DNA: Duchenne's Not Allowed

Team 14

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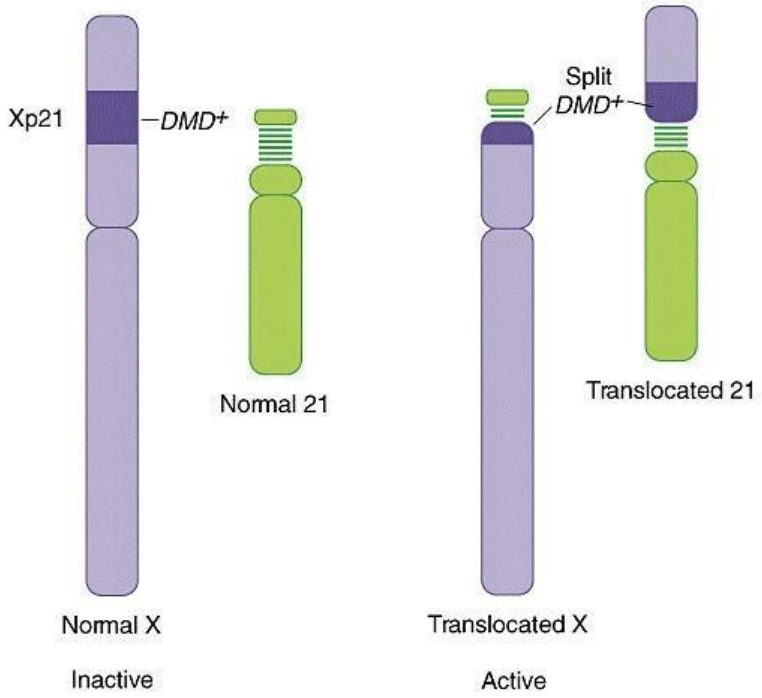
I. Introduction

Team 14 from Artesia High School is comprised of Kathryn Moore, senior, and Jessica MacKinnon, junior.

The project focused on the Biochemistry and Genetics of Duchenne Muscular Dystrophy. Duchenne Muscular Dystrophy, an x-linked recessive disease also known as DMD, targets young males. James Moore, Kathryn Moore's younger brother, inherited this disease from his mother, Sondra. Kathryn does not have Duchenne Muscular Dystrophy because she is female, and therefore she inherited two x chromosomes. The second x chromosome protects the female from the disease because it, in essence, "replaces" the defective DNA in the first chromosome. The DMD defective chromosome is shown below:



The time it takes to search through each individual base pair for Duchenne-causing mutations in the DNA drastically increases the time needed to find a potential treatment for DMD. To erase the need for this tedious step, Team 14 created a Java algorithm to find the mutations in the DNA and "print" out the mutated gene sequences in order of occurrence.

II. Duchenne Muscular Dystrophy

A.Cause

Mutation(s) in the DNA coding sequence for the Dystrophin gene cause Duchenne Muscular Dystrophy. An individual inflicted with DMD possesses one or more of the following mutations:

A missing base pair in the DNA code causes a frame-shift mutation, which causes the nucleotides that form messenger RNAs (mRNAs) to "skip" that base-pair and continue to the next base pair. Why is that a problem? Skipping a base pair is exactly like skipping a bubble on a standardized test; all the following code is misplaced by one. This incorrect code will not form a functional dystrophin gene. It will either produce a gene which makes too little Dystrophin or a gene which makes no Dystrophin at all.

An incorrect base pair in the DNA code causes a missense mutation. Although no base pairs are missing, an incorrect base pair in place of a true base pair prevents the correct continuation of the mRNA.The mutated sequence may be skipped and the coding continued further down the DNA strand, but this process will not produce functional Dystrophin.

An added base pair in the DNA also causes a missense mutation. However,

the base-pairs are no longer one behind their respective matches as in a frame-shift mutation but one ahead. This produces a mangled dystrophin gene with little or no function.

A premature stop triplet, called a stop codon, halts the reading of the DNA, and therefore stops the making of the Dystrophin gene. On the other hand, the absence of a stop codon allows further reading of the gene past the end of the Dystrophin coding sequence. This does not produce dystrophin, but rather a tangled mass of amino acids which has no function whatsoever.

