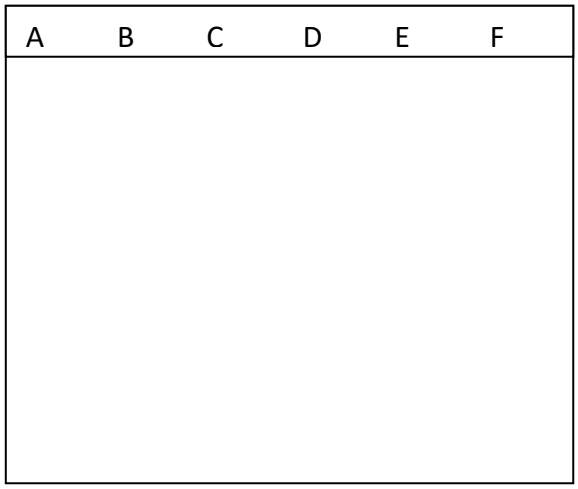
Staining: Wear Latex or nitrile gloves to protect hands!

6. Remove the gel by sliding it gently into the Ziploc bag. Place an instastain paper on top of the gel with enough buffer to cover the gel. Seal the bag and gently press the stain paper on the gel. This is the overnight staining method which requires no destaining.
7. Place the gel in the refrigerator for overnight staining.

Analysis: Wear latex or nitrile gloves!

1. Remove the gel by sliding it out of the bag and into a weigh boat or flat container. If the gel is very dark with stain, add about 10ml of warm water and gently rinse and pour off.
2. Place the gel on the lightbox or an overhead projector. Bands should appear beneath the wells at various distances.
3. Refer to Part IV to recall the contents of each well.
4. Determine the results of each patient by looking at the control well and compare.
5. Determine which patient is heterozygous, normal, and homozygous for the FH mutation. Write your results for each lane.

Take a photo of your gel as it appears after staining to include in your lab write up.



Citation for Lab Given:

Edvo-Kit#118. "Cholesterol Diagnostics." *Edvo-Kit#118* (n.d.): n. pag. *Edvotek*. Edvotek, 1989-2014. Web. 11 Feb. 2016. <http://www.edvotek.com/site/pdf/118.pdf>.

DNA Electrophoresis Lab

Lab Write Up

Title

- Appropriate title to describe the lab
- Proper Grammar and Spelling

Background

- Included description of Cholesterol, LDL's, HDL's, Restriction Enzymes, DNA Electrophoresis (include DNA Ladder of Standards), and Familial Hypercholesterolemia
- Works Cited and In-Text Citations (parenthetical)
- Proper Grammar and Spelling

Purpose

- Proper Grammar and Spelling

Materials

- Properly referenced
- Proper Grammar and Spelling

Procedure

- Properly referenced
- Proper Grammar and Spelling

Data / Results

- Picture of Gel: Labeled Figure 1 with a Title and Key

Analysis

- Explains the results of the lab referring to appropriate figure(s)
    - Each column (A through E) should be discussed and explained!
- Error and Expansion
- Proper Grammar and Spelling

Conclusion

- Restate the purpose of the lab and explain how the purpose was or was not met
- Proper Grammar and Spelling

Format:

- 1.5 Spacing and 12 Point Font
- Extra Space Between Sections
- Roman Numerals and Section Titles
- Text Starts Below Section Titles (Title is the exception)