CHAPTER 3
How Genes and the Environment Influence Our Health

SECTION E
How Does a Genetic Counselor Detect Mutant Genes?
Chapter 3: Section E Background

DUCHENNE MUSCULAR DYSTROPHY (DMD), which is the subject of the following exercise, is a relatively common sex-linked disease. It affects about 1 boy in 3000, most of whom appear to be healthy until age 4 or 5, whereupon they begin to develop muscular weakness. Frequently, the first symptoms are problems with running and climbing stairs. Affected individuals are usually wheelchair-bound before they reach their teens and few survive into their twenties, most frequently dying from lung or heart failure. Fewer than 10% of carrier females exhibit any muscular weakness as a consequence of having one mutant allele, and female homozygotes are extremely rare, since very few affected males ever become fathers. There are currently no effective treatments for this disease.

DMD can result from any one of a variety of mutations in the gene coding for dystrophin, an important structural protein in the muscle, heart, and brain. (Dystrophin’s essential role in the brain is pointed to by the fact that patients with DMD often show a decline in IQ scores as the disease progresses.) A milder form of the disease, called Becker’s muscular dystrophy results from mutations that cause production of a partially functional dystrophin molecule.

The dystrophin gene is the largest human gene that has been studied to date and occupies almost 2% of the entire X chromosome. Perhaps because of its size, this gene has an extremely high mutation rate, and nearly one third of all cases of DMD are the result of new mutations that occurred by chance during formation of the egg from which the affected boy developed. In such cases, the mother is not a carrier for DMD and is very unlikely to have a second child with a defective DMD allele. However, because very few boys with DMD ever live long enough – or are healthy enough – to produce children, new mutant DMD alleles that show up in boys usually disappear from the population in one generation.

New DMD mutations that show up in girls persist much longer, on average, because such girls become carriers, who have a 50% chance of passing their mutant gene on to each of their offspring. Moreover, because girls receive an X chromosome from each parent, they’re about twice as likely as boys to end up with a novel DMD mutation – one that occurred during formation of either the egg or the sperm from which they received their genes. It is estimated that an average genetically normal male produces a sperm cell with a new mutation in the dystrophin gene every 10-11 seconds. (As high as this mutation rate may sound, the rate of sperm production in a healthy male is so enormous that only a tiny fraction of all sperm carry a new DMD mutation.)

Most dystrophin gene defects resulting in DMD are deletions (the absence of some normal portion of a chromosome) of varying sizes. These deletions are the basis for many of the available diagnostic tests. Frequently, DNA samples from patients and family members are analyzed by PCR amplification of several different portions of the dystrophin gene where deletions are known to occur, followed by separation of the resulting DNA fragments on an agarose gel. (PCR amplification is explained in the next section.) Individuals with deletions
will either lack certain DNA bands or will exhibit smaller bands than family members without the defect.

**BACKGROUND REFERENCES**


**HOW PCR IS USED TO AMPLIFY A GENE FRAGMENT OF INTEREST**

The polymerase chain reaction (PCR) referred to above is one of the most important, most powerful and most widely used techniques in modern biology. PCR is used routinely for a wide range of purposes by research biologists and genetic counselors (as is simulated in the following exercise). It also has become the most important method used by law enforcement agencies for personal identification. With it, a single hair or a single drop of blood found at a crime scene can be used to trap the guilty or free the innocent. Information in *Jurassic Park* notwithstanding, PCR has not yet been used to bring back the dinosaurs, nor is it likely to. However, PCR has been used to examine DNA sequences of tiny bits of plants and animals that lived long ago – including insects trapped in amber for more than 100 million years. Indeed, PCR has become so important in many areas of biology and medicine that Kary Mullis was awarded the Nobel Prize in Chemistry for inventing it.

PCR is based on one simple but important fact about DNA polymerase, the enzyme that replicates DNA in cells before each round of cell division. This fact is that in order for DNA polymerase to replicate any target DNA molecule (which is called its template), it must have a short piece of nucleic acid, called a primer, that is complementary in its base sequence to part of the template. The primer base-pairs to the template and acts as the starting place for DNA synthesis. DNA polymerase then functions by adding nucleotides to the primer in the sequence that is specified by the template.