No chemicals or other biological substances induce Duchenne Muscular Dystrophy, it is solely caused by the hereditary traits passed down to the individual from the mother and father.

B. Symptoms & Course

The symptoms of Duchenne Muscular express themselves in young boys between the ages of six and eight. These symptoms are (in order):

Gowers sign

Curved Spine (Scoliosis)

Difficulty Walking

Slurred Speech

Fatigue

Hypertrophy of the Calf Muscle (Gastrocnemius)

Loss of strength

Falling

Inability to Walk

Loss of Primary Motor Function

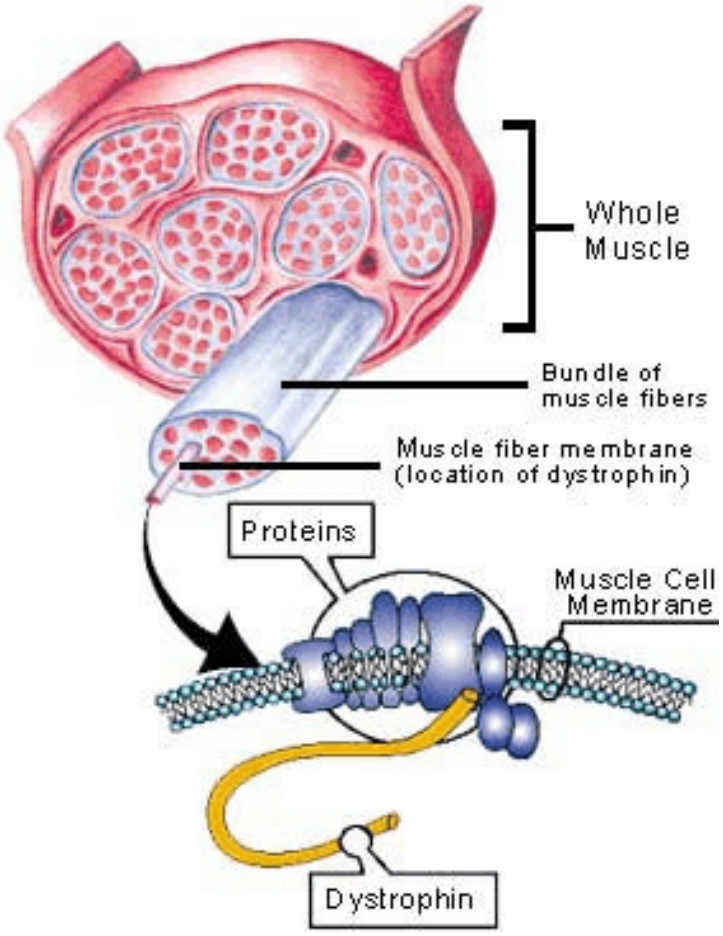
Weakness of Neck Muscles (Trapezius, Sternocleidomastoid)

Loss of Secondary Motor Function

Coma

Death (Respiratory/Cardiac failure)

What is Dystrophin?



Dystrophin is the "sticky" substance which holds the muscle fibers together that form the functional muscles we know as the Biceps or Pectoralis Major. The picture below show the location of dystrophin in the muscle:

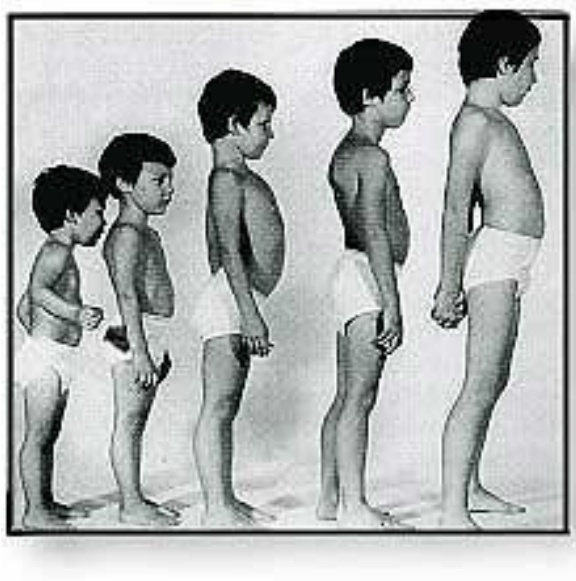
Without dystrophin, the muscle fibers could not bind to each other and therefore could not work together. This means that the muscle would have no function in moving the body, which is what happens in the progression of Duchenne Muscular Dystrophy.

