WARD'S

Paternity Testing Lab Activity Student Study Guide

BACKGROUND

In 1983, a geneticist at the University of Leicester in England named Alec Jeffreys began studying inherited genetic variation between individuals and the evolution of contemporary genes. He achieved this by looking at a genetic peculiarity known as the intron. Introns, also referred to as junk DNA, are "nonsense" DNA base sequences that, to the best of our knowledge, do not carry any genetic information, although they may have been useful to our ancestors over 100,000 years ago.

DID YOU KNOW?

DNA paternity testing could not be used to determine which of two identical twins fathered a child since identical twins

Through his research, Jeffreys determined that some introns are contain the same repeating DNA base pair sequences, but the number of repetitions varied from person to person. For example, in one person a particular base pair sequence (e.g., ATCGATCGATCGATCGATCG) may be repeated ten times, while in another person the same sequence may be repeated 25 times). Although the sequence of genes is fairly constant from person to person, introns are unique to each person, except in the case of identical twins.

Because of the uniqueness of each person's pattern, these variable DNA base pair sequences can be used to distinguish one person from another. They can be separated using electrophoresis, then matched with complementary radioactive probes to create an individual-specific DNA banding pattern. This technique is called "DNA fingerprinting".

DNA Fingerprinting

Of the three billion nucleotides in human DNA, more than 99% are identical among all individuals. The remaining 1% that is different, however, adds up to a significant amount of code variations between individuals, making each person's DNA profile as unique as a fingerprint. Due to the very large number of possible variations, no two people (with the exception of identical twins) have the same DNA sequence.

For every 1,000 nucleotides inherited, there is one site of variation, or polymorphism. These DNA polymorphisms change the length of the DNA fragments produced by the digestion with restriction enzymes. The exact number and size of fragments produced by a specific re-

striction enzyme digestion varies from person to person. The resulting fragments, called Restriction Fragment Length Polymorphisms (RFLPs), can be separated and their size determined by electrophoresis.

Most of the DNA in a chromosome is not used to code for genes. It is uncertain what, if any, use this "unused" DNA may have. Because these regions are not essential to an organism's development, it is more likely that changes will be found in these nonessential regions. These regions contain nucleotide sequences (e.g., GTCAGTCAGTCAGTCA) that repeat from 20 to 100 times. These restriction enzymes that flank these repeating sequences cut the DNA strand creating RFLPs.

The differences in the fragments can be quantified to create a "DNA fingerprint". Distinct RFLP patterns can be used to trace the inheritance of chromosomal regions with genetic disorders or to identify the origin of a blood sample in a criminal investigation. Scientists have identified more than 3,000 RFLPs in the human genome, many of which are highly variable among individuals. It is this large number of variable yet identifiable factors that allow scientists to identify individuals by the number and size of their various RFLPs.

DID YOU KNOW?

DNA paternity testing can establish a probability of over 99% that a man has fathered a child. The only way to establish 100% probability would be to perform a DNA test on every male on Earth.

This technique is being used more and more frequently in legal matters. DNA fingerprints can positively exclude someone but only establish a probability to include someone. Using DNA fingerprinting, the identity of a person who has committed a violent crime can be determined from minute quantities of DNA left at the scene of the crime in the form of blood, semen, hair, or saliva. The DNA fingerprint matched to a suspect can be accurate to within one in 10 billion people, which is about twice the total population of the world. Certain limitations in the technique prevent two samples from being identified as a "perfect match", yet it is possible to measure the statistical probability of two samples coming from the same individual based on the number of known RFLPs that exist in a given population.

DNA fingerprinting has many other applications. Since half of a person's genome comes from each parent, DNA fingerprinting can be used to determine familial relationships. It has a much higher certainty than a blood test when used to determine fatherhood in a paternity suit. DNA fingerprinting can be used to track hereditary diseases passed down family lines and can be used to find the closest possible matches for organ transplants. It can also be used to ascertain the level of inbreeding of endangered animals, aiding in the development of breeding programs to increase animals' genetic health and diversity.

