Name _____

Date _____ Hour _____

Detecting the Duchenne Muscular Dystrophy (DMD) Mutation

IN THIS EXERCISE WE will pretend that you are a technician in a genetic counselor's laboratory. You will perform a DNA screening test that is similar to the one used by real genetic counselors, and then you will interpret the results and decide how to present this genetic information to the family that is involved.

The situation you will be dealing with is the following: 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 had never heard of DMD before, so they asked the doctor a lot of questions and went to the library for more information. They learned that DMD 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 DMD is male. A girl may inherit an X chromosome with a defective copy of the DMD gene from her mother and like her mother, she will be a carrier for DMD. But the girl most likely will be protected from developing DMD by the normal X chromosome she gets from her father. In contrast, a boy does not get an X chromosome from his father, and if the X chromosome he gets from his mother carries a defective copy of the DMD gene, he will develop DMD.

Usually boys with DMD are healthy until the age of 4 or 5, at which time their muscles start to weaken. The doctor told the Smiths that Daniel would probably need a wheelchair in a few years, and that he would probably die before the age of 21. Although scientists are working to find a cure for this disease, there is no effective treatment for DMD now.

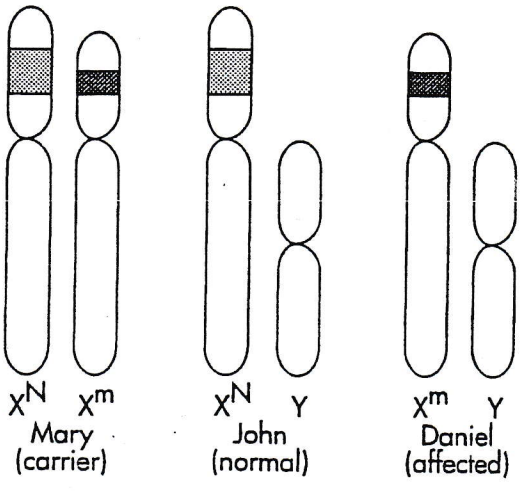
Obviously, Mary and John were very upset by this news about Daniel. Then they began to worry about their other children. Because Mary was a carrier for DMD, it was possible that Michael would develop DMD also, although he was too young to show any symptoms yet. They also worried about their unborn child and whether he or she might be at risk for DMD. Finally, even though Alice would not develop DMD, 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 DMD, Mary and John decided that they wanted this information. So the doctor sent them to a genetic counseling center where the tests could be done.

First the genetic counselor met with Mary and John and reviewed with them all of the information about DMD that they had received earlier. She tried to make sure that they understood the nature of the test that was to be performed, what kinds of information it could provide them, and how they might choose to use that information once they had it. When the Smiths assured their counselor that they understood, and that they did want to have the test performed, you, as lab technician, took a small drop of blood from a finger of each family member. Mary also went through a process of fetal blood sampling, so that the DNA from her unborn child could also be tested. The genetic counselor told Mary and John to come back in a week to find out the test results.

You took the Smith's blood samples and isolated DNA from the white blood cells in each of them. But the DMD gene that the Smith family wished you to analyze was only one of many thousand genes in each of the resulting DNA samples. So, to get enough of this particular gene to study, you used a procedure called **PCR** (short for **polymerase chain reaction**) to amplify (make many copies of) the tiny section of DNA that contained the DMD gene.

PCR 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. A single round of replication takes only about two minutes. Then both the copies made in that first round can be replicated again, and so on. At this rate of doubling, PCR can produce over a billion copies of a piece of DNA in about an hour. With so 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

You have used PCR to make billions of copies of the DMD genes from the DNA sample taken from each member of the Smith family. Now your next job is to analyze the samples and determine which ones contain only copies of the wild-type DMD allele, which ones contain only copies of the mutant DMD allele, and which ones contain copies of both DMD alleles. As an expert, you know that most mutations that cause this disease involve a deletion of part of the DNA from the DMD 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 DMD, has one normal and one short (mutant) allele at the DMD locus. Since John, being a male, has only one X chromosome and does not have DMD, his DMD allele must be of normal length. Daniel has the DMD disorder, so he must have received the X chromosome with the defective DMD gene from his mother.



Now the question is, Which DMD alleles do the other family members have? That's what you will try to determine.

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 DMD gene. In a real diagnostic test, PCR-amplified DNA samples derived from the blood cells of the various members would be subjected to gel electrophoresis, and then the gel would be stained to make the DNA fragments visible. 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. From this information, the counselor would then determine whether each individual had only the normal allele, only the mutant allele, or one of each (and therefore was a carrier).

