**Gel Electrophoresis Analysis: Lab Directions**


**Introduction:**
Mary and John Smith have three children: Daniel, age 5; Alice, age 4; and Michael, age 1. Mary is two months pregnant. Recently Mary and John noticed that Daniel was having trouble climbing the stairs. He also complained several times that he was really tired after playing tag with his sister. Daniel’s doctor suggested some medical tests, which brought the family some bad news: Daniel has a disease called Duchenne muscular dystrophy (DMD).

Mary and John learned that MD is a sex-linked genetic disease, which means that it results from damage to a gene on the X chromosome. That is why almost everyone with MD is male, as they need only one defective copy of the X chromosome to have the disease. A girl may inherit an X chromosome with a defective copy of the MD gene from her mother and like her mother; she will be a carrier for MD. Usually boys with MD are healthy until the age of 4 or 5, at which time their muscles start to weaken. Obviously, Mary and John were very upset by this news about Daniel. Then they began to worry about their other children. Because Mary was a carrier for MD, it was possible that Michael would develop MD also, although he was too young to show any symptoms yet. They also worried about their unborn child and whether he or she might be at risk for MD. Finally, even though Alice would not develop MD, the Smiths wanted to find out whether she was a carrier like her mother. When the doctor explained that there is a genetic test that could determine whether each family member had the defective gene for MD, Mary and John decided that they wanted this information. The family submitted the blood of each member for testing. DNA was isolated from the white blood cells, but the MD gene is only one of many thousands of genes.

**Restriction enzymes** are used to cut DNA at specific, known sequences in order to first isolate a particular gene of interest, like the MD gene. In this case, the enzymes will cut or “cleave” the DNA before and after the MD gene to isolate it. Then, a procedure called **PCR (polymerase chain reaction)** is used to amplify the tiny section of DNA that contains the MD gene. The technique uses DNA polymerase (the same enzyme that cells use to replicate their DNA before they divide) to replicate, over and over again, a particular DNA region of interest. With many copies of that gene in each tube, it becomes much easier to compare genes from different individuals and see if they are the same or different.

Your job is to analyze the samples and determine which ones contain only copies of the wild-type MD allele, which ones contain only copies of the mutant MD allele, and which ones contain copies of both MD alleles. As an expert, you know that the mutation that causes this disease involves a deletion of part of the NA from the MD gene, so that the mutant alleles will be shorter than the wild-type alleles. As illustrated in the diagram, this implies that Mary, who is a carrier for MD, has one normal and one short (mutant) allele at the MD locus. Since John, being a male, has only one X chromosome and does not have MD, his MD allele must be of normal length. Daniel has the DMD disorder, so he must have received the X chromosome with the defective MD gene from his mother.

DNA molecules that differ in length can be separated and analyzed by a process known as agarose gel electrophoresis. This method uses an electric current to push DNA molecules through a gel-like substance called agarose. Small DNA molecules move through the gel faster than large ones.

You will use agarose gel electrophoresis to simulate the procedure that the genetic counseling lab would use to determine the genotypes of a set of family members with respect to a gene of interest – such as the MD gene. Each family member’s DNA sample would then be seen to contain one or both of two different sizes of DNA fragments: small or large. Other extraneous DNA may be present and many need to be differentiated from the gene of interest. Also, gel techniques can be different for different disorders or compared to a known genetic “key” to see if a disease is present. From this information, the counselor would then determine whether each individual had only normal genes, only a mutant allele, or one of each (and therefore was a carrier).
While this lab includes only certain parts of the DNA analysis process, the full process is detailed below:

**I. Isolating DNA:**
First, DNA must be isolated from a cell. This is easily done by dissolving the phospholipid cell membrane with mild chemical detergents and using the slightly negative charge of the DNA to clump it together and precipitate it out of the cell mixture. Then, if a specific area of DNA is going to be studied, that area must be isolated. This is done with restriction enzymes.

**II. Copying DNA**
After isolating DNA or finding a DNA sample for analysis, the DNA needs to be replicated so there are ample amounts of the DNA to work with. Each DNA sample can be multiplied using the polymerase chain reaction (PCR), a process that heads the DNA to break the hydrogen bonds holding the double helix together and then uses the polymerase enzyme to copy the DNA. The process is easily repeated to create large amounts of DNA.