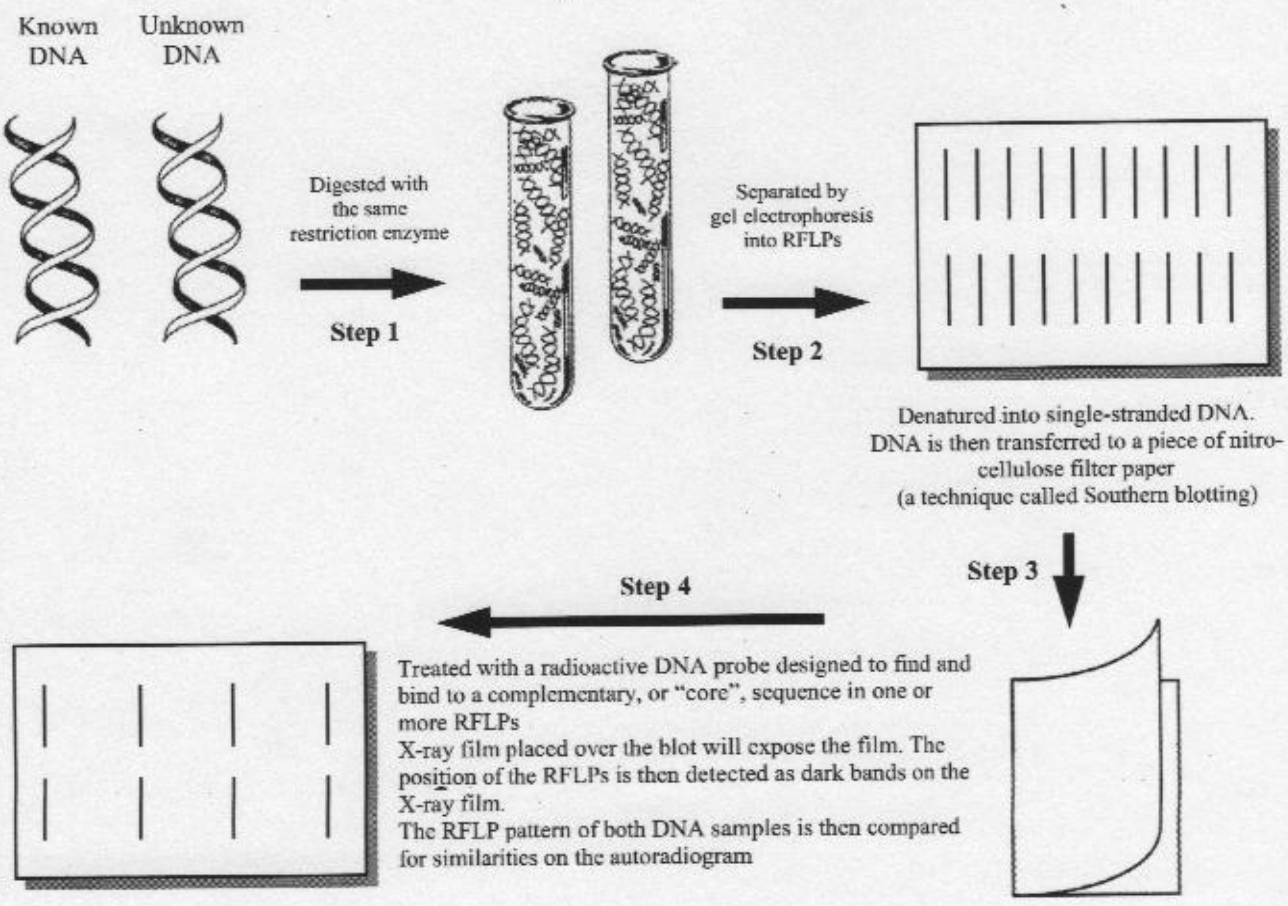
DNA Fragment Length Determination

Under a given set of electrophoretic conditions such as pH, voltage, time, gel type, concentration, etc., the electrophoretic mobility of a DNA fragment molecule is standard. The length of a given DNA fragment can be determined by comparing its electrophoretic mobility

on an agarose gel with that of a DNA marker sample of known length. The smaller the DNA fragment, the faster it will move down the gel during electrophoresis.

Using a technique called Southern blotting, the separated fragments are transferred to nitrocellulose paper, labeled with a radioactive probe, and developed against X-ray film. The probe, which is coded to bind to specific RFLPs being tested, will develop the film. The greater the concentration of DNA in that particular band, the darker the band will be. The resulting image, called an autoradiogram, shows a series of dark and light bands. This pattern is the DNA fingerprint of the tested individual. Comparing the distances between the bands in different samples determines the similarities between the samples.

DNA Fingerprinting Protocol



DID YOU KNOW?

Unlike regular fingerprints, which can be altered and only occur on fingers, DNA fingerprints are the same for every cell of a person, whether it be skin, hair, bone, blood, etc.