Gowers sign:

This is the process by which the afflicted individual picks himself up off of the floor. It is a slow process of many steps, but is exhibited by all boys with Duchenne Muscular Dystrophy. This process is illustrated by the picture below:

Curved Spine (Scoliosis):

At the onslaught of the disease, scoliosis, or a curvature of the spine, sets in. The progression of scoliosis follows the progression of the disease, which is evidenced below:



Difficulty Walking:

As the muscles grow steadily weaker from Dystrophin depravation, It becomes increasingly difficult for the child to walk normally. Scoliosis in the spine alters the center of gravity and weak muscles struggle to lift bones once so easily lifted. This is the stage at which most cases of DMD are noticed and diagnosed.

Slurred Speech:

The muscles which control the action of speech are sometimes targeted by Duchenne. The result of this targeting is the lisped s and stuttering, sometimes even inability to fully control the muscles of speech. Not all DMD victims inherit this trait, however. Many are fully capable of normal speech.

Fatigue:

Muscles need oxygen to work properly. Lack of oxygen causes muscle fatigue, which slows down the fatigued individual. However, muscles are also involved in

breathing. These muscles, the intercostals and the diaphragm, are sometimes broken down by DMD. This means that the rest of the muscles i n the body do not receive the required amount of oxygen for the amount of tasks laid before them. The result is an exhausted individual.

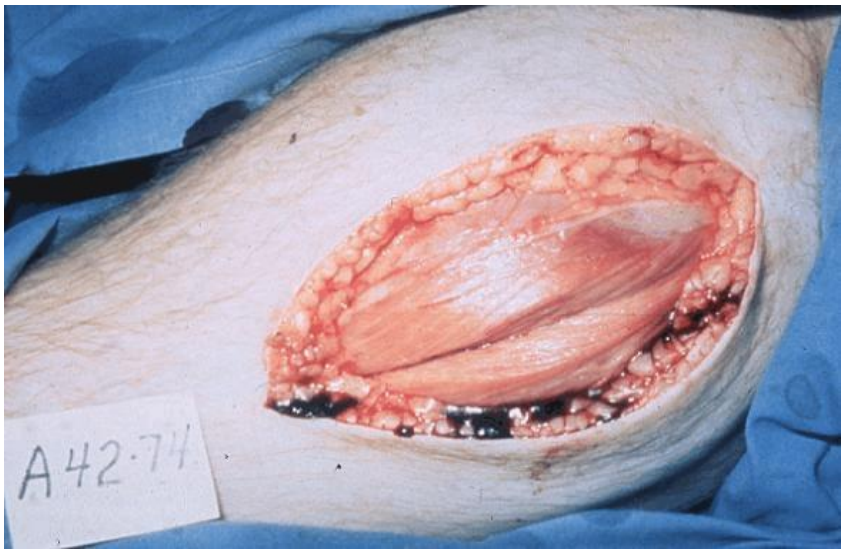
Pseudohypertrophy of the Calf Muscle:

The calf muscle (Gastrocnemius) is the largest muscle in the lower leg. In Duchenne, this area bulges and becomes psuedeohypertrophic. Psuedohypertrophy literally means "false growth."

When a person "works out," he or she stresses the muscles until the fibers rupture. This allows for growth of the muscle. However, in Duchenne patients, the torn muscle is filled in with fat (adipose tissue) rather than new muscle tissue.

The picture below illustrates the psuedohypertrophic Gastrocnemius.

Loss of Strength:



The progression of Duchenne Muscular Dystrophy is marked by a symmetric loss of strength beginning at the proximal muscle tissue. Many of the larger muscles, such as the Latissimus Dorsi, Trapezius, and Pectoralis Major, noticeably lose strength at this stage.

Falling:

Loss of strength in DMD victims allows them to fall more often. Every fall

holds risk for potential injury, which could prevent them from walking.

Inability to Walk:

Usually caused by an injury or lack of muscle strength, the inability to walk strikes DMD patients around age ten. This leaves them confined to a wheelchair. In the case of James Moore, he was able to continue walking until age thirteen, when a foot injury ended his walking ability.