In living cells, a whole set of other proteins are required to find the location on a chromosome where replication is to begin and to make a primer that DNA polymerase can use to get started. The details of how this is accomplished need not concern us here. What is important is to recognize that if DNA polymerase has a primer and a template it can begin replicating the DNA. But if DNA polymerase has no primer, it is incapable of copying DNA. Therefore, one can get DNA polymerase to copy just exactly the part of a template DNA molecule that one is interested in by providing it with primers that define the desired starting places on both strands of the double-stranded molecule of interest. (See the diagram on the next page.)

Of course, one must know the DNA sequence in the region of interest in order to design useful primers. In the diagram, the primers shown are ten nucleotides long (and with pointed ends indicating where DNA polymerase will add nucleotides). In actual practice longer primers must be used to assure that they will base pair with – and initiate DNA replication from – only the desired sites in the template DNA molecules. That is because a site comple-
mentary to a primer only 10 nucleotides long will occur by chance about once in every $4^{10}$ (equal to about one million) base pairs, or about three thousand times in the human genome, which is $3 \times 10^9$ base pairs long. In contrast, a site complementary to a primer 25 nucleotides long will occur by chance only about once in every $4^{25}$, or $10^{15}$, base pairs.

In the diagram, the starting DNA sample is represented by a sequence of bases in the region of interest superimposed on an arrow (which is meant to indicate that the template goes on and on). In the first round of replication, only the initial DNA serves as a template. But then in the second round the new DNA molecules can also be used as template. So in each round of synthesis the number of template molecules used, and the number of new molecules produced is doubled. This is where the chain reaction part of the term PCR comes from. In 30 rounds of replication, $2^{30}$ (more than a billion) molecules of the gene of interest are produced for every starting double-stranded molecule present in the original sample. This process produces more than enough of the DNA needed to allow for its study in a variety of ways. This includes gel electrophoresis, the process your students will simulate.
POLYMERASE CHAIN REACTION DIAGRAM

1. Identify and sequence the genetic region of interest

2. Combine your DNA sample (the “template”) with two primers complementary to regions near the ends of the genetic region of interest

3. Replicate once with DNA polymerase

4. Add more primer

5. Replicate again with DNA polymerase

Repeat steps 4 and 5 over and over…

Modern Genetics for All Students
Detecting the Duchenne Muscular Dystrophy (DMD) Mutation

LESSON OVERVIEW

In this electrophoresis exercise, your students will pretend to be technicians in a genetic counseling lab. They will simulate the technique that would be used in such a lab to determine which family members might share the mutant dystrophin gene that has caused one of the Smith boys to develop Duchenne muscular dystrophy. To circumvent all sorts of logistic problems, in this simulation we will use two dyes with different electrophoretic mobilities to simulate two PCR fragments of different lengths - the normal allele and the DMD allele of the dystrophin gene.

Key concepts explored in this activity include a) electrophoresis as a way to separate charged molecules from one another, b) DNA analysis as a way to detect genetic abnormalities, c) X-linked genetic diseases, d) genetic counseling, and e) the personal implications of DNA analyses. It is an excellent complement to Exercise D.4, in which the students will prepare a pamphlet for use by a genetic counselor. Thus, you should schedule this activity for the period of time during which your students will gather the information for their pamphlets.

TIMELINE

There are at least three different ways you can schedule this exercise. Options 1 and 2 require that you have at least as many gel combs and at least half as many gel casting trays as you will have lab groups in all of your classes combined. If you have more than one class but only enough gel casting trays for one class, Option 3 is your only choice. Option 1 takes the least class time but requires more advance preparation on your part.

**Option 1**

You pour the gels in advance; the students complete the rest of the exercise, including analysis and discussion, in one class period (50 minutes).

**Option 2**

Day 1: One period is devoted primarily to discussion of various topics related to this exercise, such as the nature of sex-linked diseases in general and DMD in particular, the principles of electrophoresis, and genetic diagnosis and counseling. The students also pour their gels, wrap them in plastic wrap, and store them in the refrigerator overnight.

Day 2: Students load their gels (5 minutes), run the electrophoresis (15 minutes), and then record, analyze, and discuss their results (30 minutes).
Option 3

Day 1: Students perform the entire lab exercise in one class period, including pouring their gels, loading and running them as soon as the agarose has solidified, and recording the results on their work sheets. This will take most of a 50 minute period, but there will be two 10-15 minute intervals (while the gels are solidifying and while the electrophoresis is occurring) when some discussion will be possible.