Prior to the advent of DNA fingerprinting technology, paternity testing was based on blood type, since the blood type of an offspring is based on the blood types of the parents. But blood types can only be used to exclude possible biological parents rather than to actually prove familial relationships with a high degree of certainty. DNA fingerprinting is used routinely to determine familial relationships. Paternity determination uses the principles of Mendelian genetics to infer the identity of the true biological father of an offspring, since typically the mother is known. Because half of a person's genome comes from each parent, any gene found in an offspring, not inherited from its mother, must have come from its father. The exact numbers of probes needed to resolve any particular case depends on gene frequencies and local breeding history. On the average, a typical paternity case will require four or five DNA probes for a decisive resolution.

OBJECTIVES

- Learn the process of agarose gel electrophoresis
- Perform the electrophoresis procedure
- Identify the most probable DNA match between two alleged fathers

MATERIALS

MATERIALS NEEDED PER GROUP

1 0.8% Agarose gel, on gel tray
TBE running buffer 1X, 350 mL
DNA stain
Staining tray
Micropipets

SHARED MATERIALS

DNA Samples:
Mother
Child
Alleged father #1
Alleged father #2
Agarose electrophoresis chambers
Micropipets with tips
Power supplies
Waterbath or microwave
lab markers
UV transilluminator (36 W 9951)
UV goggles (36 W 9951)
Biohazard bag (36 W 9951)

DID YOU KNOW?

Besides agarose, other types of semi-solid media used for electrophoresis are cellulose acetate and polyacrylamide gel.

SCENARIO

Two men claim to be the biological father of a child. The courts have obtained DNA samples from the mother, child, and the two alleged fathers. As the director of a genetic testing laboratory, you will analyze the samples using the DNA fingerprinting technique to help settle the paternity dispute.

Loading and Running a Gel

1. Place the gel, on the gel tray, in the center of the electrophoresis chamber with the wells closer to the negative (black) electrode.

2. Add approximately 350 mL of 1X TBE running buffer to the chamber. **Slowly** pour buffer from a beaker into one side of the chamber until the buffer is level with the top of the gel. Add buffer to the other side of the chamber until the buffer is level with the top of the gel. Continue to **slowly** add buffer until the level is approximately 2-3 mm above the top of the gel.

If you are running two gels, place the first gel in the chamber and add buffer until the first gel is completely submerged, then load the gel. After the first gel is loaded, place the second gel with the gel tray into the electrophoresis chamber. Slowly add more buffer until it reaches a level that is 2-3 mm above the second gel and load the second gel. Do not overfill the chamber. Wipe off any spills.

3. Load 10 µL of each DNA sample into the corresponding lane of your gel. Do not pierce the bottom of the wells with the micropipet tip. Do not overload wells.

   Lane #1: Mother
   Lane #2: Child
   Lane #3: Alleged Father #1
   Lane #4: Alleged Father #2

The amount of DNA in the reaction tubes is extremely small. Demonstrate the correct procedures needed to transfer samples from these reaction tubes to the wells on the gel.

DID YOU KNOW?

The first time DNA was used as evidence in a trial was in 1985.

The first time DNA evidence actually sent someone to jail was in 1988.

Preparation Notes

The power supply produces a high enough voltage to cause severe electrical shock if handled improperly. For safe operation, follow all directions and precautions.

- Examine all components of the electrophoresis apparatus prior to each use: all cords, plugs, jacks, the electrophoresis chamber itself, and the power supply.

- Do not operate electrophoresis apparatus in a damp or humid environment; any condensed moisture may short out electrical components. You may wish to designate one area of the laboratory specifically for electrophoresis equipment, where cells and power supplies are connected. Ensure that power cords and patch cords are free from moisture and that any wall outlet is properly wired; i.e., that correct polarity exists (use a circuit tester).

- Be sure that students are well acquainted with the correct procedure for making electrical connections. Students should be supervised at all times when performing this investigation.

- Do not come in personal contact with or allow metal or any conductive material to come in contact with the reservoir buffer or the electrophoretic cell while the power supply is on.

4. Make sure the cover, as well as the female jacks and the plugs, are dry, then slide the cover onto the electrophoresis chamber. Wipe off any spills on the apparatus before proceeding to the next step.

5. Make sure that the patch cords attached to the cover are completely dry, then connect the red patch cord to the red electrode terminal on the power supply. Connect the black patch cord to the black electrode terminal on the power supply.

Check the connections before allowing students to proceed to the next step.

6. Plug in the power supply and set it to the desired voltage.

It is recommended that you set the power supply between 75-125 volts. The system may be run at lower voltage settings but this will increase the running time of your agarose gels.

7. Turn on the power supply. The red power light will illuminate, and bubbles will form along the platinum electrodes.

DID YOU KNOW?

Agarose is a highly purified form of agar, which is a material extracted from seaweed.

