Electrophoresis Procedure

HASPI Medical Biology Activity 10a

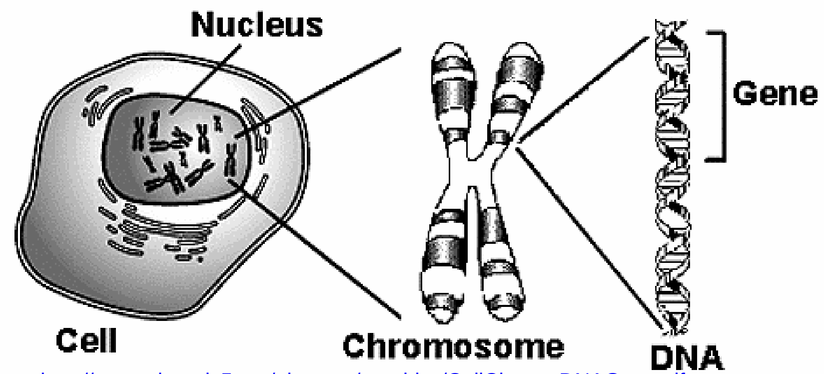
Name: ______________________________

Period: ___________ **Date:** ___________

Background

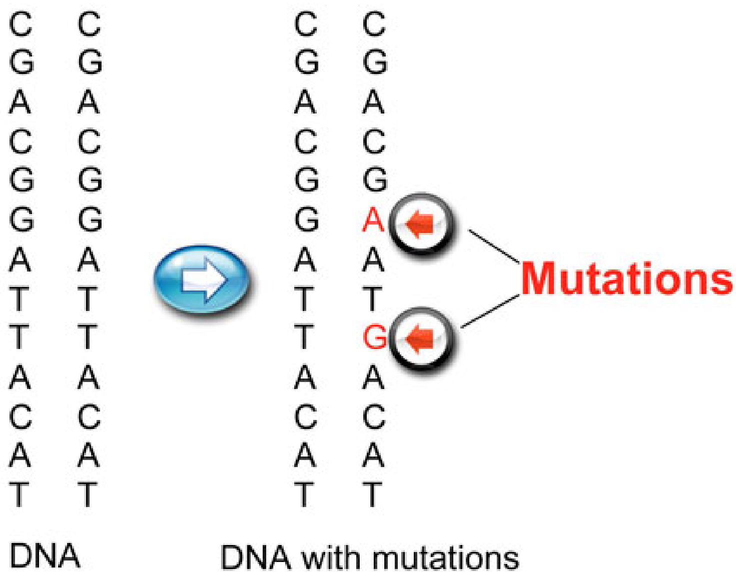
DNA, Genes, and Mutations

DNA, or deoxyribonucleic acid, is the hereditary material in humans and almost all other organisms. Nearly every cell in a person's body has the same DNA. Most DNA is located in the cell nucleus, but a small amount of DNA can also be found in the mitochondria.



http://www.phoenix5.org/glossary/graphics/CellChromoDNAGene.gif

The information in DNA is stored as a code made up of four bases: adenine (A), guanine (G), cytosine (C), and thymine (T). Human DNA consists of about 3 billion bases, and more than 99% of those bases are the same in all people. The order, or sequence, of those bases determines the information available for building and maintaining an organism, similar to the way in which letters of the alphabet appear in a certain order to form words and sentences.



