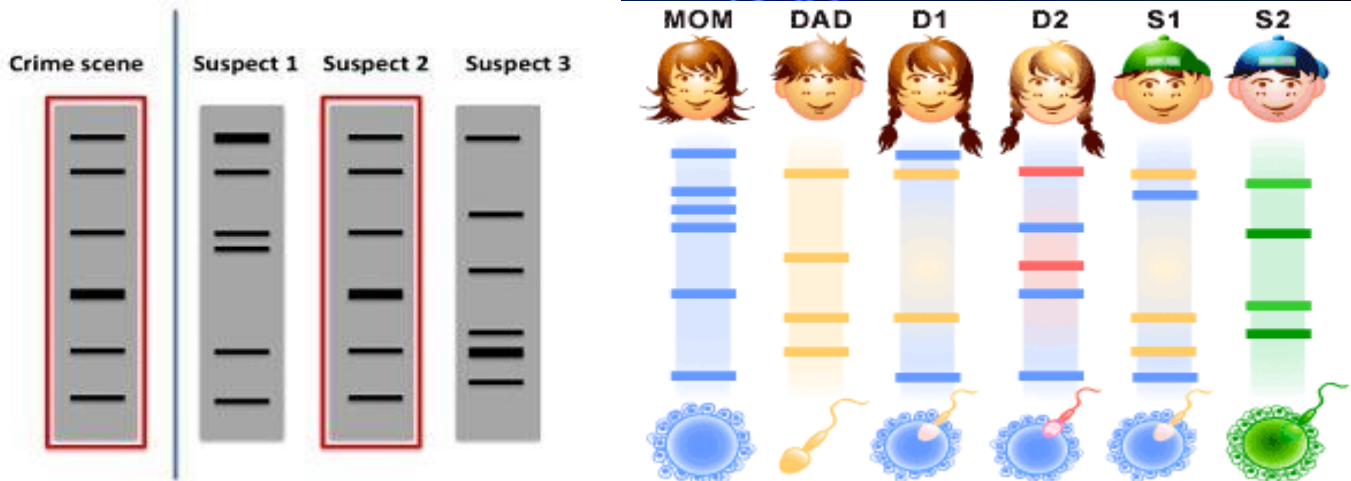


Broughton High School

Chapter 4



DNA Fingerprinting

*Broughton High School***Chapter 4 – Vocabulary Words – DNA Fingerprinting**

Vocabulary for Chapter 4

Term	Definition
1. Allele	
2. Chromosome	
3. DNA Fingerprint	
4. DNA Probe	
5. Electrophoresis	
6. Gene	
7. PCR	
8. Restriction Enzyme	
9. STR	
10. VNTR	
11. Polymorphisms	
12. DNA Analysis	
13. Annealing	
14. Denaturing	
15. DNA Polymerase	

DNA Fingerprinting

Except for identical twins, no two people on earth have the same DNA (deoxyribonucleic acid). Advances in DNA technology have allowed criminal cases to be solved that previously were thought unsolvable. Since the 1980s, DNA evidence has been used to investigate crimes, establish paternity, and identify victims of war and large-scale disasters. Because each human is unique, DNA evidence from a crime scene or from an unidentified body can be traced back to one and only one person. DNA evidence can be used to link a suspect to a crime or to eliminate a suspect. It can also be used to identify a victim, even when nobody can be found. DNA evidence has been used to identify human remains of victims of large-scale disasters, such as plane crashes, tsunamis, and hurricanes. Several types of biological evidence, such as skin, blood, saliva, urine, semen, and hair, are used in forensics for identification purposes. Biological evidence is examined for the presence of inherited traits, such as blood type or enzyme variants. Most laboratory techniques used in forensics were originally developed for other purposes, such as medical diagnosis or treatment. When human cells are present in biological evidence, their chromosomes can be examined to determine whether the evidence comes from a male or a female. The analysis of chromosomes is known as karyotyping. Blood-typing techniques, which were first developed to make transfusions safe, are now used in forensics. Blood-type information obtained from crime-scene evidence can help investigators to exclude suspects. Blood typing can also be used to determine if blood found at a crime scene comes from only one person or from multiple individuals. Because blood type is an inherited trait, blood typing is used in paternity testing.

DNA fingerprinting, also known as DNA profiling, is used in criminal and legal cases to determine identity or parentage. DNA can be extracted from relatively small amounts of biological evidence, such as a drop of blood or a single hair follicle. When DNA fingerprinting is performed and interpreted by qualified forensic scientists, the results can very accurately predict whether an individual can be linked to a crime scene or excluded as a suspect.

CASE STUDIES**Andrew's DNA Fingerprinting**

Andrew was a teenage boy living in England with his mother Christiana and her other three children. The family had originally come from Ghana. On his way back from a family visit to Ghana in 1984, immigration authorities stopped Andrew at Heathrow airport in London. They suspected that his passport had been altered or forged and that Andrew was not its true owner. Andrew was not allowed to enter the country. Because his father's whereabouts were unknown, no blood specimen could be collected from the father. Traditional biological evidence, such as blood typing, could only be used to prove that Andrew was related to Christiana, but could not prove he was her son.

In 1985, lawyers sought help with Andrew's case from Dr. Alec Jeffreys, a famous British geneticist. Dr. Jeffreys had developed the DNA fingerprinting technique in 1984. In this technique, DNA fingerprints appear as a pattern of bands on X-ray film. Except for identical twins, no two humans have identical DNA or DNA fingerprints. Because half of a child's DNA is inherited from the mother and the other half is inherited from the father, DNA fingerprints can be used to prove family relationships. Any bands in a child's DNA fingerprint that are not from the mother must be from the father. Dr. Jeffreys collected blood samples from Andrew, Christiana, and her other three children. He isolated the DNA and ran DNA fingerprints. He used the DNA fingerprints of Christiana and her three children to reconstruct the DNA fingerprint of the missing father. Each of the bands in Andrew's DNA fingerprint could be found in the DNA fingerprints of Christiana or her three children. About half of the bands in Andrew's DNA fingerprint matched Christiana's fingerprint.

The remaining bands matched the reconstructed bands matching the fingerprint of the father of Christiana's children. DNA fingerprinting provided the evidence needed to prove that Andrew was indeed a member of the family. The case against Andrew was dropped. He was allowed to enter the country and was finally reunited with his family.

Think Critically

How was DNA fingerprinting used to determine the identify of Andrew?

DNA Fingerprinting

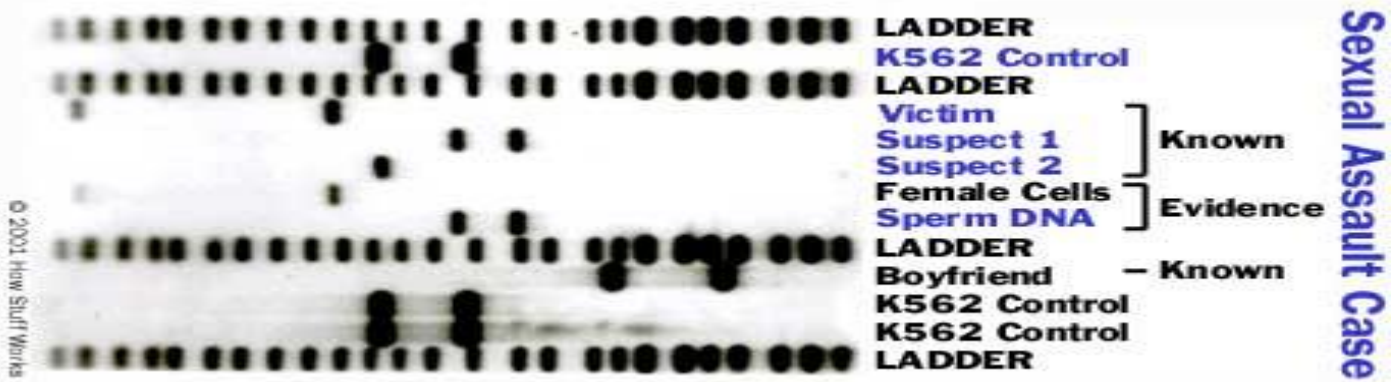
The concept of DNA fingerprinting.

Only 0.001% of DNA (about 3 million bases) differs from one person to the next. However, those small variable regions are enough for scientists to generate a DNA profile of an individual, using DNA extracted from blood, bone, hair, and other body tissues or products.

In criminal cases, DNA is extracted from both the crime scene evidence and from the suspect. Both sets of DNA are analyzed for the presence of a set of specific DNA regions (markers). Scientists find the markers in a DNA sample by designing small pieces of DNA (probes) that will each seek out and bind to a complementary DNA sequence in the sample. A series of radioactive probes bound to a DNA sample creates a distinctive pattern for an individual.

Forensic scientists compare these DNA profiles to determine whether the suspect’s sample matches the evidence sample. A single marker is not usually unique to an individual, so forensic scientists generally look at multiple markers. If the sample profiles don’t match, the person did not contribute the DNA at the crime scene, but if the two DNA samples match at multiple regions, the odds are good that the two samples come from the same person. While there is a chance that someone else has the same DNA profile for a particular probe set, the odds are exceedingly slim, especially if there are multiple probes. Four to six probes are recommended.

Pose the following question: “How small do the odds have to be when conviction of the guilty or acquittal of the innocent lies in the balance?” Tell students that many judges consider this a matter for a jury to take into consideration along with other evidence in the case. Experts point out that using DNA forensic technology is far superior to eyewitness accounts, where the odds for correct identification are only about 50:50. The more probes used in DNA analysis, the greater the odds for a unique pattern and against a coincidental match, but each additional probe adds greatly to the time and expense of testing.



This is a realistic depiction of a sexual assault case with gel electrophoresis. It could be seen that suspect 1's DNA band matches with the sperm DNA evidence. This means that the results are conclusive: the sexual offender is suspect 1 as suspect 2 and the boyfriend do not have DNA that matches with the collected Evidence. However, in different cases when there are no matches, suspects are able to be eliminated. In some cases, the results may be inconclusive as the process may not be successful or not enough DNA was collected.