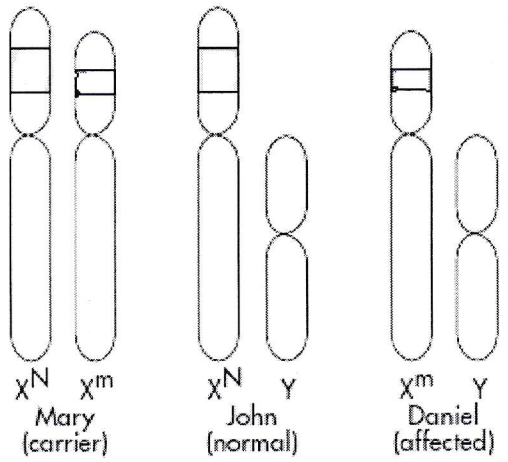
Because we do not have a real Smith family, a real PCR machine, or a real PCR technician available in our classroom, we will use two dyes of slightly different color to represent the mutant and normal DNA fragments of interest. Each sample you receive for electrophoresis will contain one or both of these dyes. The dyes are ones that (like DNA molecules of different lengths) move through a gel at different rates when subjected to an electric field. The faster-moving dye will represent the mutant DMD allele, and the slower moving one will represent the wild-type allele. Your job will be to determine which allele(s) each member of the Smith family possesses, by inspecting the gel at the end of the electrophoresis.

DMD Diagnosis Worksheet

What is Duchenne's Muscular Dystrophy?_____

_____

On the diagram below, color the defective alleles (mutated) purple and the normal alleles blue.

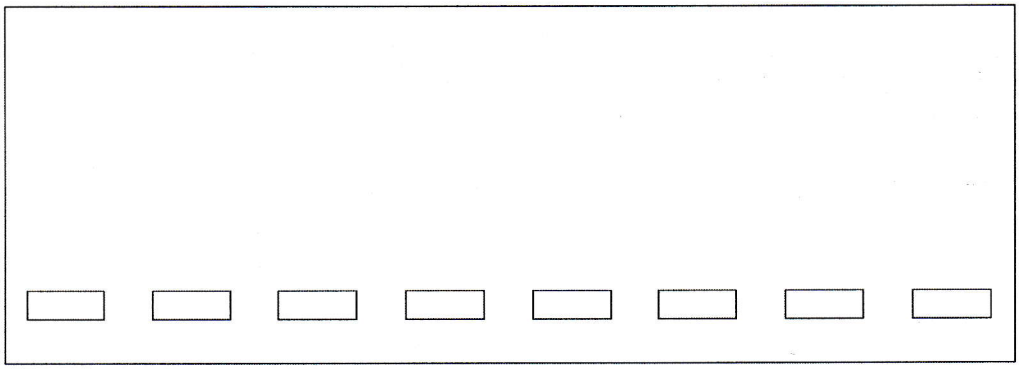


Questions

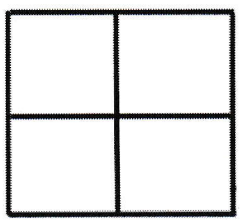
1. What is different between the X and the Y?
2. What do you notice about the X chromosome with the mutation (m) versus the X chromosome without?
3. What do you notice about the gene on the X chromosome for the mutation (m) versus the X chromosome without (N)?

4. Number of alleles a females must inherit to have a recessive X-linked trait _____ Number of alleles a male must have to inherit an X-linked trait _____

5. On the diagram below above each well write the name that was on the sample that was loaded into the well. Once the results are finalized draw results with the number of bands and color. The wells should be loaded in the following order: Mary (mom), John (dad), Daniel, Alice, Michael, Fetus.



6. Complete the Punnett Square for the mother and father to predict the chance of their offspring having DMD.



1. What is the probability the Alice will be a carrier? _____
2. What is the probability that Michael will have DMD?

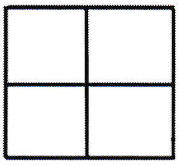
Results of Gel Electrophoresis: Smith Family

Family Member	# of total alleles	# of mutated Alleles	# of alleles required to have DMD	Genotype	Status
Mother Mary					
Father John					
Son Daniel					
Daughter Alice					
Son Michael					
Fetus					

3. Is the fetus a male or a female? How do you know?

4. Describe how gel electrophoresis works using complete sentences.

5. What situation would have to be present for Alice to have DMD? Do this Punnett square and explain. Explain the results to the Smith Family.



6. Does Michael have DMD? Explain the results to the Smith family. (how can you tell and what does it mean)

7. How would the results be different for cystic fibrosis with is an autosomal recessive disorder. Do the Punnett Square and explain.

8. On another sheet of paper draw a pedigree for this family. Include the possible genotypes for grandparents.