http://www.uic.edu/com/dom/gastro/fgicu/assets/images/Mutations_chart.jpg

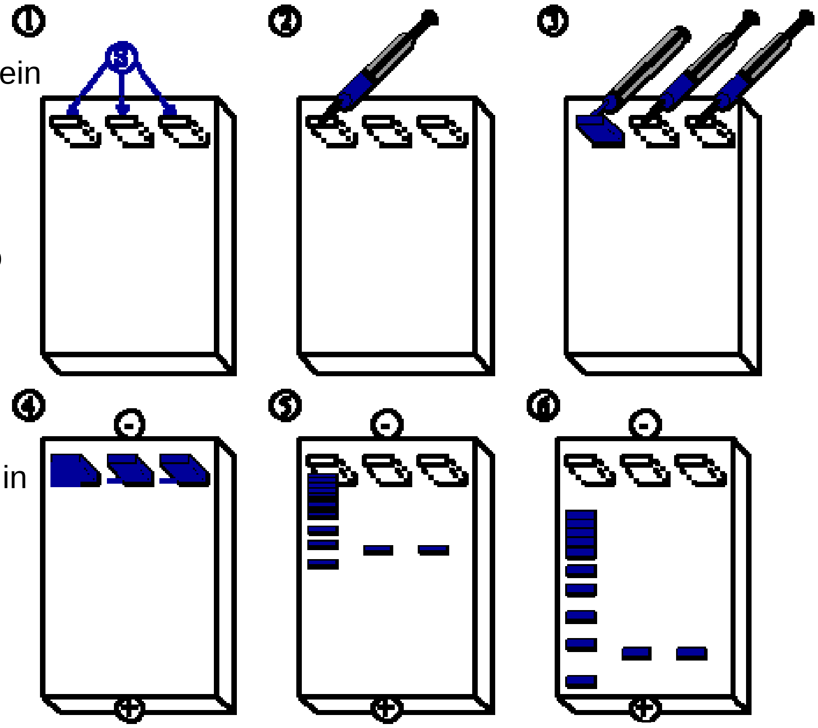
Just like a sentence has a meaning or directions, so does the order of DNA bases. For example, a sentence may read, *"I need you to pick up milk from the store, and then stop and fill the car up with gas."* This sentence has meaning and a set of directions. If the same words were used in a sentence but in a different order, it would not have the same meaning. For example, it could read, *"Stop from milk car need I you up then and and fill store gas the with pick to, the."* Obviously, this does not make any sense and does not relay the same directions even though all of the words are the same.

The order of the 4 DNA bases is the same. The organization of the bases has meaning and is actually a set of directions to make a protein. This set of directions for a specific protein is called a **gene**. When a gene has the incorrect order of bases, the directions are wrong and mistakes in how the protein is made occur. These are called **genetic mutations** that result in genetic disease. For example, the protein that gives color to our skin is called melanin. Individuals who are albino have a mistake in the gene that makes this protein.

NIH. 2011. What is DNA and How Do Genes Work? Genetics Home Reference, National Institutes of Health. http://ghr.nlm.nih.gov/handbook/basics/dna

Name: ______________________________ Date: __________ Period:__________

Electrophoresis

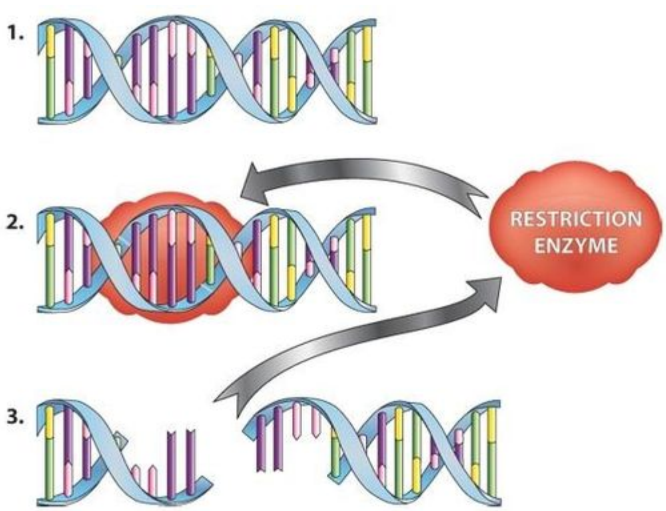


http://www.websters-online-dictionary.org/images/wiki/wikipedia/commons/1/11/Agarose-Gelelektrophorese.png

In order to diagnose a genetic disease, the presence of the incorrect gene or wrong protein must be identified. One method that can be used to do this is called electrophoresis. **Electrophoresis** is a technique that uses electricity to separate pieces of DNA by size through a gel. DNA is negatively charged so it moves through the gel to the positive pole. Larger pieces move slower than smaller pieces, which causes them to separate.