Loss of Primary Motor Function:

At this stage, the young men start losing other functions such as the ability to pick up the leg off of a chair, the ease of raising the hand, and most all other major motor functions. This leaves the afflicted not only confined to a wheelchair, but helpless too.

Weakness of Neck Muscles:

At this point, the muscles in the neck cannot hold up the head any longer. This causes the patients head to fall backward and he soon loses the ability to move his head.

Loss of Secondary Motor Function:

Here the individual loses the ability to write, type, and speak. The small

movements of the hand and foot are ceased, and the finer movements of the face are also stopped.

Coma:

The lack of strength in the muscles that support the neck causes damage to the spinal cord. Long term wear damages the spinal cord to the extent that the DMD victim falls into a coma.

Death:

The victims usually die of Respiratory or Cardiac failure.

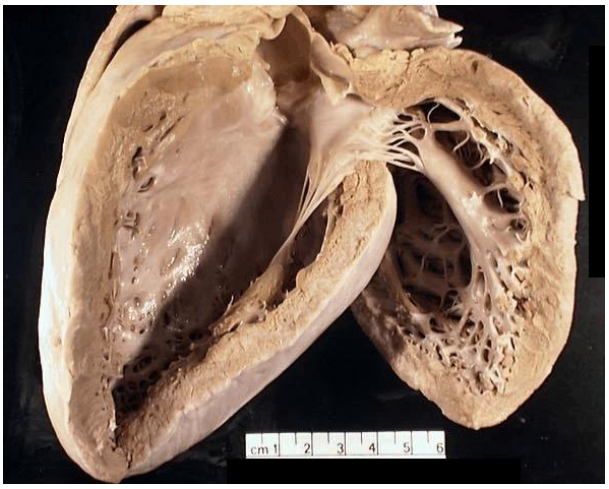
In Respiratory failure, the diaphragm is no longer strong enough to support the actions of breathing. Therefore, not enough oxygen enters the body via the lungs. The lung tissue begins to die followed by the rest of the tissues.

Fig. 4 - Hemorrhagic infarcts in the middle and lower lobes of the right lung.

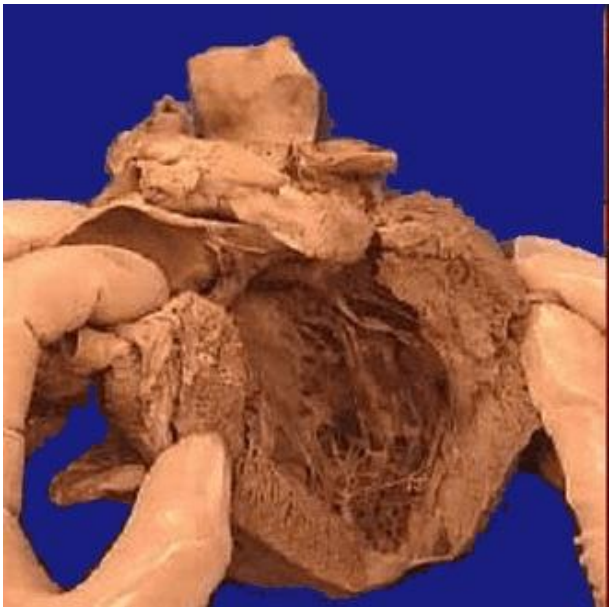
In Cardiac Failure, psuedohypertrophy of the myocardium inhibits conductivity of the heart as a whole and leads to its failure. The pictures below

illustrate a healthy heart and a psuedohypertrophic heart.