DNA Fingerprinting

The process of DNA fingerprinting is used to identify certain people and to also find out if people are related. The process includes the extraction of DNA as the first step. Stains and tissue is collected in order to extract DNA such as blood and saliva through buccal swabs (cheek). Depending on the type of stain or the tissue that is collected, the method of extraction and purification would vary. Epithelial tissues are known to be one of the easiest types of tissue of deal with while bone tissue may be slightly more difficult. DNA extraction with semen mixed in with other components such as vaginal epithelial cells (usually in sexual assault cases) has a particular method in extraction as the process must not mix the substances or cells together. This process is known as differential extraction. Differential extraction is based on the knowledge that sperm cells have protein disulphide bonds around them, making them resistant to enzymes that break up cell membranes that are usually used in DNA extraction for epithelial tissues. To break the bonds, an agent called Diatheothreitol is used since it is a "de-protecting" or disulphide bond reducing substance for thiolated DNA. In this process, Diatheothreitol is added to the DNA of the sperm and they are separated by filtration or Chromatography, depending on the type of Diatheothreitol used (solid catalyst or liquid form). After collecting the DNA, the amount of DNA collected is then recorded as this is crucial because the amount of DNA collect can vary widely. To retrieve samples of STR that are in the DNA, the PCR or the Polymerase Chain Reaction method can exponentially amplify certain sections of DNA (in this case, STR) so that the scientist can have thousands to millions of copies of the STR that are the same. The PCR method is based upon the method of thermal cycling, which is the process of heating and cooling down in a cycle. The PCR method consists of around 20-30 cycles. Short synthetic pieces of DNA called primers flank the DNA and indicate the sections of DNA that would be copied during the process. When starting, if the equipment was used and not cleaned up properly, it is heated until all the DNA and the primers have melted. The first stage is called **melting or denaturing**. The DNA will then be heated at a certain temperature so that the hydrogen bonds that connect the strand together would break and the DNA would separate into two and the primers would have been separated. The next step is called the **annealing** step which is when the temperature is cooled down to about so that the primers can attach to each of the DNA strands. The last step is called **elongation or synthesizing**. The DNA-Polymerase then fills in the missing strands by starting at the primer. The temperature at this stage depends on the DNA-Polymerase itself as well as the length of the DNA that is going to be amplified. The cycle is then repeated numerous times with new primers replaced and fresh Polymerase until many samples are replicated. The DNA-Polymerase could be added at the initial point of the process as well. This process is very effective in replicating copies of DNA. However, it should be ensured that the DNA is not replicated too much as this would affect the quality of the DNA strand as well.

The extracted and duplicated STR samples then go through a process called gel electrophoresis so that the DNA samples can be sorted and measured by length. The scientist slang for gel electrophoresis is "running the gel". This procedure features an agarose gel tray. The gel is made of hydrocolloid that is extracted from seaweed. At the top of the tray, there are small holes. The first step is to drop the DNA samples in each of the holes. Then, an positively charged electrical current is applied to the other end without the holes and negatively charged electrical current is applied to the end with the sample. As DNA is negatively charged because of its phosphate ions, the DNA would travel to the other end of the tray, which has a positive electrical charge. As shorter strands are less heavier than longer strands, they would travel faster and end up being closer to the end of the tray. DNA strands that have the same length and mass would end up being group together at the same area. As a single DNA strand cannot be seen by the naked eye, by using an electric current, DNA would end up being in groups that are called **bands** and therefore, can be seen. In order to increase the visibility of the bands, coloured dye or stains are added- preferably ethidium bromide, which is a chemical that can bind to DNA. The result can then be examined by the eye in order to find out various things such as whose father or mother is the child's or to link up DNA from the crime scene with a suspect. If the bands match each other, this means that the suspect was at the crime scene. In the circumstances of finding parents, the father and the mother's DNA strands would each match half of the child's DNA and so, if an alleged father has DNA that does not match the band of the child's, they are not the father.



Broughton High School

Most of the human genome is the same in all humans, but some variation exists among individuals. Scientists are able to identify individuals based on this variation. Much of this variation is found in the noncoding DNA. What is interesting about noncoding DNA is that much of it is in the form of repeated base sequences. Individuals have unique patterns of repeated base sequences in the noncoded DNA, and certain base sequences may be repeated many times. DNA sequences have different lengths and different sequences of the bases in different individuals. Within a human population, these differences in DNA sequences are called *polymorphisms*.

In 1984, Dr. Alec Jeffreys at the University of Leicester observed that DNA from different individuals contains different polymorphisms. His laboratory developed a technique for isolating and analyzing these variable areas that is known as DNA fingerprinting, or DNA profiling. A DNA fingerprint appears as a pattern of bands on X-ray film. When DNA fingerprinting is used to analyze biological evidence, variable regions appear as a pattern of bands. The unique patterns of repeated base pairs can be analyzed and used to identify an individual. Because the number and location of polymorphisms are unique in each individual, each individual's DNA has a unique band pattern. The examination of DNA profiles can help forensic scientists to decide if two or more DNA samples are from the same individual, related individuals, or unrelated individuals.

Different repeated sequences appear in different places in the genome of each individual. These repeats may be studied to aid in the identification of individuals. Forensic scientists focus on two types of repeating DNA sequences in the noncoding sections of DNA known as VNTRs (variable numbers of tandem repeats) and STRs (short tandem repeats) found in the DNA.

VNTR

Within the noncoding sections of DNA, certain short sequences of DNA are repeated multiple times. The number of copies of the same repeated base sequence in the DNA can vary among individuals. For example, if the repeated base sequence is CATAACAGAC, there might be three copies (CATAACAGAC CATAACAGAC CATAACAGAC) in the DNA of one individual, while another individual might have seven copies. Because the number of repeats varies from one person to another, these multiple tandem repeats are known as **variable number of tandem repeats (VNTR)**. The length of a VNTR can be from 9 to 80 bases in length.

STR

DNA sequences with a high degree of polymorphism are most useful for DNA analysis. A **short tandem repeat (STR)** is a short sequence of DNA, usually only two to five base pairs in length, within the noncoding DNA. An STR is much shorter than a VNTR. For example, an STR GATA repeats four times in an individual with the sequence GATAGATAGATAGATA. The polymorphisms in STRs result from the different number of copies of the repeat element that occur in a population. The use of STRs is becoming the preferred method of analysis because of its accuracy and because small and partially degraded DNA samples may be analyzed to identify individuals. Because the repeated units in VNTRs are longer, the resulting strands of DNA being compared among individuals are also much longer than with STRs. This greater length makes it more difficult to separate the VNTR sequences.



Broughton High School

DNA is found in the nucleus of cells in the human body. A perpetrator may leave biological evidence, such as saliva, blood, seminal fluid, skin, or hair, at a crime scene. This biological evidence contains the perpetrator's unique DNA. Because this evidence is capable of identifying a specific person, it is known as *individual evidence*. A saliva sample can be collected from an envelope, a toothbrush, or a bite wound. DNA can be isolated from a sample of biological evidence as small as a drop of blood or a hair follicle.

When the amount of evidence left at a crime scene is very small, it is considered to be *trace evidence*. One of the problems encountered in dealing with trace evidence is that the evidence may be totally consumed during forensic testing. The use of the **polymerase chain reaction (PCR)** technique helps resolve this problem. Dr. Kary Mullis invented the PCR technique, for which he shared the Nobel Prize in 1993. PCR generates multiple identical copies from trace amounts of original DNA evidence. This enables forensic scientists to make billions of DNA copies from small amounts of DNA in just a few hours. The DNA produced with PCR can be analyzed using DNA fingerprinting techniques.

Steps of DNA Fingerprinting

Several steps are necessary before DNA samples can be analyzed and compared. These steps are summarized as follows and then expanded upon in more detail following the summary:

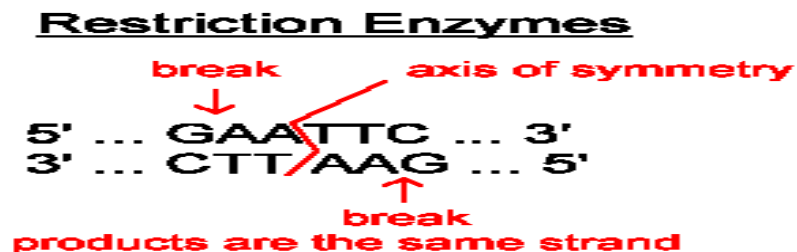
1. Extraction. DNA is extracted from cells.
2. Restriction fragments. In some VNTR analyses, DNA is cut by restriction enzymes. **Restriction enzymes** recognize a unique pattern of DNA bases (restriction sites) and will cut the DNA at that specific location. Restriction fragments of varying lengths are formed when the DNA is cut.
3. Amplification. In the case of other VNTR analyses analysis, specifically chosen DNA fragments are amplified using polymerase chain reaction.
4. Electrophoresis. DNA is loaded into the wells found in an agarose gel. When an electric current is passed through the gel, the negatively charged DNA fragments (pieces of DNA) migrate toward the positive end of the gel. DNA fragments are separated by size, with the smallest DNA fragments moving the fastest through the gel.

Extraction

The first step in preparing a sample for DNA fingerprinting is to extract the DNA from the cell nucleus. Cells are isolated from tissue and are then disrupted to release the DNA from the nuclear and cell membrane as well as from proteins and other cell components.

Restriction Fragments

In the case of some DNA profiles, after the DNA is extracted, the sample is mixed with a restriction enzyme to cut the long strands of DNA into smaller pieces called DNA restriction fragments. Restriction enzymes are “molecular scissors” that cut DNA at specific base sequences. Restriction enzymes are often produced and used by bacteria to defend themselves against invading viruses. There are many different restriction enzymes, and each one binds to a specific recognition site. Moreover, the enzyme cuts the DNA strand at specific locations within that restriction site. For example, the restriction enzyme *Hind III* recognizes the AACGTT base sequence. The *Hind III* restriction enzyme cuts the DNA between the two AA bases. When restriction enzymes cut DNA into pieces, fragments of many different lengths are produced. Within some of these fragments are unique sequences called VNTRs. Several different restriction enzymes may be used to cut the DNA in a sample.