**III. Cleaving DNA:**
The ability to cut and paste, or cleave and ligate, a functional piece of DNA—predictably and precisely—is what enables biotechnologists to analyze or recombine DNA-molecules to make recombinant DNA. The first step in DNA-splicing is to locate a specific gene of interest on a chromosome. A restriction-enzyme is then used to cut out the targeted gene from the rest of the chromosome. - **Restriction enzymes** are proteins that cut DNA at specific sites. Restriction-enzymes, also known as restriction endonucleases, recognize specific sequences-of DNA base pairs and cut, or chemically separate, DNA at that specific-arrangement of base pairs. They were first identified in and isolated from bacteria—that use them as a natural defense mechanism to cut up the invading DNA of bacteriophages — viruses that infect bacteria. Any foreign DNA encountering a-restriction enzyme will be digested, or cut into many fragments, and rendered-ineffective. These enzymes in bacteria make up the first biological immune system.-There are thousands of restriction enzymes, and each is named after the bacterium-from which it is isolated. For example:
- EcoRI = The first restriction enzyme isolated from Escherichia coli bacteria
- HindIII = The third restriction enzyme isolated from Haemophilus influenzae bacteria
- PstI = The first restriction enzyme isolated from Providencia stuartii bacteria
Each restriction enzyme recognizes a specific nucleotide sequence in the DNA,- called a restriction site, and cuts the DNA molecule at only that specific sequence.-Many restriction enzymes leave a short length of unpaired bases, called a “sticky”-end, at the DNA site where they cut, whereas other restriction enzymes make a cut-across both strands creating double-stranded DNA fragments with “blunt” ends. In general, restriction sites are palindromic, meaning the sequence of bases reads the-same forwards as it does backwards on the opposite DNA strand. The specific restriction enzyme will cut all its palindromes , no matter what species the DNA comes from.
For example, the image is a list of enzymes and the sites where they cut.

**IV. Electrophoretic Analysis of Restriction Fragments**
A restriction enzyme acts like molecular scissors, making cuts at the specific-sequence of base pairs that it recognizes. The three-dimensional structure or shape of a restriction enzyme allows it to fit perfectly in the groove formed by the-two strands of a DNA molecule. When attached to the DNA, the enzyme slides-along the double helix until it recognizes a specific sequence of base pairs which signals the enzyme to stop sliding. The enzyme then chemically separates, or cuts,-the DNA molecule at that site — called a restriction site.

If a specific restriction site occurs in more than one location on a DNA-molecule, a restriction enzyme will make a cut at each of those sites, resulting in multiple fragments of DNA. Therefore, if a given piece of linear DNA is cut with a-restriction enzyme whose specific recognition sequence is found...
at five different-locations on the DNA molecule, the result will be six fragments of different lengths.-
The length of each fragment will depend upon the location of restriction sites on-the DNA molecule.

A DNA fragment that has been cut with restriction enzymes can be separated-using a process
known as agarose gel electrophoresis. The term electrophoresis-means to carry with electricity.
Agarose gel electrophoresis separates DNA-fragments by size. DNA fragments are loaded into an agarose gel slab, which is-
placed into a chamber filled with a conductive buffer solution. A direct current is-passed between wire electrodes at each end of the chamber. Since DNA-fragments are negatively charged, they will be drawn toward the positive pole-(anode) when placed in an electric field. The matrix of the agarose gel acts as a-
molecular sieve through which smaller DNA fragments can move more easily than-larger ones. Therefore, the rate at which a DNA fragment migrates through the gel-is inversely proportional to its size in base pairs. Over a period of time, smaller-DNA fragments will travel farther than larger ones. Fragments of the same size-stay together and migrate in single bands of DNA. These bands will be seen in the gel after the DNA is stained.

An analogous situation is one where all the desks and chairs in the classroom-have been randomly pushed together. An individual student can wind his/her way-through the maze quickly and with little difficulty, whereas a string of four students-would require more time and have difficulty working their way through the maze.

Making DNA Visible

DNA is colorless so DNA fragments in the gel cannot be seen during-electrophoresis. A loading dye containing two blue dyes is added to the DNA-solution. The loading dye does not stain the DNA itself but makes it easier to load-the gels and monitor the progress of the DNA electrophoresis. The dye fronts-migrate toward the positive end of the gel, just like the DNA fragments. The “faster”-dye comigrates with DNA fragments of approximately 500 bp, while the “slower”-dye comigrates with DNA fragments approximately 5 kb in size. Staining the DNA-pinpoints its location on the gel. When the gel is immersed in Fast Blast DNA stain,-the stain molecules attach to the DNA trapped in the agarose gel. When the bands-are visible, your students can compare the DNA restriction patterns of the different-samples of DNA.

This Lab:
You will be provided with “DNA” samples that has, in theory, has the gene of interest isolated already, gone through PCR already, and been cleaved with restriction enzymes. You DNA samples are already loaded with color-coded dye to eliminate the need for staining and speed up the process. The DNA restriction analysis that you are about to perform is fundamental to a variety of genetic engineering techniques, including gene splicing, DNA sequencing, gene localization, forensic DNA matching, or DNA fingerprinting.