Psuedohypertrophic



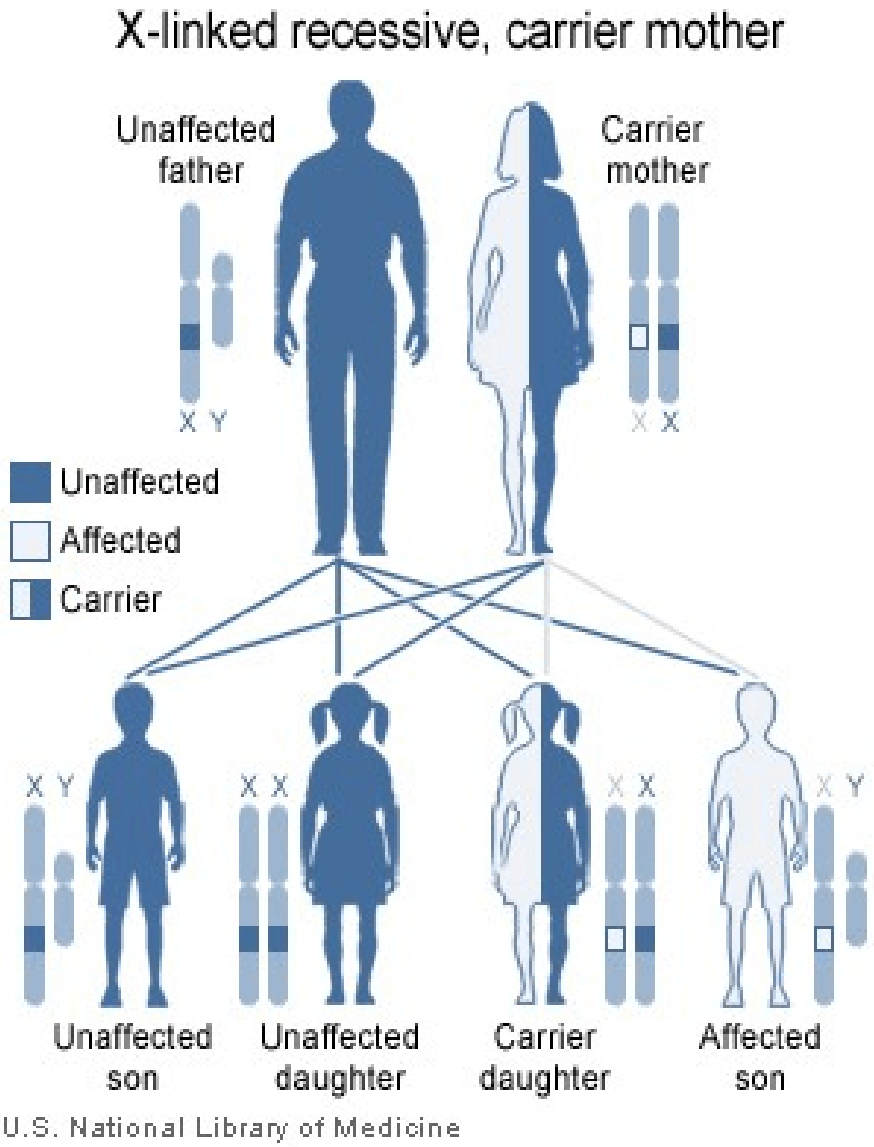
Healthy Human



C. Victims

Duchenne Muscular Dystrophy targets male children. In the picture below,

the genetics behind the passing of DMD are below.