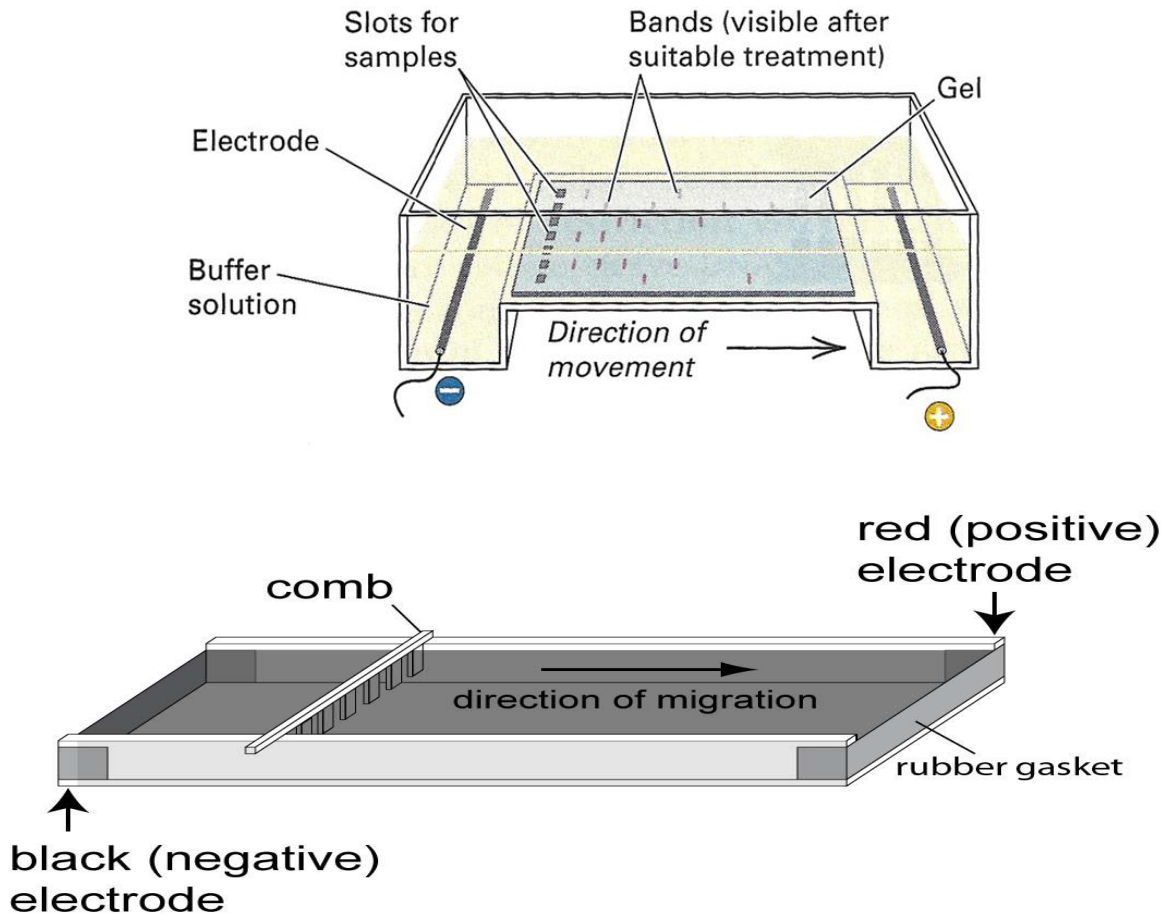
Amplification

With some DNA analyses, polymerase chain reaction (PCR) can be used to amplify certain pieces of the DNA that contain the VNTRs. In STR profiles, restriction enzymes are unnecessary and PCR allows the amplification of the strands with the STR sequences.

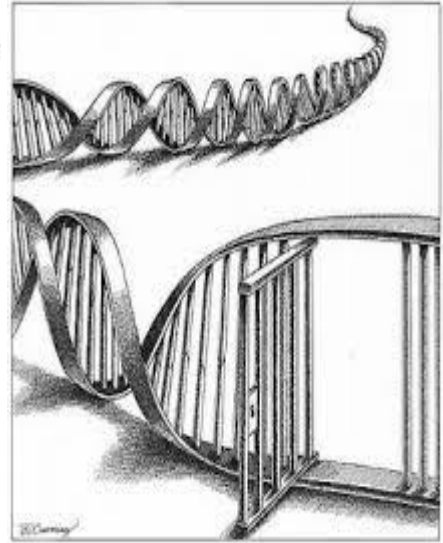
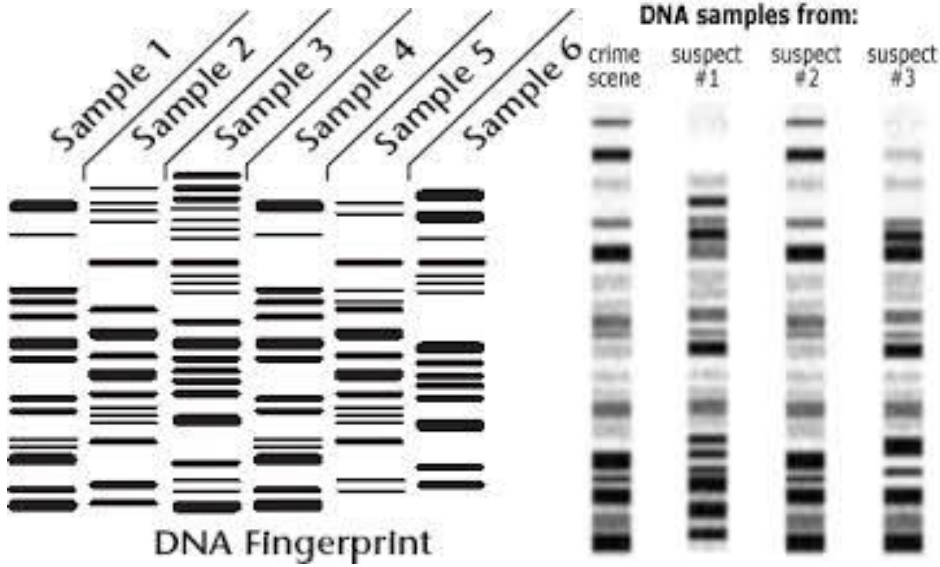
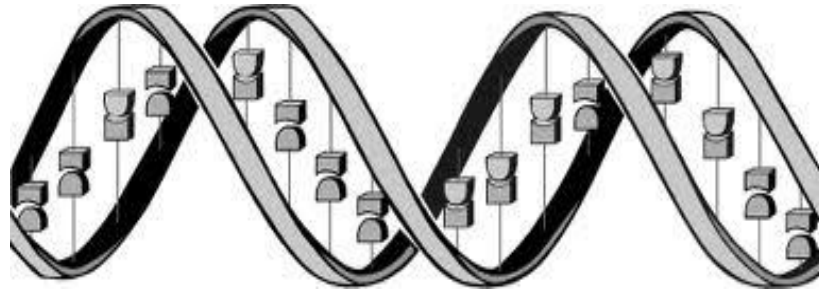
Electrophoresis

The fragments of cut DNA are separated by gel electrophoresis. A gel is the matrix (usually agarose) used to separate DNA molecules. Electrophoresis is the method of separating the molecules within an electric field based on the size of the DNA fragments. The gel forms a solid but porous matrix for the DNA fragments to move through. For this technique, the gel is placed into a gel electrophoresis chamber. Then, each DNA sample containing the amplified fragments is drawn up into a micropipette and placed into a separate well or chamber along the top of a gel. One well contains a control, a solution containing DNA fragments of known lengths called a DNA Ladder or Standard DNA. An electric current is passed through the gel. The negatively charged fragments of DNA in the wells move toward the positively charged opposite end of the gel. DNA fragments of different sizes are separated as the smaller DNA fragments move easily from the negative end of the gel toward the positive end of the agarose gel. All of the DNA fragments line up in bands along the length of the gel, with the shortest fragments forming bands closest to the positive end of the gel and the longest fragments forming bands closest to the negatively charged end.

Agarose gel electrophoresis of DNA



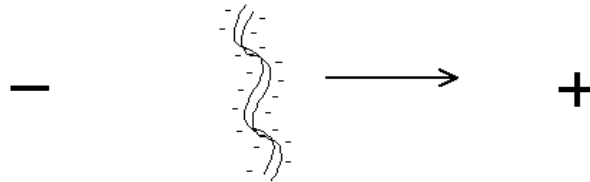
Forensic DNA Fingerprinting



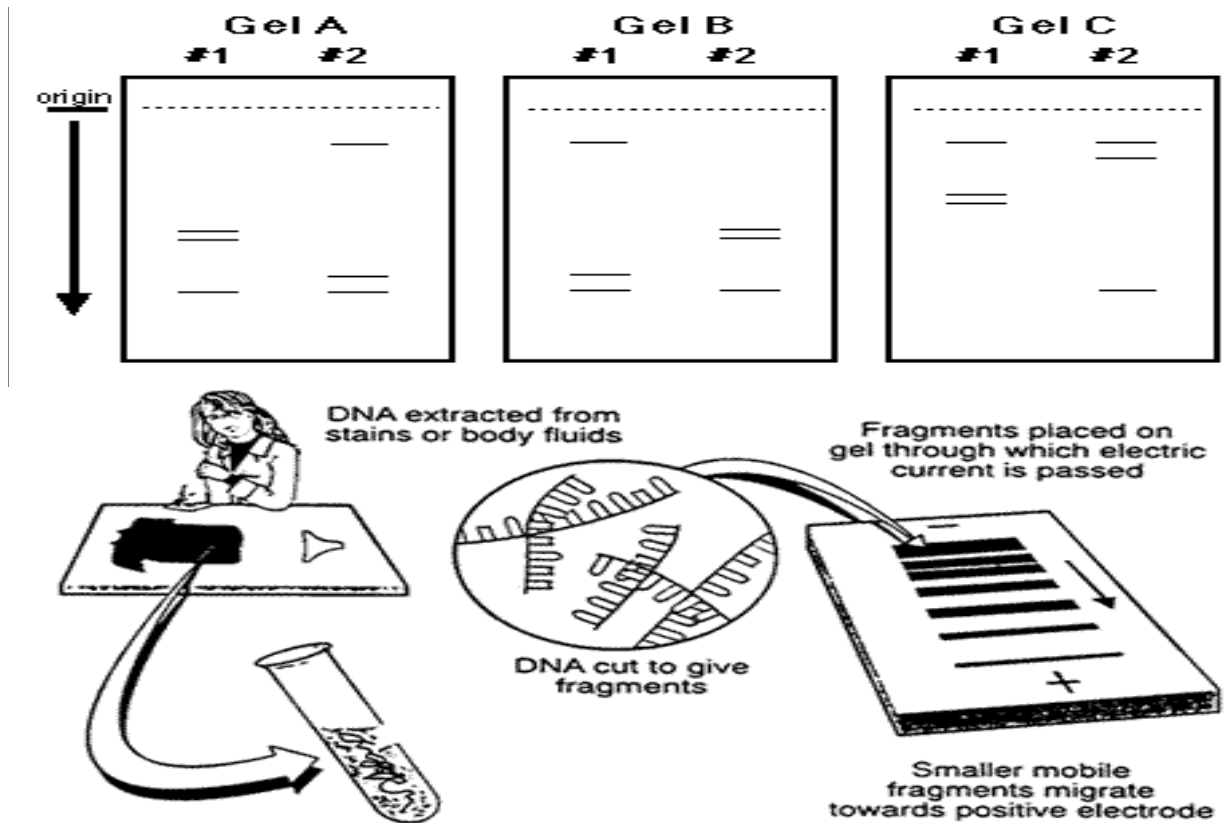
DNA Gel Electrophoresis

Agarose gel electrophoresis is an easy way to separate DNA fragments by their sizes and visualize them. It is a common diagnostic procedure used in molecular biological labs.