* answer all pre-lab questions in lab report

Materials:
- 20 ul vial of DNA fragments prepared using restriction enzymes
- rack for holding samples
- micro pipette and tips
- permanent marker
- gel electrophoresis chamber
- power supply
- staining tray
- 0.8% agarose solution (or prepared gel)
- 1x TAE (tris-acetate-EDTA) buffer
- camera for photo of gel
Part A: Practice Using Micropipets
Practice using a micropipette with water or food coloring. Practice pipetting drops of water onto a paper towel with hand-drawn rectangle to represent wells in the gel.

1. Look at the micropipette to determine the volume range.
2. Twist the dial on the micropipette to set the desired volume to 5 ul (0.05).
3. Affix a tip to the pipettor by firmly jamming the end of the pipettor into a tip in the rack.
4. Press the micropipette plunger to the first (soft) stop.
5. Insert the pipet tip into the solution to be transferred.
6. Slowly release the plunger to retrieve the liquid.
7. Insert the pipet tip into the desired tube.
8. Press the plunger past the first stop to the second (hard) stop to transfer the liquid. Make sure to keep the plunger pressed when lifting the pipet tip out of the tube.
9. Eject the tip from the pipettor after using it. Always do this after loading each sample, otherwise the samples will be contaminated.
   ✗ Be careful that you do NOT push to the second stop when drawing in liquid, only when expelling liquid.

Part 2: Agarose Gel Electrophoresis
* To save time, the gels and DNA have already been prepared. This lab uses dye to represent DNA of different fragments. For the sake of the simulation, it should be assumed that the DNA has already been isolated, amplified, and the gene of interest has already been cut out of the X chromosome using restriction enzymes.

WARNING
Although the dyes and stains are nontoxic and noncarcinogenic, avoid skin contact to keep hands from becoming stained blue or wear latex or vinyl gloves while handling the stain or stained gels. Lab coats or other protective clothing can be worn to avoid staining clothes.

A. Set Up Your Gel Electrophoresis Chamber
1. Obtain your agarose gel. Remove the tape if it is still on your gel tray.
2. Place the casting tray, with the solidified gel in it, onto the central platform in the gel box. The wells should be at the negative (cathode) end of the box where the black electrical lead is connected. Again, the end containing the comb is toward the black electrode.
3. Pour about 275 ml of electrophoresis TAE buffer into the electrophoresis chamber, alternating the side you pour into so it is even. There should be enough buffer in the box until it just covers the wells of the gel by 1–2 mm.
4. Very carefully remove the comb from the gel by gently pulling it straight up.

B. Load your Samples and Run them by Electrophoresis
5. Locate the DNA samples A through F and the known DNA samples.
6. Pipet 3 µl from each tube (A through F) into separate wells in the gel chamber. Use a fresh tip for each tube. If enough space, skip a well between each sample. Gels are read from left to right. To keep things straight, the first sample is typically loaded in the well at the upper left-hand corner of the gel. If you notice that a well in the gel has been compromised (the wall between it has been broken), skip that well and use the next one.
7. After loading samples A through F, and if there is space, load the DNA samples of the known normal and known mutated sample to the last wells. See loading chart below:

<table>
<thead>
<tr>
<th>Lane</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
<td>F</td>
<td>Xn</td>
<td>Xm</td>
</tr>
<tr>
<td>Person</td>
<td>Mother</td>
<td>Father</td>
<td>Daniel</td>
<td>Alice</td>
<td>Michael</td>
<td>Fetus</td>
<td>Normal</td>
<td>MD</td>
</tr>
</tbody>
</table>

Optional Demo: At this point, if available, you can also add extra unknown DNA samples or unknown dye samples that simulated unknown DNA to the extra lanes on the other side of your gel. This will be provided by your teacher. Make sure to skip as many lanes as you can between your DNA and these sample, starting on the right side of your gel instead of the left. Your teacher may do this for you.

<table>
<thead>
<tr>
<th>Lane</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample:</td>
<td>suspect 1</td>
<td>suspect 2</td>
<td>crime scene unknown</td>
</tr>
</tbody>
</table>

8. Slide the cover of the chamber into place, and connect electrical leads to the power supply, anode to anode (red to red) and cathode to cathode (black to black). Make sure both electrical leads are attached to the same channel of the power supply.

9. Plug the gel chamber into the power supply, black to black and red to red. Turn the power on and set the voltage to 110V and press the “run” button.

10. Electrophorese at 110 V for 15–35 minutes (ask instructor as needed). If it is working, small bubble can be seen coming up through the chamber. Shortly after the current is applied, th dye can be seen moving through the gel.

Waiting? Take a look at the analysis/ conclusion questions, as some can be done now.

C. After electrophoresis:

11. When electrophoresis is complete, turn off the power supply, disconnect the leads from the inputs, and remove the top of gel chamber.