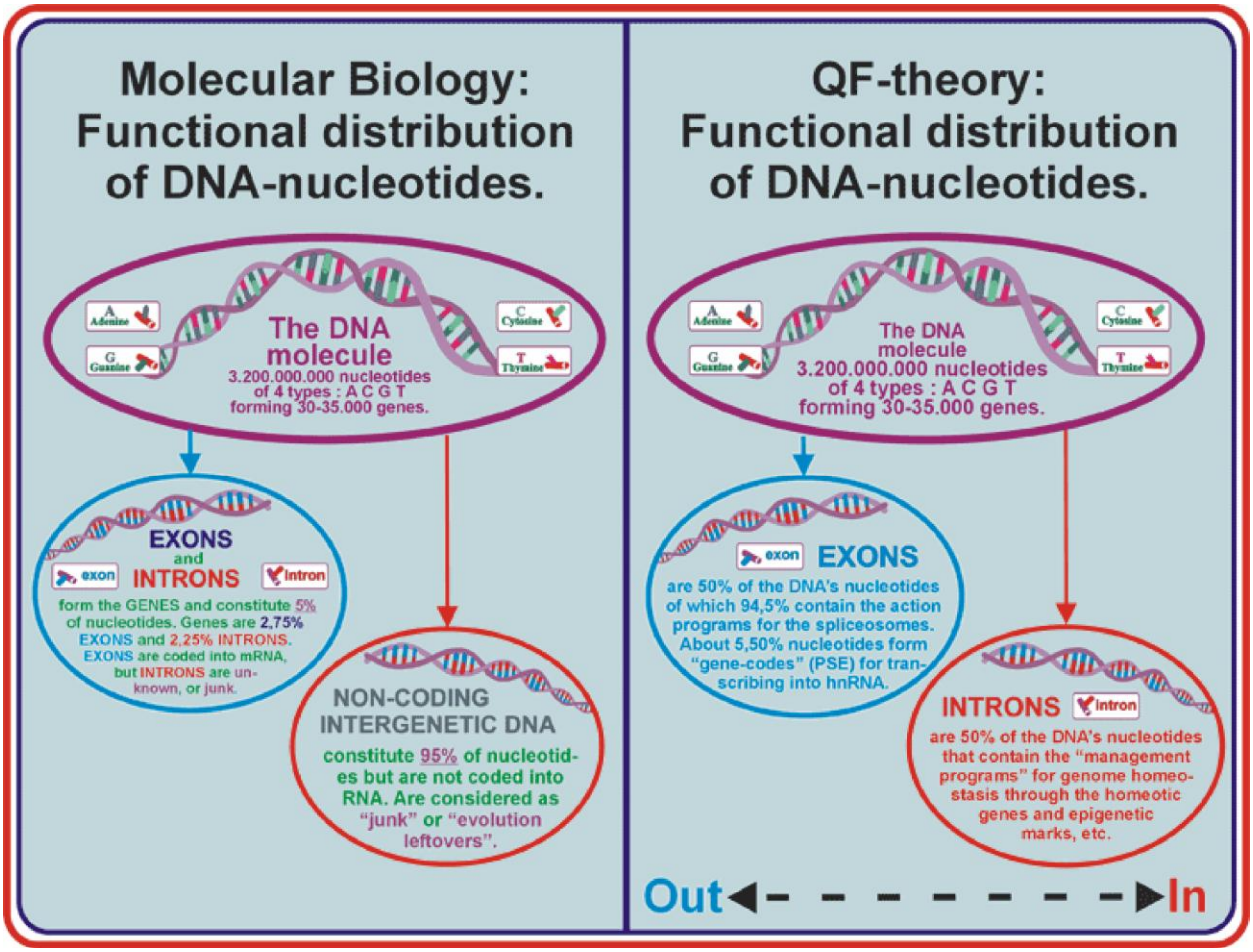
Only the males contract Duchenne Muscular Dystrophy because they have only one x chromosome. This means that half of the male's DNA is corrupted.

D. Potential Treatments

There are several promising potential treatments, the most prominent-being Exon Skipping and-Viral integration of the corrected gene sequence.

Exon Skipping:

Exon skipping is the skipping of exons in order to induce dystrophin. As is illustrated by the graphic below, an exon is a section of DNA.



A chemical induces exon skipping of a specific exon. In order to treat Duchenne Muscular Dystrophy, exon 47 is skipped. Instead of completely halting production of the dystrophin gene, skipping exon 47 induces production of a completely functional and healthy dystrophin gene.

Viral Integration:

In Viral Integration, a corrected gene sequence is injected into a virus which targets the dystrophin gene. Once injected into the patient, the virus integrates its own DNA into the mutated section. This effectively corrects the error and restores normal production of dystrophin.

III. Statement of Purpose

Our purpose in this project is to aid in the search of a cure for Duchenne Muscular Dystrophy by providing a simple algorithm that will find the mutations in the dystrophin DNA sequence.

The main reason we chose to focus on Duchenne Muscular Dystrophy was James Moore, Kathryn Moore's younger brother. He is a candidate for gene therapy as soon as it is released to the doctors. This algorithm will speed up the process of releasing the cure to the doctors.

IV. Progression of Project

As we started out our project, we didn't know exactly what we were doing. During the Kickoff conference, we began to develop our idea. Soon, we wrote a summary of what our program would accomplish. We produced a pseudo-code to help guide our project.

We began to write code. The program was based from the Sieve of Eratosthenes, which finds all prime numbers up to a given amount. We hoped to use this ability to find patterns in our project. With this program, we would be able to find introns, exons, and specific segments of gene.