Detailed Steps

1. Wells, or holes, in the gel are created in order to have a space for the DNA to be placed.
2. A micropipette is used to place small amounts of DNA into the wells.
3. A DNA sample with standard sized pieces of DNA for comparison, a mutated gene for comparison, and the patient sample are loaded into the wells.
4. An electrical power source is used to run an electrical current through the gel.
5. The DNA moves from negative to positive over time.
6. The smallest pieces of DNA move the farthest and the larger pieces of DNA move the shortest distance. The distance each section of DNA moves can be measured and the number of bases in the DNA can be calculated.



http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/R/RestrictionEnzymes.html

Restriction Enzymes

In order to identify and separate out a specific gene through electrophoresis, the gene has to be separated, or "cut", out of the DNA. **Restriction enzymes** are enzymes found in bacteria that are capable of "cutting" the DNA into smaller fragments. Each restriction enzyme is named for the bacteria from which it was taken. There are several types of restriction enzymes that cut DNA at the same place every time. For example, the bacteria *Haemophilus aegypticus* produces a restriction enzyme called **HaeIII** that cuts DNA everywhere it sees the following sequence of nucleotides – "GGCC". Some other restriction enzymes include:

Enzyme	Bacteria Source	Cuts at:
EcoRI	*Escherichia coli*	GAATTC
BamHI	*Bacillus amyloliquefaciens*	GGATCC
HindIII	*Haemophilus influenzae*	AAGCTT

Materials

24.5 cm Mother DNA (red)	*24.5 cm Daughter DNA (yellow)*	*Ruler*
24.5 cm BRCA1/BRCA2 DNA (blue)	*Scissors*	*Tape or glue*

Procedure

Purpose: The goal of this lab will be to learn the use of restriction enzymes and electrophoresis while testing whether a mother and daughter may have the gene for breast cancer present in their DNA.

5-10% of breast cancer cases are inherited. There are two genes associated with inherited breast cancer: BRCA1 (BReast CAncer gene 1) and BRCA2 (BReast CAncer gene 2). The normal function of this gene is to maintain the function and growth of breast cells. If these genes are mutated they do not control the normal growth of breast cells, which increases the risk of breast cancer. On average, 12% of women are at risk of developing breast cancer, but those with an abnormal BRCA1 or BRCA2 gene have an 80% risk of developing breast cancer.

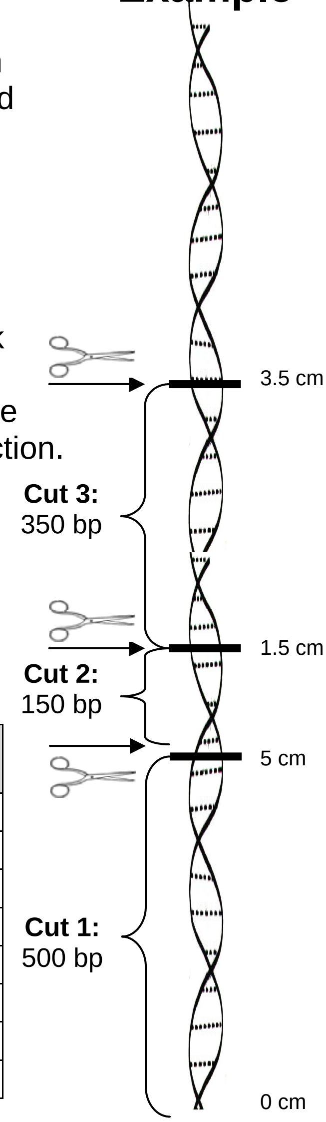
A female patient has come to HASPI Genetic Horizons Inc. to be tested to see if she or her daughter may have the BRCA1 or BRCA2 gene. Her own mother has recently passed away from breast cancer, so she believes she and her daughter may be at risk. You have been tasked with testing the mother and daughter DNA samples for the presence of these genes.

Part A: Restriction Enzyme

The restriction enzyme EcoRI was used to cut the DNA samples taken from the mother and daughter. A DNA sample that contains both the BRCA1 and BRCA2 gene for breast cancer was also used for comparison.

1. Obtain the three DNA sample strips – BRCA1/BRCA2 DNA is blue, mother DNA is red, and daughter DNA is yellow.
2. ***Data Table 1*** below shows where the restriction enzyme EcoRI cuts each DNA sample by the number of base pairs.
3. **1 cm = 100 base pairs (bp):** Using the ruler, measure out and mark where EcoRI will cut each DNA sample. There will be 8 cut sites for each of the 3 strips according to the table below. Make a mark on the DNA strip at each cut site and record the number of bps for each section.
4. Use scissors to cut the DNA strip at each measured mark.
5. Repeat this for each DNA strip – BRCA1/BRCA2, Mother, and Daughter. See the **Example** showing the first 3 cuts for the BRCA1/BRCA2 DNA sample.
6. Continue on to Part B: Electrophoresis with the cut DNA samples.