Electrophoresis: The technique of electrophoresis is based on the fact that DNA is negatively charged at neutral pH due to its phosphate backbone. For this reason, when an electrical potential is placed on the DNA it will move toward the positive pole:



The rate at which the DNA will move toward the positive pole is slowed by making the DNA move through an agarose gel. This is a buffer solution (which maintains the proper pH and salt concentration) with 0.75% to 2.0% agarose added. The agarose forms a porous lattice in the buffer solution and the DNA must slip through the holes in the lattice in order to move toward the positive pole. This slows the molecule down. Larger molecules will be slowed down more than smaller molecules, since the smaller molecules can fit through the holes easier. As a result, a mixture of large and small fragments of DNA that has been run through an agarose gel will be separated by size. This is a graphic representation of an agarose gel made by "running" DNA molecular weight markers, an isolated plasmid, and the same plasmid after linearization with a restriction enzyme:



Worksheet No. 11

To Catch a Jewel Thief...**DNA Fingerprinting****Purpose**

To learn how to set up DNA digestions with restriction enzymes in order to create DNA fingerprints for analysis. To gain an understanding of restriction endonucleases critical for genetic engineering and biotechnology.

Importance (Application)

DNA Fingerprinting is a procedure whereby the genetic information, called DNA, in a person's cells is analyzed and identified. The word fingerprinting is used because, just like a fingerprint, no two person's genetic code is exactly the same. This makes [DNA fingerprinting](#) a very useful tool for our modern society.

It can be used to:

- **Determine Family Relationship** - DNA can help find out who a person's parents or siblings are. Prenatal paternity tests are available to mothers who need to identify the father of their unborn babies.
- **Detect Inherited Diseases** - Your genetic code can be tested to determine your likelihood of getting certain diseases.
- **Prove Guilt or Innocence** - DNA left at the scene of a crime can be matched with a sample from a suspect.
- **Identify a Dead Body** - The DNA from an unidentified body can be matched with a person in a government DNA database

Background**Introduction to DNA Gel Electrophoresis**

In order to analysis DNA fragments restriction enzymes are used. Restriction enzymes are proteins that cut long DNA pieces into smaller pieces or sections. Restriction enzymes cut DNA segments at particular sections or specific sites in order to make the DNA fragments smaller. Restriction enzymes act like microscopic scissors and cut DNA into segments. When DNA Electrophoresis labs are actually performed, restriction enzymes are put into a small tube with DNA samples. The restriction enzymes digest the DNA into smaller fragments. After the reaction is finished, it looks like a clear fluid.

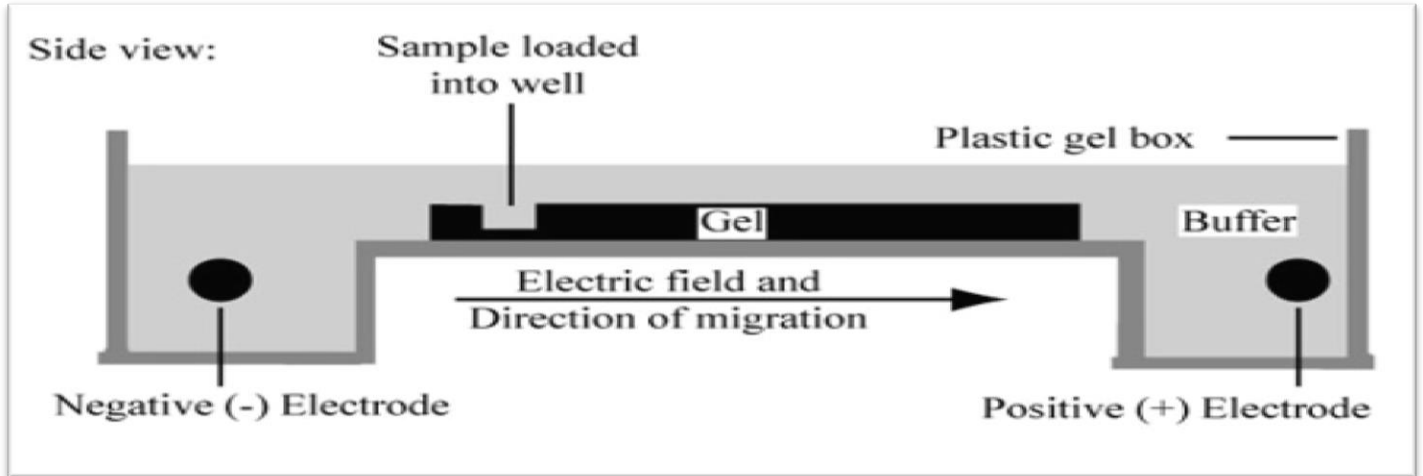
In order for restriction enzymes digestion to mean much, you have to be able to somehow see the different DNA fragments that are produced. So scientist separate DNA fragments or smaller pieces of DNA so that they can look at the result of the restriction digestion by a process called Gel Electrophoresis. Gel Electrophoresis takes advantage of the unique chemistry of DNA to be able to separate the DNA fragments.

Under normal conditions, the phosphate groups in the backbone of DNA are negatively charged. According to the laws of electrical charges, opposites attract, so the negatively charged DNA molecules are very much attracted to anything that is positively charged.

Broughton High School

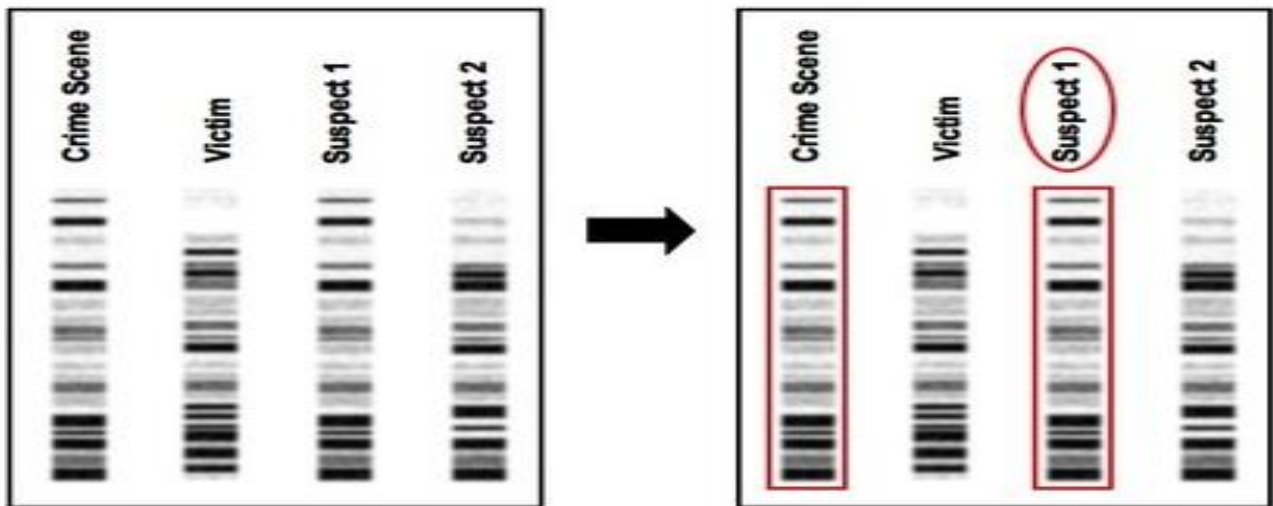
The electric fields makes the DNA fragments move. To separate the DNA fragments and make them easier to look at, the whole process is carried out on a gel. If you have ever eaten Jell-O, you have experience with a gel.

Scientist often use a gel material called agarose. Agarose gel is similar to Jell-O.



For electrophoresis, the entire gel is placed in a solution of salt water. A small electric current is applied across the tank. As the electric current flows the DNA molecules begin to move or migrate toward the positive poles of the electric field. After a period of time, the large DNA fragments or pieces move slightly toward the positive poles. The smaller DNA fragments move further away from the poles and closer toward the positive poles.

Forensic Investigation



Thinking Critically

How can you probe that suspect 1 is the perpetrator?

To Catch a Jewel Thief: DNA Fingerprinting

Objective:

Read the passage below. Use the description to predict which suspect committed the crime:
(Stealing the Golden Key)

The Set Up

The Key

On Friday October 6, 2017 the mayor of Raleigh, NC presented a large antique Golden Key to the City to the students at SMART Weekend for their outstanding academic achievement and citizenship. The Key was quite large and studded with semi-precious stones including amethyst and topaz. After a ceremony with Mr. Mitchell and the mayor awarding the key to the science scholars and SMART weekend students, the key was placed on a velvet cloth in a substantial display case just outside Broughton High School main office.

The Crime

Later that day, a great crash was heard from the hall outside Broughton High School main office. When several students went to investigate, all they found was an empty office and a broken display case. The GOLDEN Key to the City was gone!

The Evidence

The SMART Weekend students and the science scholars noticed that the intruder has cut him or herself on the broken glass of the display case. Here was a small puddle of blood left behind in the case. Being excellent science scholars, the students collected the blood for DNA analysis. The students knew how to get a DNA fingerprint of the suspect with the evidence. The students had performed a similar lab procedure with Mr. Davis just a week ago. The students took statements and collected DNA samples from each suspect. It worth noting that each suspect had a recent cut on his or her arm.

The Suspects

Suspect 1 - CIS President: The CIS President claims he was not in the office because he was returning from a late lunch. The cut on his right index finger was supposedly from a mishap serving birthday cake to a staff at lunch today.