For the Project Evaluations, we also created a StarLogo demonstration of the effects of the Human Endogenous Retroviruses (which will be displayed in our HTML report). However, at the Evaluations, the model failed to work, and our code didn't impress the judges.

Scrapping the code, we sought help from Mr. Nick Bennett. With his aid, we created the code that we now have.

V. Code

1. Java

This code sets up our program.

```
/*
 * Finder.java
 *
 * Created on March 5, 2007, 2:23 AM
 *
 * To change this template, choose Tools | Template Manager
 * and open the template in the editor.
 */
package edu.artesiahs.scc.duchenne;
import java.util.ArrayList;
enum SearchResult {
    FoundGoodGene,
    FoundBadGeneLength,
    FoundBadGeneData,
    FoundStart,
    EndOfText
}
/**
 *
 * @author artesiascc
 */
```

```
public class Finder {
    private String start;
    private ArrayList<String> stop = new ArrayList<String>();
    private String gene;
    private StringBuffer dna = new StringBuffer ();
    private int endGene = 0;
    private long discarded = 0;
    private int startPosition;
    private int stopPosition;
    private String currentStop;

    /** Creates a new instance of Finder */
    public Finder() {
    }
    public SearchResult detect() {
        SearchResult result = SearchResult.EndOfText;
        startPosition = dna.indexOf(start, endGene);
        if (startPosition != -1) {
            dna.delete(0, startPosition);
            discarded += startPosition;
            startPosition = 0;
            endGene = 0;
            stopPosition = -1;
```

```
for (String testStop : stop) {
    int testPosition = dna.indexOf(testStop, startPosition + start.length());
    if (testPosition != -1) {
        if (testPosition < stopPosition || stopPosition == -1) {
            stopPosition = testPosition;
            currentStop = testStop;
        }
    }
}
if (stopPosition != -1) {
    endGene = stopPosition + currentStop.length();
    String testGene = dna.substring(startPosition + start.length(),
 stopPosition);
    if (testGene.length() == gene.length()) {
        if (testGene.equalsIgnoreCase(gene)) {
            result = SearchResult.FoundGoodGene;
        }
        else {
            result = SearchResult.FoundBadGeneData;
        }
    }
    else {
        result = SearchResult.FoundBadGeneLength;
```

```
            }
        }
        else {
            result = SearchResult.FoundStart;
        }
    }
    else {
        discarded += dna.length();
        dna = new StringBuffer ();
        endGene = 0;
    }
    return result;
}
public String getStart() {
    return start;
}

public void setStart(String start) {
    this.start = start;
}
public String getStop(int index) {
    return stop.get(index);
}
```

```
public void addStop(String stop) {
    this.stop.add(stop);
}
public String getGene() {
    return gene;
}
public void setGene(String gene) {
    this.gene = gene;
}
public void setDna(String dna) {
    this.dna = new StringBuffer (dna);
    endGene = 0;
    discarded = 0;
}
public void addDna (String dna) {
    this.dna.append(dna);
}
public long getStartPosition() {
    return (startPosition > -1) ? startPosition + discarded : -1;
}
public String getFoundGene() {
    String result = null;
    if (startPosition > -1 && stopPosition > -1) {
```

```
            result = dna.substring(startPosition, stopPosition + currentStop.length());
        }
        return result;
    }
}
```