Day 2: Students analyze and discuss their results.

MATERIALS

For each class:
   - electrophoresis power supplies
   - gel electrophoresis chambers

For each group of students (group size to be determined by equipment availability):
   - 1 precast agarose gel, or 1 gel-casting tray plus masking tape, 1 or 2 gel-casting combs and 50 ml of 0.8% agarose in water
   - a small container of tap water
   - 1 20 µl micropipettor
   - 6 dye samples labeled A-F
   - 6 pipette tips

The number of electrophoresis chambers and power supplies that you have available will determine the maximum number of groups that can perform this exercise at once. A power supply that will run two gel electrophoresis chambers at once works well for this exercise. The number of groups that can run samples simultaneously with one such power supply is increased to four if extra combs are available. This will allow two sets of samples to be run in each gel. The combs and trays are inexpensive relative to the cost of the rest of the equipment, and they greatly increase flexibility. If you will be running two sets of samples per gel, you or the students who are preparing the gel will need to place two combs in each tray before pouring the agarose. One comb should be placed about 1 cm from one end and the second comb should be placed just beyond the middle of the tray.

The above mentioned materials can be ordered from:
Carolina Biological
(800) 334-5551
www.carolina.com
Electrophoresis Power Supply- catalog # BA-21-3672
Gel Electrophoresis Chamber- catalog # BA-21-3668
Extra Comb- catalog # BA-21-3666
Extra Gel Casting Tray- catalog # BA-21-3667

Agarose can be ordered from:
Carolina Biological
Agarose- catalog # BA-21-7080
ADVANCE PREPARATION

1. **Prepare 0.8% agarose solution, 50 ml per group.** The following recipe is for 200 ml (4 groups):
   - To 200 ml water in a 500 ml flask add 1.6 grams agarose.
   - Cap flask with foil and heat carefully on a hot plate, or cap with plastic wrap and heat carefully in a microwave oven for 3-5 minutes.
   - **Safety Note:** Agarose solution can superheat and either boil over during heating or erupt violently when the flask is touched. Handle hot agarose very carefully, wearing safety goggles and heavy gloves.
   - Swirl flask and make sure that all agarose has dissolved.
   - If gels are to be poured right away, cool flask to about 60°C before pouring.
   - If gels are to be poured later the same day, hold flask in a 60°C water bath until use.
   - If gels are to be poured another day, store solution covered and refrigerated. (Keeps for several weeks.) Then, well in advance of scheduled use, reheat agarose carefully on a hot plate or in a microwave oven until it is completely melted. Then bring it to about 60°C before pouring gels.

   If you will be pouring the gels yourself, follow the instructions given on the student pages for this exercise. When the gels have solidified, wrap each one in plastic wrap to prevent it from drying out.

2. **Prepare stock solutions of bromphenol blue and xylene cyanole.** In this exercise we will simulate the DNA samples of the various Smith family members with a pair of dyes, bromphenol blue and xylene cyanole. Bromphenol blue (BB) travels faster in an agarose gel, so it will be used to represent the "defective allele" that is a partially deleted version of the dystrophin (DMD) gene. Xylene cyanole (XC) travels more slowly in the gel, and so it will be used to represent the "normal" dystrophin allele. Prepare stock solutions as follows:
   - Label two small beakers or flasks BB and XC. Add 10 ml of deionized water and 1 ml of glycerol to each. Swirl to mix.

   **Safety Note:** Certain dyes can be dangerous to your health when they are in the dry state but become harmless once they have been dissolved. So weigh out all dyes in a fume hood, and wear a dust mask, goggles, and gloves until they are in solution.
• Weigh out 0.025 grams (25 mg) of bromphenol blue and add it to the BB container.
• Weigh out 0.025 grams (25 mg) of xylene cyanole and add it to the XC container.
• Carefully stir or swirl both containers until the dyes are thoroughly dissolved.

3. **Prepare simulated Smith family DNA samples from the stock dye solutions.** Set up six microcentrifuge tubes, lettered A–F, for each group. Add dyes as shown in the table below. Then cap the tubes and place each set of six in a sandwich baggie or other small container to hand out to each lab group. As you begin the experiment, inform the students which family member is represented by which letter, but do not provide the other information in the table.