8. Observe the migration of the loading dye down the gel toward the red electrode. Turn off the power when the loading dye has reached the end of the gel. Unplug the power supply.

The loading dye is a special dye added to the DNA samples prior to performing electrophoresis and serves two purposes. It is heavier than the electrophoresis buffer, causing the DNA samples to sink into the wells. It is also smaller than most of the DNA fragments in the samples so it runs to the end of the gel faster than the DNA, giving an indication of when to end the electrophoresis run.

9. Wait approximately 10 seconds and then disconnect the patch cords from the power supply. Remove the cover from the electrophoresis chamber.

10. Carefully remove the gel, on the casting tray, from the electrophoresis chamber.

DID YOU KNOW?

If all of the DNA molecules found in a single human cell were lined up end-to-end, they would reach a distance of about two meters. Yet, all of these molecules are packed into a nucleus 10 millionths of a meter in diameter.

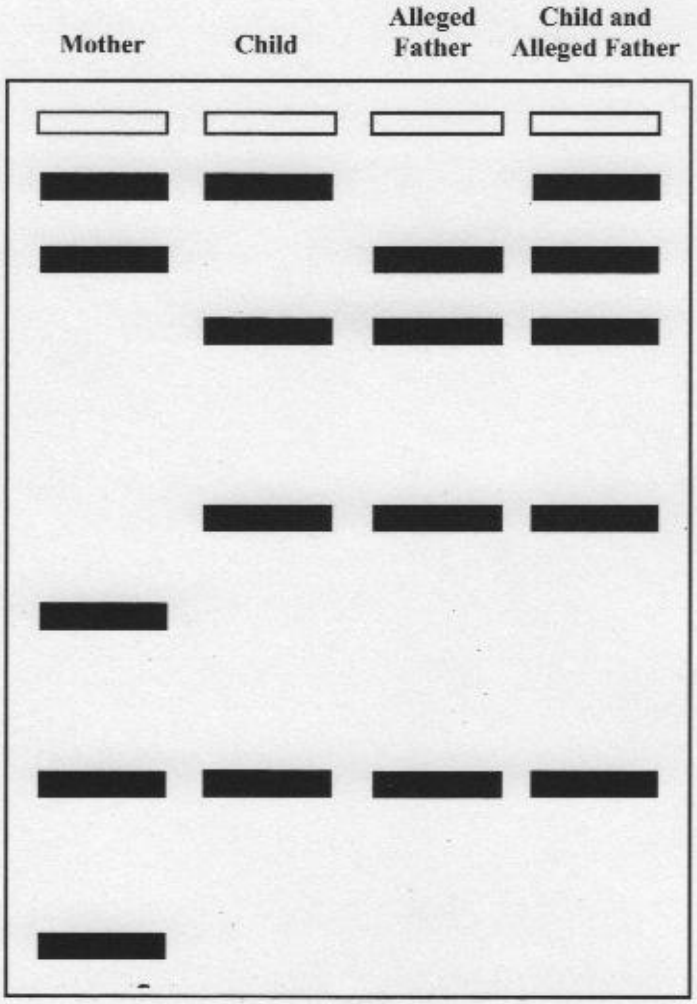
Optional stopping point:

If the lab period does not allow time to stain and destain the gel, place the gel in a resealable bag and add 1-2 mL of 1X TBE buffer and refrigerate the gel until the next lab period.