Suspect 2 – Senior Director: The Senior Director was the only person alone in the office at the time of the break-in. She had a bright red stain on her wrist. She told the students it was from assisting Mr. Davis in a science experiment earlier in the day.

Suspect 3 – Director: The Director was delivering books to classrooms located down the hall from the main office. Therefore, she was in the general area at the time of the crime. It is a well-known fact that she passionately collects jewelry containing semi-precious stones. The Director had a Band-Aid on her right hand.

Suspect 4 - Lead Teacher: The lead teacher was wearing a jacket although it was a very warm day. The jacket appeared to conceal a bandage. It was possible the antique key was of interest to their collection of artifacts. The lead teacher's coffee break was about the time the key was stolen. The lead teacher had ample opportunity to conduct the heist.

Thinking Critically

Who stole the Golden Key?



CASE STUDIES

Ian Simms (1988)

Helen McCourt was last seen alive as she boarded a bus on her way home from work in Liverpool, England. Evidence found in the apartment of Ian Simms, a local pub owner, linked him to McCourt’s disappearance. His apartment was covered with blood, and part of McCourt’s earring was found there. The rest of her earring was found in the trunk of his car. Bloody clothing belonging to McCourt was found on the banks of a nearby river. Her body was never recovered. Dr. Alec Jeffreys analyzed the blood found in Simms’s apartment and matched it to blood from her parents. Dr. Jeffreys determined that there was a high probability that the blood found in Simms’s apartment matched that of Helen McCourt. Simms was found guilty of murder, and sentenced to life imprisonment. This was the first time DNA evidence was used to convict a murderer in a case where the victim’s body was not found.

Kirk Bloodsworth (1984)

Dawn Hamilton, age nine, was found raped and beaten to death in a wooded area near her home in 1984. In 1985, Kirk Bloodsworth was accused and convicted of the crime, despite evidence supporting his alibi. Because of a legal technicality, his case was retried, and he was again found guilty in 1986. He was sentenced to three terms of lifetime imprisonment. Bloodsworth continued to maintain his innocence. In 1992, a semen sample from the victim’s clothing was analyzed by both a private laboratory and the FBI laboratory. Using PCR and DNA fingerprinting, both laboratories determined that Bloodsworth’s DNA did not match the DNA evidence from the crime scene. He was pardoned after spending nine years in prison.

Think Critically

Review the Case Studies and the information on DNA Fingerprinting. Should DNA evidence alone be sufficient to convict when there is no corroborative evidence? State your opinion and provide support for it.

Crime-Scene Investigation – Case 1

Crime-Scene Investigation – Case 2

CASE STUDIES

Colin Pitchfork (1983)

Two schoolgirls in the small British town of Narborough in Leicestershire, United Kingdom, had been raped and murdered three years apart. The methods used were the same for both cases. Blood-type testing revealed that semen samples collected from both victims were from a person with type A blood. This blood type matched 10 percent of the adult male population in the area, but without further information, no suspects could be identified. The noted geneticist Dr. Alec Jeffreys, developer of the DNA fingerprinting technique, was consulted. To match a suspect to the DNA found in the semen, Dr. Jeffreys suggested that police launch the first-ever DNA-based manhunt. Every young man in the entire community was asked to submit a blood or saliva sample. Blood group testing was performed and DNA fingerprinting was carried out on the 10 percent of men with type A blood. At first no DNA match to the crime-scene evidence was found among the 5,000 samples collected. Then it was discovered that Colin Pitchfork, a local bakery worker, had asked a friend to give a blood sample on his behalf. The police then forced Pitchfork to give a blood sample. His DNA matched the DNA evidence found on both victims. He confessed to the crimes and was sentenced to life in prison. This was the first time DNA fingerprinting was ever used to solve a crime. Joseph Wambaugh’s book, *The Blooding*, is based on this case.

Tommie Lee Andrews (1986)

Nancy Hodge, 27, worked at Disney World in Florida. She was attacked and raped at knifepoint in her apartment. Her attacker’s face was covered (concealed). A series of rapes followed, and police suspected that up to 27 attacks could be attributed to the same man. Tommie Lee Andrews was apprehended and linked to the rapes by conventional fingerprint and DNA profile evidence. He was sentenced to more than 100 years in prison. This was the first time DNA evidence was used in the United States to convict a criminal.

Think Critically

Review the Case Studies and the information on DNA Fingerprinting. Should DNA evidence alone be sufficient to convict when there is no corroborative evidence? State your opinion and provide support for it.

Crime-Scene Investigation – Case 3

Crime-Scene Investigation – Case 4

Mini Lab No. 2 - DNA Gel Electrophoresis**Kool-Aid Gel Electrophoresis****Introduction**

In this lab, gel electrophoresis will be used to separate the dyes present in different flavors of Kool-Aid. In this experiment students will be introduced to the basis of gel electrophoresis. Students will learn about the basis of color theory and how the eye perceives color.

1. Students will use dyes from Kool-Aid that will be exposed to an electrical current and a buffer solution.
2. The Kool-Aid and the buffer solution will become ionized and take on a structure that will respond to an electrical charge. This lab contains eight different flavors of Kool-Aid: grape, black cherry, cherry, strawberry, tropical punch, lemon-lime, orange, and lemon.

Materials:

Each group of 6 students will perform the experiment. The materials needed to perform this lab include:

- Disposal cups
- Agars gel solution
- Set of Kool-Aid samples with 50% glycerol
- 5 syringes, 1cc
- 5 yellow tips
- Electrophoresis tray with 1% agarose gel solution
- Set of (5) 9Volt batteries
- Set of Alligator clips –(one red cable & one black cable)
- Paper towels
- Gel Electrophoresis Diagram

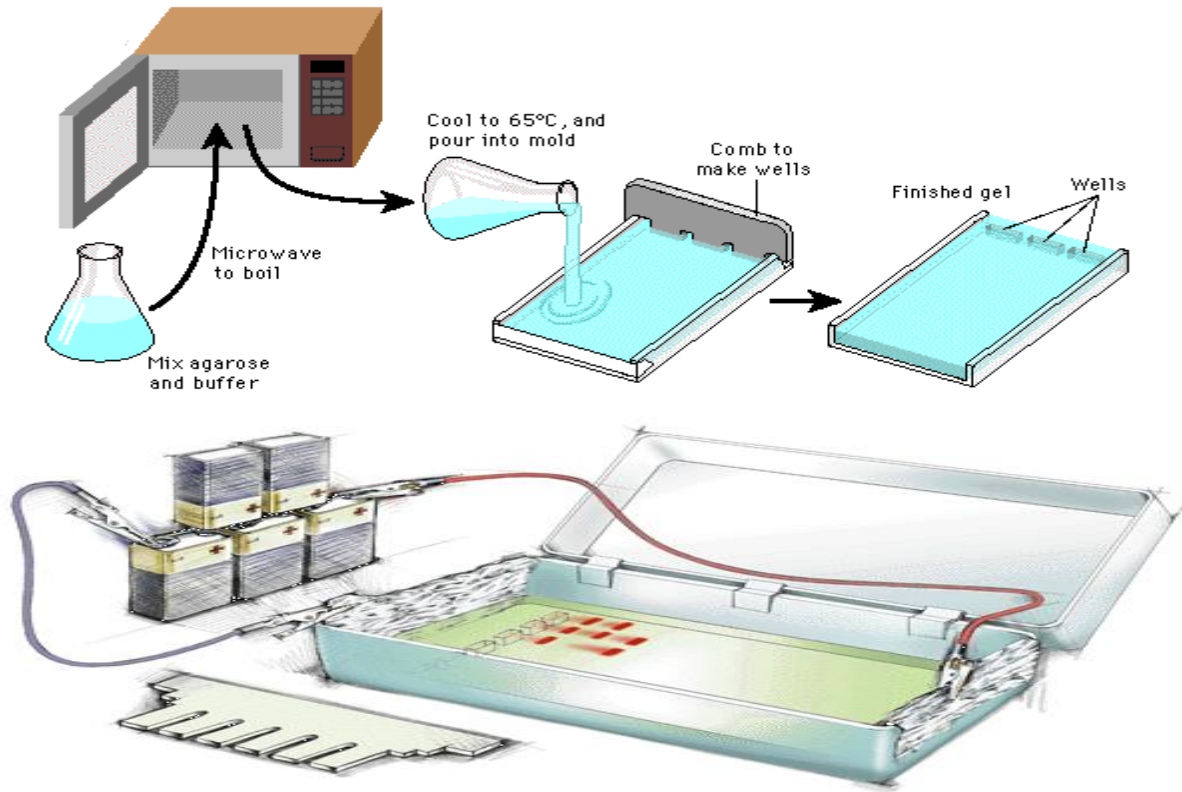
**Procedure:**

1. Collect the necessary equipment from the lab table
2. Remove the plastic wrap from the 1% agarose gel
3. Remove a 1cc syringe from the color plastic cup
4. Carefully place a yellow pipette tip on the syringe, make sure the small plastic tube surrounding the syringe tips is not pushed to far up on the syringe
5. Place 50 ml of water into the colored container
6. Practice removing about 0.05 mls (1 drop) of water into and out of the syringe
7. Use the 1-cc syringe and a yellow tip to load about 10 μ L into separate wells on the gel. Load each Kool-Aid sample only once. The first line on the yellow pipet tip is 10 μ L.