<table>
<thead>
<tr>
<th>Tube</th>
<th>Family member</th>
<th>Genetic condition</th>
<th>Dye sample used</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Mother</td>
<td>Carrier</td>
<td>25 µl BB &amp; 25 µl XC</td>
</tr>
<tr>
<td>B</td>
<td>Father</td>
<td>Normal</td>
<td>50 µl BB</td>
</tr>
<tr>
<td>C</td>
<td>Daniel</td>
<td>Affected</td>
<td>50 µl XC</td>
</tr>
<tr>
<td>D</td>
<td>Alice</td>
<td>Carrier</td>
<td>25 µl BB &amp; 25 µl XC</td>
</tr>
<tr>
<td>E</td>
<td>Michael</td>
<td>Normal</td>
<td>50 µl BB</td>
</tr>
<tr>
<td>F</td>
<td>Fetus</td>
<td>Carrier</td>
<td>25 µl BB &amp; 25 µl XC</td>
</tr>
</tbody>
</table>

4. **Check that each group of students has established proper electrical polarity before giving them permission to turn on their power supply.** The only way that this experiment can work is if the students electrophorese their dye samples in the correct direction, which is from the black electrode (cathode) toward the red electrode (anode). Emphasize (a) that their gel should be placed in the electrophoresis chamber so that the wells will be at the end with the black electrode, and (b) that they should call you to check things over before they turn on their power supply. If you find a gel in the wrong orientation, do not try to move it, or all of the samples will be lost. Instead, switch the wires at the power supply, so that the black wire is connected to the red terminal and vice versa, thereby ensuring that current coming from the black terminal on the power supply will run to the end of the gel where the samples are located.
1. On the diagram below, color the defective alleles purple and the normal alleles blue.

2. On the diagram below
   a. Above each well on this drawing of the gel, put the letter that was on the sample that was loaded into that well.
   b. Using colored pencils, draw all bands that you observed on your gel after electrophoresis.

3. Complete each of the blanks in the data table below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Family Member</th>
<th># of DMD Alleles</th>
<th>Genotype</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tube A</td>
<td>Mother Mary</td>
<td>2</td>
<td>XN Xm</td>
<td>Carrier</td>
</tr>
<tr>
<td>Tube B</td>
<td>Father John</td>
<td>1</td>
<td>Y XN</td>
<td>Healthy</td>
</tr>
<tr>
<td>Tube C</td>
<td>Son Daniel</td>
<td>1</td>
<td>Y Xm</td>
<td>Has DMD</td>
</tr>
<tr>
<td>Tube D</td>
<td>Daughter Alice</td>
<td>2</td>
<td>XN Xm</td>
<td>Carrier</td>
</tr>
<tr>
<td>Tube E</td>
<td>Son Michael</td>
<td>1</td>
<td>Y XN</td>
<td>Healthy</td>
</tr>
<tr>
<td>Tube F</td>
<td>Fetus M or F</td>
<td>2</td>
<td>XN Xm</td>
<td>Carrier</td>
</tr>
</tbody>
</table>
4. Which allele moves further into the gel, the normal (XN) or mutant (Xm) allele? Why?
   The mutant (Xm) allele. It has undergone a partial deletion, so it is shorter, and shorter DNA fragments migrate faster than longer ones in gel electrophoresis.

5. Is the daughter, Alice, a carrier for DMD? How can you tell?
   Yes. Her DNA contains two fragments equal in size to the fragments representing the normal and mutant alleles present in her mother's DNA.

6. Does Michael have DMD? How can you tell?
   No. He has only the wild-type (normal) DMD allele.

7. What can you tell the Smith family about their unborn child?
   It is female, and like Mary and Alice, it will be a carrier for DMD.

8. Why are most patients with DMD male?
   A female with DMD is only produced in the extremely rare (1/10,000,000) cases when a carrier female produces an egg bearing the mutant DMD allele and that egg is fertilized by a sperm carrying a brand-new DNA mutation that arose during sperm formation.

9. Can a boy be a carrier for DMD without having the disease? Why or why not?
   No. Boys only have one X chromosome, so if they inherit a mutant DMD allele they get the disease.

10. If you were the genetic counselor in this case, what would you tell the Smiths about their test results?
    That there is some good news and some bad news. The good news is that neither Michael nor the unborn child will get DMD. The bad news is that both Alice and the unborn daughter are carriers.