Example



Data Table 1 – EcoRI Restriction Enzyme

Cut Number	BRCA1/BRCA2 DNA	Mother DNA	Daughter DNA
1	500 bp	100 bp	70 bp
2	150 bp	350 bp	130 bp
3	350 bp	450 bp	350 bp
4	90 bp	300 bp	550 bp
5	210 bp	50 bp	400 bp
6	470 bp	650 bp	450 bp
7	230 bp	220 bp	200 bp
8	450 bp	330 bp	300 bp

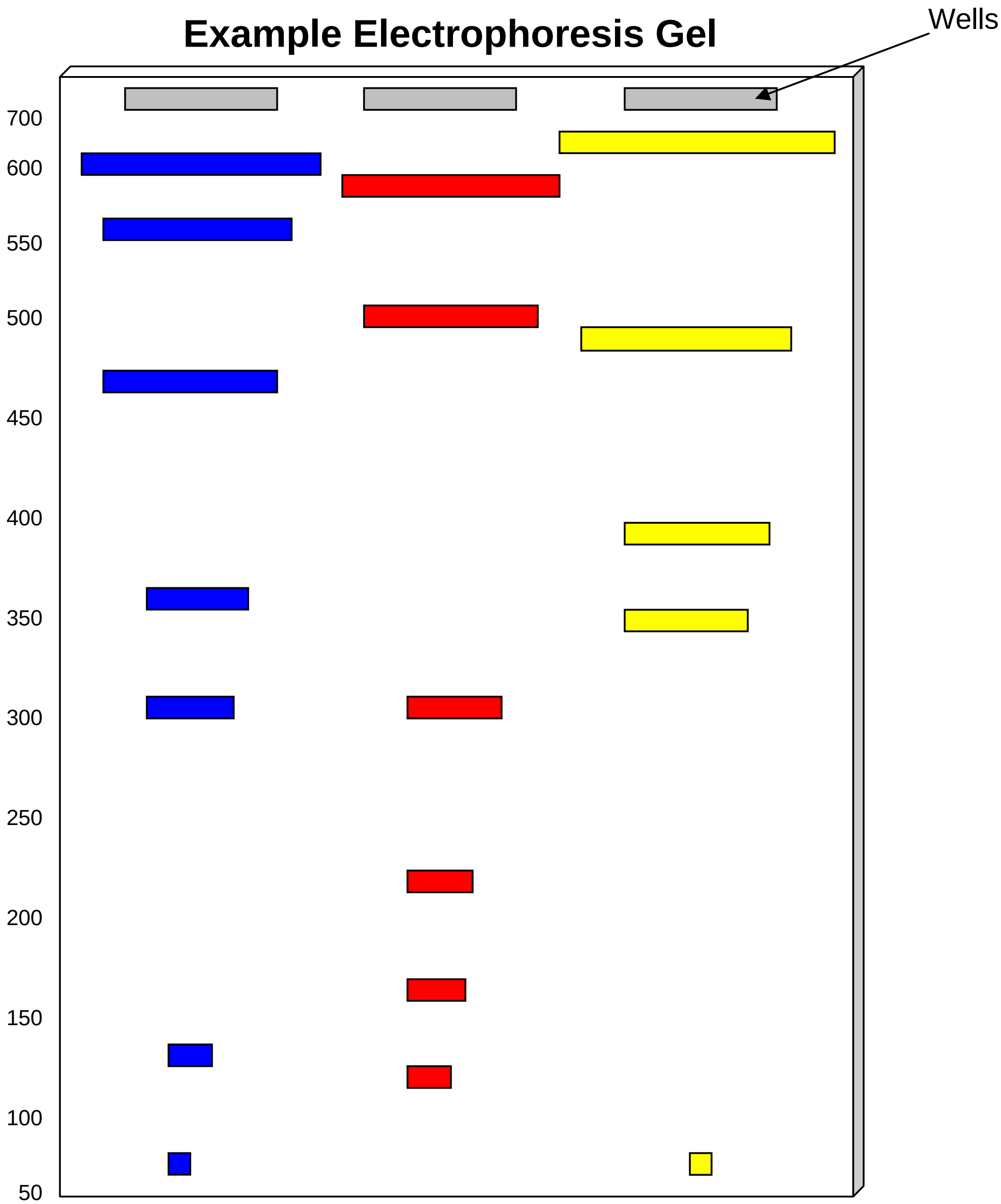
Name: ____________________________________ Date: __________ Period: __________

Part B: Electrophoresis

Now that the DNA samples have been cut by the restriction enzyme, they need to be placed on the electrophoresis gel according to how far they would move. Remember: larger pieces of DNA (with more base pairs) do not move far, while shorter pieces of DNA (with less base pairs) move the farthest.