TIPS

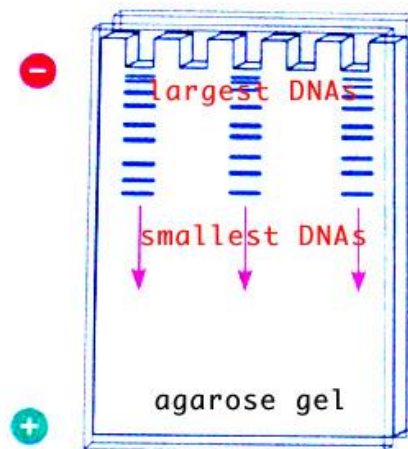
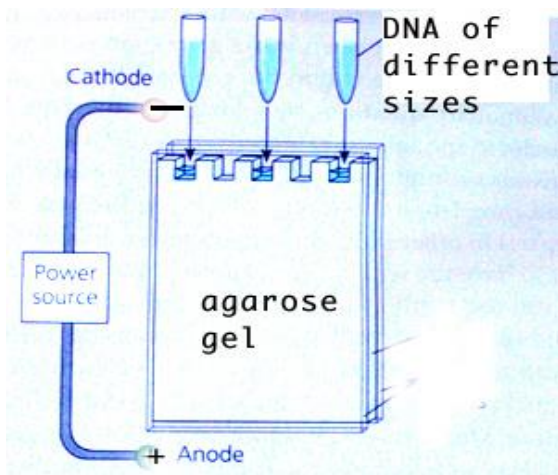
- Steady syringe over well using two hands
- Be careful to expel any air in the pipet tip end before loading the gel. (If air bubbles form “cap” over well, sample will flow into buffer around edges of well.)
- Dip pipet tip through surface of buffer, position it over well, and slowly expel the mixture. Glycerol in the Kool-Aid solution weighs down the sample, causing it to sink to the bottom of the well.
- **Be careful not to punch tip of the pipet through bottom of gel.**

DNA Gel Electrophoresis Technique



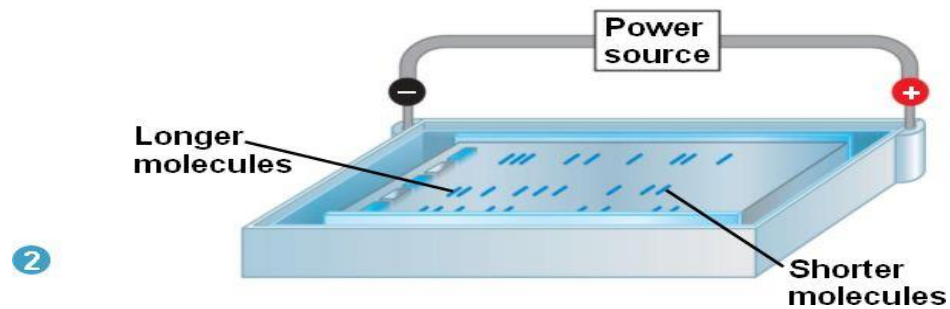
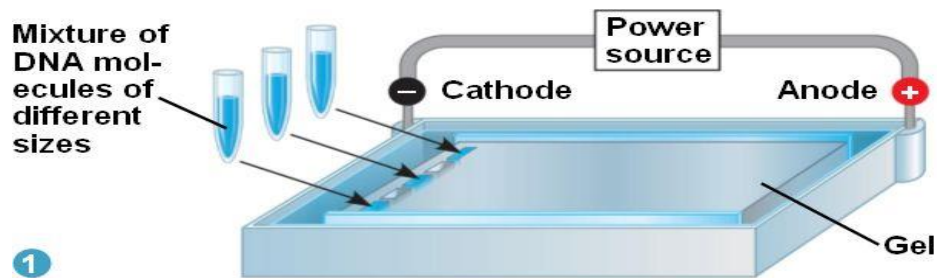
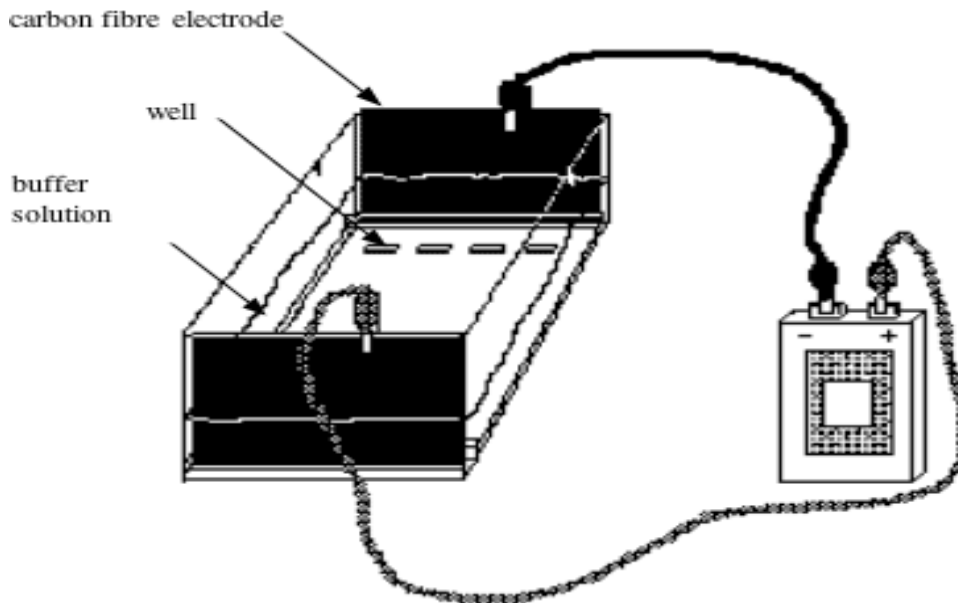
The term "gel" in this instance refers to the matrix used to contain, then separate the target molecules. In most cases, the gel is a cross linked polymer whose composition and porosity is chosen based on the specific weight and composition of the target to be analyzed.

Agarose gels are easily cast and handled compared to other matrices, because the gel setting is a physical rather than chemical change. Samples are also easily recovered. After the experiment is finished, the resulting gel can be stored in a plastic bag in a refrigerator



Broughton High School

Gel Electrophoresis Techniques



The negatively charged DNAs move through the gel, toward the positive electrode (anode) the smallest DNA move fastest and furthest down the gel and the largest DNAs move the least

Broughton High School

Agarose Gel Electrophoresis and the Koolaid Gang

Pretend that you own a cherry orchard. The Koolaid gang is composed of brothers who live in the house next to yours. They are known for their highly sugary fruit drinks. Imagine, that one morning, you wake up and find that your cherries have been stolen. You suspect the Koolaid gang. After a thorough investigation, only a piece of skin could be found on one of the cherry trees.

1. *How can we find the culprit?*
2. *Can each Koolaid brother be suspected of having committed the theft?*
3. *How can we find out which one of those did it?*

Remember that DNA is contained in the cells of the skin and that the DNA is characteristic for the individual that the tissue stems from. If we can find a way to process the DNA of the skin that was found and compare it to the DNA of the suspects, we will be able to identify which Koolaid brother did it.

Agarose gel electrophoresis can resolve molecules based on charge, size, and shape. In this laboratory you will use gel electrophoresis to separate molecules present in different samples from the Koolaid brothers.

Materials and Equipment

For each team:

Koolaid brother samples in microcentrifuge tubes:

1. _____
2. _____
3. _____
4. _____
5. _____
6. _____
7. _____

Graduate micropipets and tips to load samples

For the class:

Electrophoresis units and power supplies
 2% agarose in 1X TAE (hot liquid!)
 1X TAE for electrophoresis units
 Hot water bath to keep agarose liquid

Preparing the agarose gel

1. Record your electrophoresis unit number on your data sheet. Set up black casting dams and combs in electrophoresis unit. Place one comb in the slot near the red (+) electrode and the other comb in the middle slot.
2. Pour agarose into gel deck. Pour close to the top of the gel deck, but do not overfill.
3. Practice using micropipet and unmarked samples while agarose is solidifying.
4. Remove black casting dams, add 1X TAE to electrophoresis unit, and remove combs.

Broughton High School

Loading the samples

1. Label a microfuge tube for each brother's sample.
2. Add 5 μ l of supersaturated sucrose to each tube. (This will give the sample weight so it stays in the gel wells)
3. Transfer 10 μ l from the stock solution into the respective sample tube.
4. Flick the tube to mix. Pulse the samples down in the microcentrifuge so the sample is pooled at the bottom of the tube.
5. Record on data sheet where you will load the samples.
6. Load 10 μ l of each sample into the respective gel well.
7. Connect electrophoresis unit to power supply.
8. Turn on the power supply, and set voltage to ~100 V. The samples will start resolving toward both the negative and positive poles
9. Run samples for ~10 minutes. Turn off power supply, disconnect power cords from the chamber, and remove top of electrophoresis chamber.

Predictions (Answer the following)

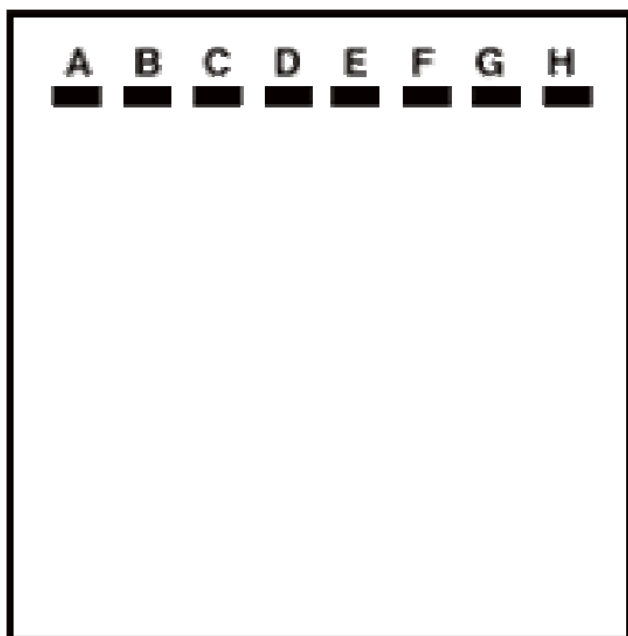
1. What do you think will happen to the different samples in the electrophoresis chamber?
2. Will the brothers have the same DNA fingerprint?

Analyzing the gel

1. Carefully remove casting tray with gel. Place gel in large weigh tray
2. Draw a diagram on your gel sheet of the banding pattern of the samples.