This code runs the program. Since we were unable to complete entirely the program, it is simply a functioning test program.

```
/*
 * TestFinder.java
 *
 * Created on March 5, 2007, 4:16 AM
 *
 * To change this template, choose Tools | Template Manager
 * and open the template in the editor.
 */
package edu.artesiahs.scc.duchenne;
import java.io.FileReader;
import java.nio.CharBuffer;
/**
 *
 * @author artesiascc
 */
public class TestFinder {
```

```
private static final int BUFFER_LENGTH = 1000;
private static final String SOURCE_FILE = "/home/artesiascc/XDNA/test subject 1.dna";
/** Creates a new instance of TestFinder */
public TestFinder() {
}
/**
 * @param args the command line arguments
 */
public static void main(String[] args) {
    try {
        Finder jessica = new Finder();
        /*
         *This is an example.
        String[] dna = new String[] {"AAAAAAXXGOODGENEYY",
                                     "BBBBBBBBBBBBXXHIJE",
                                     "SSHOWAREYOUCCCCCXX",
                                     "BADGENEYYDDDDDDXXG",
                                     "OODGENEZZEEEEEEEEE",
                                     "RKHAHAHADMSDONTHAV",
                                     "ELEVELSYYXDARKHAHA",
                                     "XXENDISNERZZBYEBYE"};
         */
        FileReader reader = new FileReader(SOURCE_FILE);
```

```
    CharBuffer buffer = CharBuffer.allocate(BUFFER_LENGTH);
    int charactersRead;
    jessica.setStart("gac");
    jessica.addStop("tac");
    //jessica.addStop("ZZ");
    jessica.setGene("GOODGENE");
    while ((charactersRead = reader.read(buffer)) != -1) {
        SearchResult result;
        buffer.position(0);
        jessica.addDna(buffer.toString().substring(0, charactersRead));
        result = jessica.detect();
        System.out.println(result);
        switch (result) {
            case FoundGoodGene:
            case FoundBadGeneLength:
            case FoundBadGeneData:
                System.out.println(jessica.getFoundGene());
            case FoundStart:
                System.out.println(jessica.getStartPosition());
            case EndOfText:
        }
    }
}
```

```
        catch (Exception e) {
            // Do nothing
        }
    }
}
```

B. StarLogo

Observer Code

```
to setup

ca

intro-bad

intro-virus

end

to intro-bad
crt 500
ask-turtles
[setxy (random screen-height)
(random screen-width)
stamp blue]
ct
ask-patches
[if pc = black
[sprout
[setbreed badcell
setc red
setshape cell]]]
cp
end
to intro-virus
create-virus 20
ask-virus
[setc yellow
setage random 3
setshape yar]
end
```

Turtle Code

```
breeds [goodcell badcell virus]
turtles-own [age]

to go
produce
move
infect
old
```

```
end

to produce
if breed = goodcell
[if age < 10
[hatch
[setbreed virus
setc yellow
setage 0
setshape yar]]]
end

to move
if breed = virus
[fd 1
rt random 360
lt random 360]
end

to infect
if breed = badcell
[if count-virus-here > 0
[setbreed goodcell
setc blue
setage 0
setshape cell]]
end

to old
if breed = virus
[setage age + 1
if age > 3
[die]]
if breed = goodcell
[setage age + 1]
end
```

V. Functions of Code

Our Java program is meant to find the mutated sections of a gene.

Basically, the program loads the provided gene sequence into its database and begins to seek out start and stop codons. Between starts and stops, Java counts the number of base pairs. If the number is more or less than 13,973 base pairs, the gene is printed out on the screen to show its mutation. Java also compares the gene side by side with the correct sequence, and if there are any alterations, that gene is printed out, as well. If the gene is correct, the program shows that it is correct and does not print out the sequence.

VII. Conclusion

Duchenne Muscular Dystrophy kills boys in their youth without giving them any chance of "normalcy" before they die. This Java algorithm will aid in the search for the coveted cure by using the patients' own Danced finding the specific mutations in that DNA. This ensures that the manufactured treatment causes production of functional dystrophin genes. The DMD victims will be cured in this way.

VIII. Acknowledgments

Here is where we give a HUGE thank you to Mr. Gaylor. He sacrificed countless hours to make sure we could finish all of our work for our project. Thanks alot Mr.G for all those hours you could have been at home.

Next we need to thank Mr. Bennett for bailing us out of a very steep hole. Without his help, we would still have a % modulus in our code.

We also need to thank Dr. Crit Caton and Mr. Thad Phipps for supporting the Challenge at Artesia High School and for sponsoring all of our out-of-town trips. Without them we wouldn't be here.

And last, but never least, we would like to think the judges who listened to us and gave us critical feedback for us to use to better our program.

VIV. Glossary

Duchenne Muscular Dystrophy - an x-linked recessive neuromuscular disease which targets males.

DNA - Deoxyribonucleic Acid

Dystrophin - the protein that binds muscle fibers together.

Exon - a DNA "plateau"

Hypertrophy - growth (particularly of a muscle)

Intron - a DNA "valley"

Myocardium - heart muscle

Psuedohypertrophy - "false growth" especially in muscle tissue

X-Linked Recessive - on the X chromosome and it is a recessive trait (does not show up often, is not dominant).