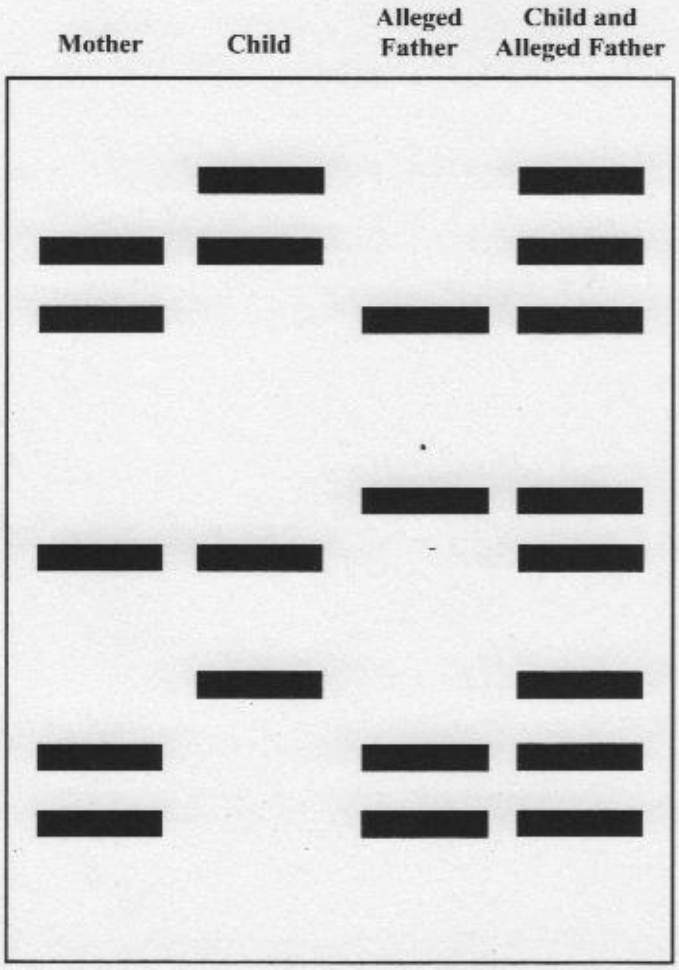
Name: _____
Group: _____
Date: _____

ASSESSMENT

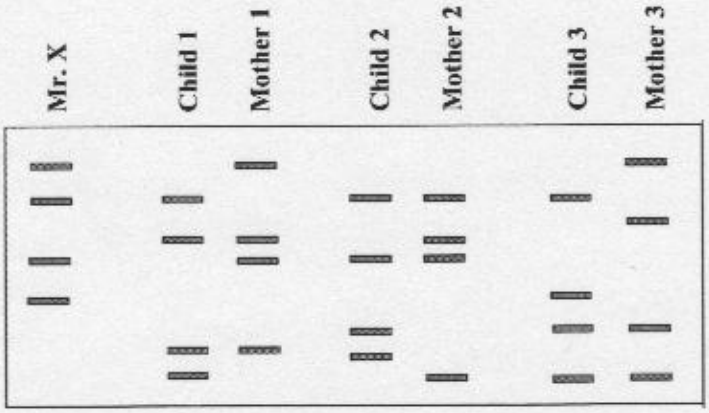
1. Below is an illustration of an autoradiogram, which is typically used in paternity disputes. Based on your knowledge of DNA fingerprinting and the fact that half a person's genome comes from each parent, is the alleged father actually the biological father of the child? Explain your answer.



2. Based on the autoradiogram below, is the alleged father the biological father of the child?



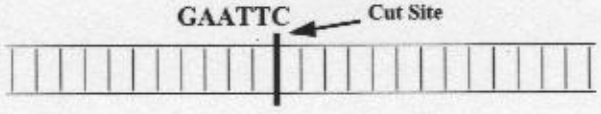
3. Three women, each of whom have one child, claim that Mr. X is the father of their children. Based on the banding patterns in the gel below, which of the children may or may not be Mr. X's?



4. *Eco*R I recognizes GAATTC, and *Hind* III recognizes AAGCTT. A student adds *Eco*R I to a linear DNA sample. To another quantity of the same DNA, she adds *Hind* III. In a third tube she adds both enzymes. She runs a gel and the following occurs:

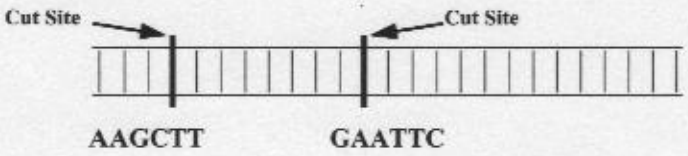
***Hind* III**	***Eco*R I**	**Double**
_____		
	_____	_____
	_____	
		_____
_____		_____

The student, after viewing the gel, draws the following map of the linear DNA



a. Explain why she placed the *Eco*R I restriction enzyme site as she did.

After a little more thought, she added another sequence to the DNA:



b. Explain how she was able to add Hind III restriction enzyme site in this position based on the results of her gel.

5. Different restriction enzymes are isolated from different types of bacteria. What advantage do you think bacteria gain by having restriction enzymes?

6. Predict what would happen if you place your gel in the electrophoresis chamber with the wells containing the DNA next to the red electrode instead of the black.

7. If you have a restriction enzyme that cuts a piece of linear DNA at two recognition sites, how many DNA fragments would you see on a gel?

8. Below is a list of the components involved in agarose gel electrophoresis. Briefly describe the purpose of each component.

Agarose gel –

TBE buffer –

Electrophoresis chamber –

Power supply –

DNA samples –

DNA stain –

9. You have used electrophoresis to examine paternal relationships and identify the most probably DNA match between two alleged fathers. What would be another use of electrophoresis?