12. This is a good time to take a picture of your gel so far.

13. Remove the casting tray from gel chamber. The gel is very slippery. Hold the tray level and place on on a paper towel or wax paper.

14. Pour the excess buffer back into the labeled container for reuse.

   *note: this is normally when staining would take place, but because of the use of dye, this is not needed for these samples.

Optional:

15. Place the gel directly upon the hydrophilic size of a piece of gel support film. (or wax paper) (Water will form beads on the hydrophobic side of a piece of gel support film.) Center the gel on the film on a paper towel and let the gel dry in a well-ventilated area, making sure to avoid direct exposure to light. As the gel dries it will bond to the film but will not shrink. If left undisturbed on the support film, the gel will dry completely at room temperature after 2–3 days. The result will be a flat, transparent, and durable record for the experiment.

   Note: Avoid extended exposure of dried gels to direct light to prevent band fading.

*answer analysis question in lab report and do your conclusion
Electrophoresis Analysis: restriction enzyme cleavage of DNA Lab Report

Prelab questions:

1. Summarize the purpose of this lab:

2. What process is used to make enough DNA copies of the DNA to use?

3. What are restriction enzymes?

4. If the DNA molecule has two restriction sites, A and B, for a specific restriction enzyme (image below), how many fragments would be produced if the DNA is cut by that enzyme?

5. What processes are used to analyze DNA fragments (the part you will actually be doing)?

6. It what direction do DNA fragments move, in reference to the charged poles in the chamber?

7. Which fragments move the farthest, small or large? Why?

8. A piece of DNA is cut into four fragments as shown in the diagram. A solution containing the four fragments is placed in a well in an agarose gel. Using the information given above, draw (to the right) how you think the fragments might be separated. Label each fragment with its corresponding letter.

Results: In the rectangle below, draw in the label of gel lanes you have and label what is in each lane by writing the name under the lane. After you gel can run, draw in the banding patterns as seen (skipped lanes are not pictures)
Analysis:

1. Indicate the genotypes and phenotypes for each of the family members
   Mother Mary = X^N X^m, carrier | Father John = X^N Y, normal
   Daniel __________________________ Alice __________________________
   Michael __________________________ Fetus __________________________

2. Which allele moves further into the gel, the normal (X^N) or mutant (X^m) allele? Why?

3. Why are most patients with DMD male? Explain how it would be possible or not possible for a female to have this disease.

4. Looking at the analysis of the DNA of the fetus, what can you tell about its genes for MD and what does that indicate about the gender of the baby?

5. Now look at the diagram of the agarose gel (below). It shows two lanes. A lane is the column below a well. The right lane contains a banding pattern from four fragments of known length (6,000, 5,000, 3,000, and 1,000 bp).
   a. Which lane contains the molecular weight standards? How do you know?
   b. Label each band in the right lane with its base-pair size.
   c. Compare the two lanes of bands. Estimate the size of the fragments in the left lane.
      Upper band: __________
      Lower band: __________

Conclusion: (attach paper to answer)

1. Summarize the process of DNA analysis. (you may use bullet points)
2. What is the overall significance of using DNA analysis techniques? How are they applied?
3. Below are three representatives of a 15,000 base pair DNA molecule from Gena. Each representation shows the locations of different types of restriction sites on Gena’s DNA, with vertical lines representing the cut site. The numbers between, show the sizes (in base pairs) of the fragments that would be generated by digesting the DNA with that enzyme. (not to scale)

<table>
<thead>
<tr>
<th>EcoRI sites</th>
<th>BamHI Sites</th>
<th>HindIII Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>4,000</td>
<td>6,000</td>
<td>8,000</td>
</tr>
<tr>
<td>3,500</td>
<td>4,000</td>
<td>4,500</td>
</tr>
<tr>
<td>2,500</td>
<td>3,000</td>
<td>2,500</td>
</tr>
<tr>
<td>5,000</td>
<td>1,840</td>
<td></td>
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</tbody>
</table>

Analysis of DNA electrophoresis has revealed that DNA fragments smaller than 300 base pairs may indicate the presence of genes that have been associated with cancer. BRCA 1, p53, and CHEK2 are three genes that have been identified in cancer research. If these fragments of DNA were run on a gel (in real life, you could not see the size until they were run on the gel) does it appear that Gena may have a cancer gene? Explain your answer.

4. There are important social and ethical implications of DNA analysis. Already, DNA testing can reveal the presence of markers of certain genetic diseases, such as Huntington’s. Suppose a DNA test that predicted your chances of getting a disease, such as cancer, were available. You take the test for cancer, and the results say you have a two in three chance of getting cancer sometime in the next 20 years. Who owns your DNA and its information? Who should have access to your genetic profile and this information? Your doctor? Health insurance companies? College admission officers? Employers? Would you want to know this information? If you were Alice (carrier for MD) would you want to have children?