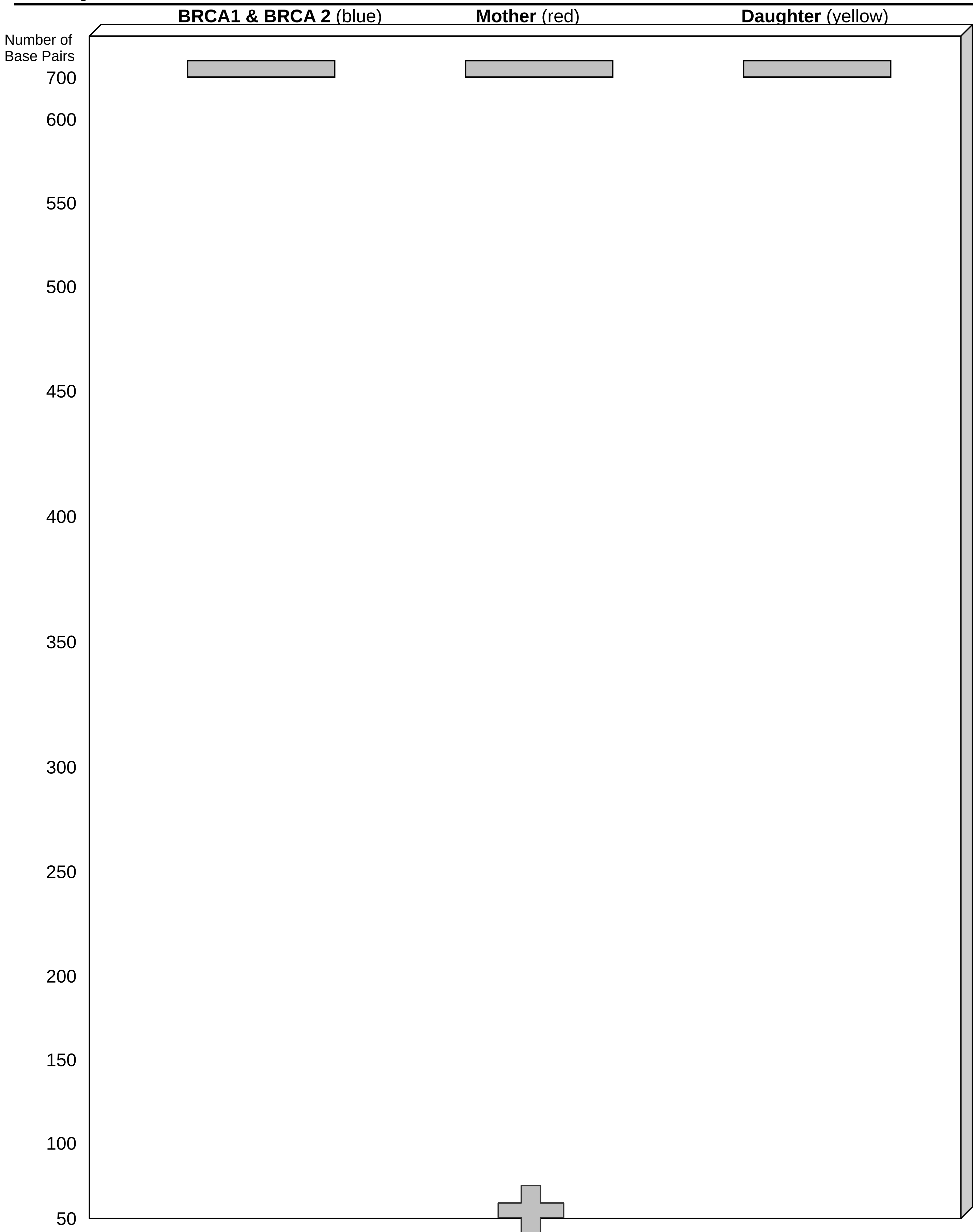
1. Go to the analysis section and locate the electrophoresis gel. The gel has three wells already labeled: BRCA1/BRCA2(blue), Mother(red), and Daughter(yellow).
2. Starting with the BRCA1/BRCA2 (blue) DNA that was cut in Part A, use tape to place each piece of DNA on the electrophoresis gel according to the "Number of Base Pairs" located on the left side of the gel.
3. Use tape to place the DNA for the Mother and Daughter DNA samples.
4. The following is an **Example** of a completed electrophoresis gel.