Results

BLACK

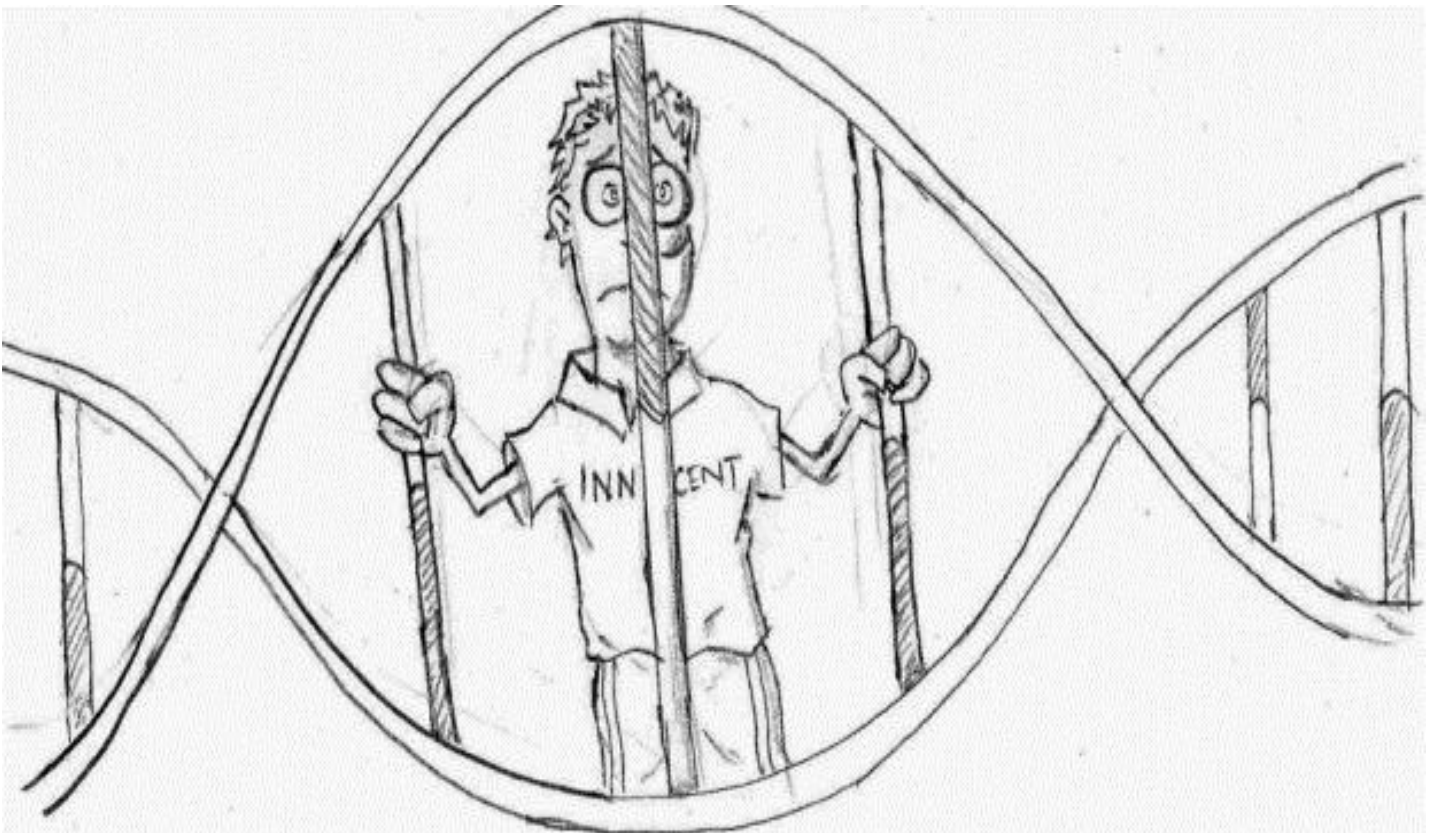


RED

Lane Number	Sample
A	_____
B	_____
C	_____
D	_____
E	_____
F	_____
G	_____
H	_____

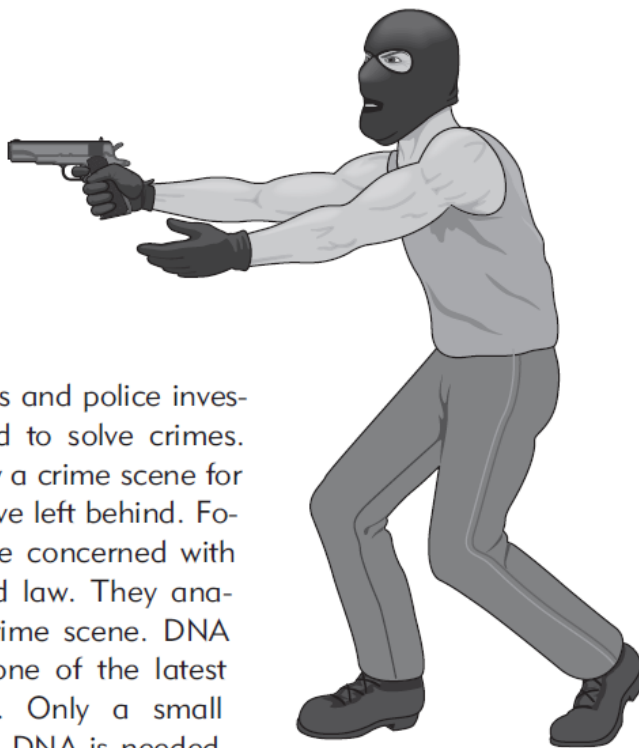
Questions

1. Which Koolaid brother is the culprit?
2. What colored dyes make up each of the brother's samples?
3. How does electrophoresis separate the dye pigment?
4. Which color molecule is the smallest? How do you know?



Activity No. 11: DNA Paper Finger Printing**Materials**

- Scissors
- Glue or tape
- Pencil
- Clear metric ruler

**Background**

Today more than ever, scientists and police investigators are working hand-in-hand to solve crimes. Police investigators carefully survey a crime scene for evidence that the criminal may have left behind. Forensic scientists are those who are concerned with relationships between science and law. They analyze the evidence found at the crime scene. DNA profiling (DNA fingerprinting) is one of the latest tools used to identify criminals. Only a small amount of a sample that contains DNA is needed to make a DNA profile.

How does the process of DNA profiling work? DNA taken from body cells can be cut into small fragments by restriction enzymes. These fragments are called *restriction fragment length polymorphs* (RFLPs), pronounced "riflips." Everyone's DNA is different and separates into fragments of different lengths and compositions. The

number and sizes of DNA fragments produced depend on the specific sequence of nucleotides in a DNA sample.

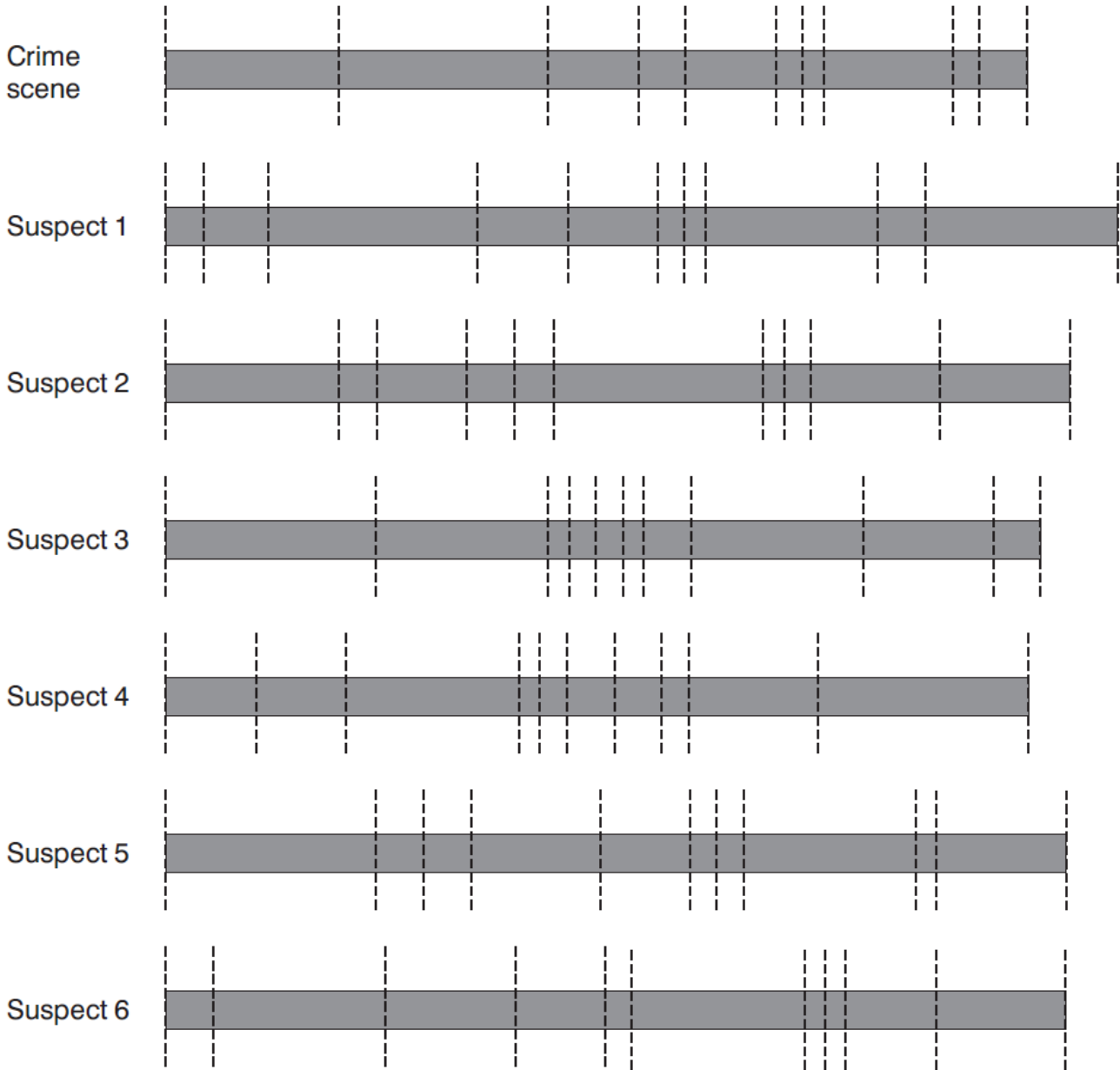
After the DNA is cut, the resulting fragments are separated by gel electrophoresis. A gel is a flat, rectangular slab of a thin, jellylike material. DNA fragments are placed in wells at one end of a gel. The gel is poured into a gel chamber that has a positive electrode at one end and a negative electrode at the other end. The gel is arranged so that the wells are located near the negatively charged electrode. DNA has a negative charge, so the fragments move through the gel toward the positive pole. The small fragments move faster and farther through the gel than the large fragments.

After the gel is stained, the DNA fragments appear as separate and distinct bands. The larger fragments are near the negative pole, and the smaller fragments are near the positive pole. Everyone's DNA produces a distinct banding pattern when cut with two or more restriction enzymes. If the banding pattern produced by a criminal suspect matches the pattern from DNA left at the crime scene, police investigators have most likely found their criminal.

Models of DNA Samples



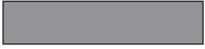







Dotted lines represent locations where restriction enzymes will cut these pieces of DNA.