Example Electrophoresis Gel



Name: ______________________________________ Date: __________ Period: __________

Analysis – *gel electrophoresis sample*



Name: ____ Date: __________ Period: __________

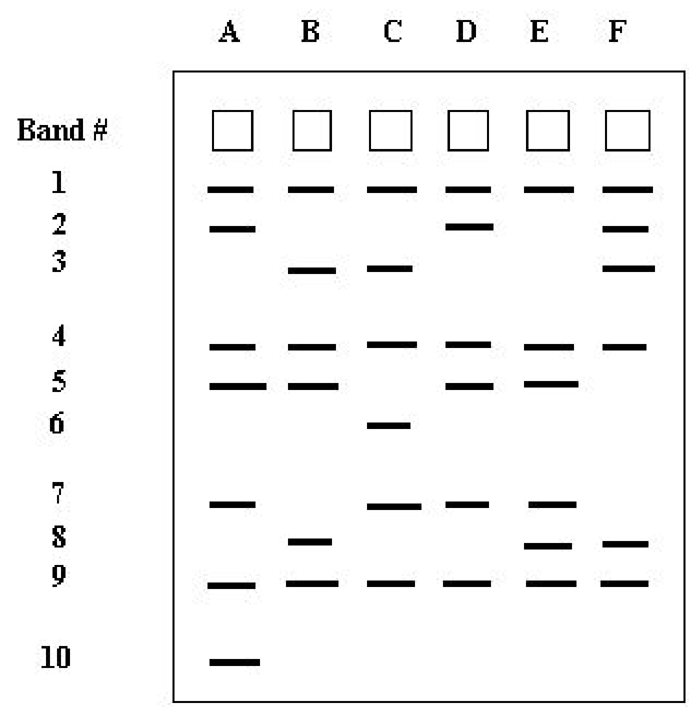
The BRCA1/BRCA2 DNA has a segment that was 50 base pairs and a segment that was 90 base pairs. The 350 base pair section of DNA indicates the BRCA1 mutation, and the 90 base pair section of DNA indicates the BRCA2 mutation. The presence of either of these segments in the Mother or Daughter DNA samples would indicate that they have this mutation.

Analysis Questions - *on a separate sheet of paper complete the following*

1. What is the normal function of the BRCA1/BRCA2 gene?
2. Examine your completed electrophoresis gel. Does the mother have either the BRCA1 or BRCA2 mutation?
3. Examine your completed electrophoresis gel. Does the daughter have either the BRCA1 or BRCA2 mutation?
4. What are the risks for breast cancer if a woman does not have the BRCA1 or BRCA2 gene? What are the risks if they do have either gene?
5. What restriction enzyme was used to cut the DNA samples?
6. What did the scissors represent in this activity?
7. Which fragments moved faster, the larger or smaller?
8. A DNA sample has the following segments: 100 base pairs, 580 base pairs, 424 base pairs, 33 base pairs, 1200 base pairs. Order the segments by which one moves the shortest distance to the farthest distance in an electrophoresis gel.
9. In electrophoresis, electricity is run through the gel. Do the DNA fragments move towards the negative or positive electrical poles? Why?
10. What would happen to the DNA fragments if the electricity (+/-) were reversed?
11. **CONCLUSION**: In 1-2 paragraphs summarize the procedure and results of this lab.

Review Questions - *on a separate sheet of paper complete the following*

1. What is DNA? Where is it found in a cell?
2. What are the four bases of DNA?
3. Explain why the sequence of DNA bases is just like a sentence.
4. How does a mutation affect the order of DNA base pairs?
5. What is electrophoresis? Summarize the steps of electrophoresis.
6. What is a restriction enzyme? Where do they come from?
7. Look at the electrophoresis sample to the right. Which DNA samples are the most similar (A-F)? What could these similarities mean?
8. If Band 8 in Sample F represents the mutated gene that causes baldness, which other DNA samples may also have the baldness gene?



http://biology.arizona.edu/sciconn/lessons2/Alongi/graphics/gel.jpg