DNA Samples



ELECTROPHORESIS

NEGATIVE ELECTRODE

Sample Sizes	Well to Deposit DNA	Well to Deposit DNA	Well to Deposit DNA
	Suspect #1	Suspect #2	Suspect #3
10 			
9 			
8 			
7 			
6 			
5 			
4 			
3 			
2 			
1 			

POSITIVE ELECTRODE

CHAMBER**NEGATIVE ELECTRODE**

Well to Deposit DNA	Well to Deposit DNA	Well to Deposit DNA	Well to Deposit DNA
Suspect #4	Suspect #5	Suspect #6	Crime Scene

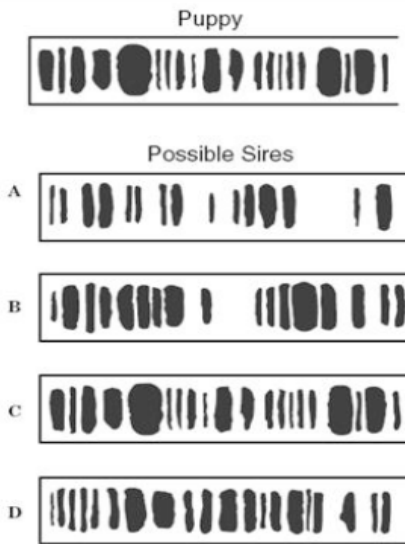
POSITIVE ELECTRODE

Activity No. 11: DNA Fingerprinting Worksheet

DNA Fingerprinting / worksheet

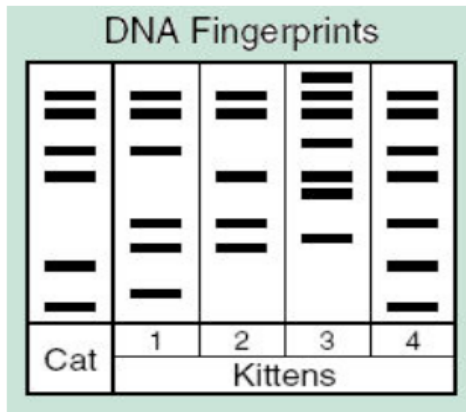
1. The DNA fingerprints were made from blood samples taken from a puppy and four possible sires of this puppy in an effort to determine the puppy's pedigree. According to this information, which sire was probably the father of this puppy? CIRCLE YOUR ANSWER

- a. A
- b. B
- c. C
- d. D

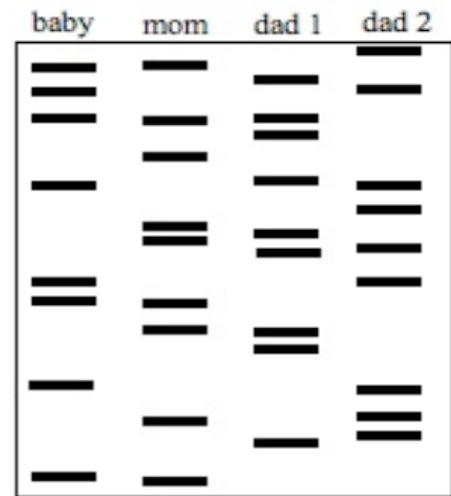


2. The picture shows a segment of DNA from a cat. Which of these is most likely the kitten of this cat? CIRCLE YOUR ANSWER.

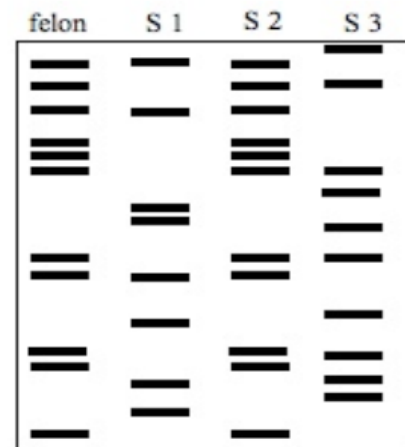
- a. 1
- b. 2
- c. 3
- d. 4



3. Mrs. Smith has a baby named Tyra. She believes one of two men can be the father of her child. A paternity test is done and the results are shown above. Which of the 2 men are baby Tyra's father? _____

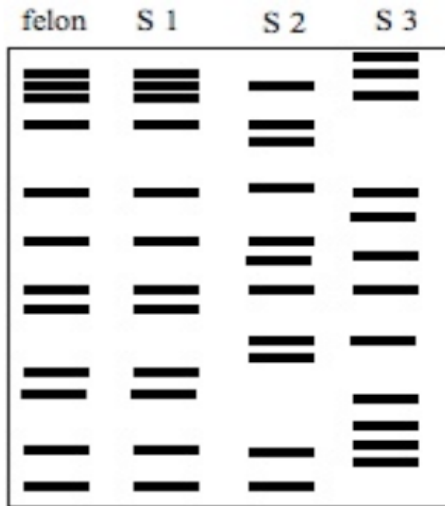


4. Lt. Russ is investigating a murder scene. The felon was scratched by his victim & some of his skin cells were found under the victim's fingernails. A DNA test was performed. Which of the suspects is the murderer? _____

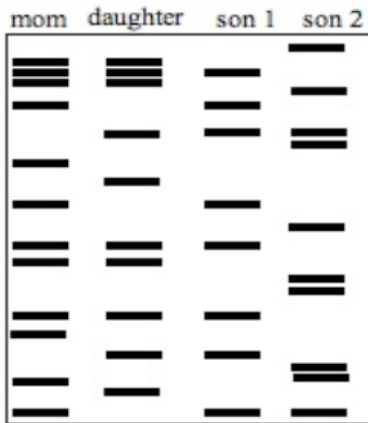


Activity No.11: DNA Fingerprinting Worksheet

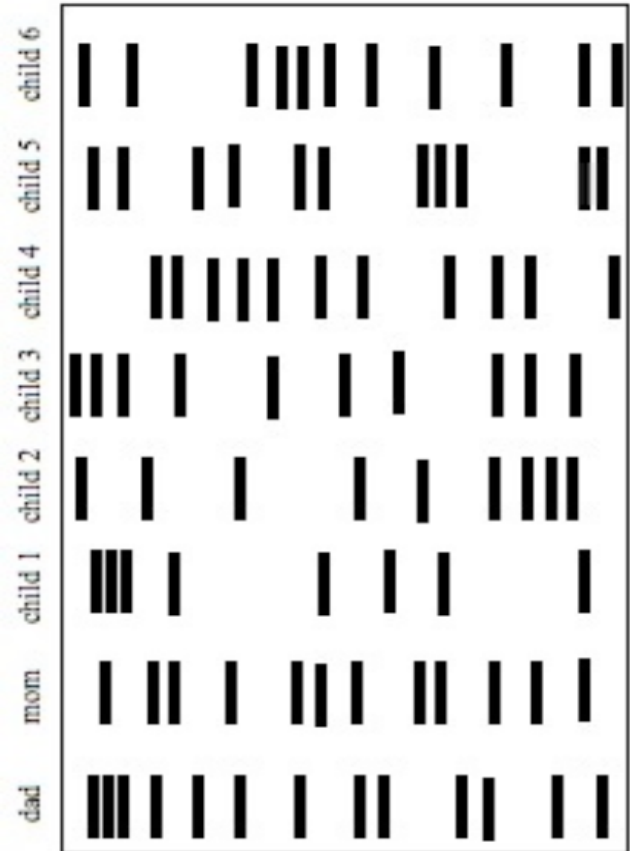
5. Suzy was assaulted in an alley and is a victim of rape. The police collected a sample of sperm that was left at the crime scene and now have 3 suspects in custody. Which of the suspects raped Suzy? _____



6. The millionaire, Mr. Big, has just died. He has left behind a wife, daughter and a large inheritance. The news of his death has brought forth 2 men who claim to be the long lost son of Mr. & Mrs. Big. Before Mr. & Mrs. Big were married they had an illegitimate child and had placed him up for adoption. They had tried to find him after they became wealthy but had no luck in locating him. A DNA sample was taken from Mrs. Big, the Big daughter and the two men who claim to be the long lost son. Which, if any, of the men are telling the truth? _____



7. Mr. & Mrs. Jones just gave birth to fraternal twins- Bob and Jane. Unfortunately, the nurse has confused the Jones twins with 4 other babies. The doctors took samples of DNA from each of the babies and Mr. & Mrs. Jones. Which of the 6 children are Mr. & Mrs. Jones twins? _____



Activity No. 12: "Who Ate the chocolate cake!"

Broughton High School

Objectives: In this simulation, you will examine crime scene evidence to determine who is responsible for eating the Queen of Heart's special imported German Chocolate cake. You will use a model of the process of electrophoresis and DNA fingerprinting to solve the crime.

Incident Data Report			
Incident Type:	Theft	Complaint Status:	Pending DNA Results
Processed by:	Chief Mares	Other Officers:	Officer Lentz

Property Data Report			
Property Code:	German Chocolate Cake	Owner's Name:	Olivia Nelson
Name:		Value:	\$2,600

Burglary Data:

Method of Entry: Unknown, no evidence of force on doors or windows.

Narrative: The German Chocolate Cake was allegedly stolen from the Queen of Heart's sitting room the night before the Broughton High School Queen of Heart's ball. The German Chocolate Cake was listed as a gift from the Tom Brown diplomat of New York City. Officer Lentz dusted for fingerprints and found none on the table or doors, the maid claimed that they had been wiped clean earlier. The wheel of German Chocolate Cake was on a platform in the sitting room, and half of it had been eaten. We took pictures of the half eaten German Chocolate Cake and sent it to the lab for further tests. Samantha Cook, the lab technician said that saliva samples could be taken from the teeth imprints of the German Chocolate Cake that was left behind.

Suspect Data

Suspect Number: 1

Name: Princess Dubbah Elix

Description of Suspicion: The princess was seen entering the sitting room earlier in the evening. She is well known for her love of German Chocolate Cake.

Suspect Number 2

Name: Electra Foresis

Description of Suspicion: Electra was recently involved in a relationship with the Manchurian diplomat that sources say ended badly. Her motive may have been to sabotage the diplomat's gift to the Queen.

Suspect Number 3

Name: Ada Nine

Description of Suspicion: Ada was the maid in charge of cleaning the sitting room. She had access to the German Chocolate Cake

Suspect Number 4

Name: Gene Tics

Description of Suspicion: Gene is the leader of the local German Chocolate Cake Guild, he may not have wished for Queen Olivia Nelson to have German Chocolate Cake from anywhere but his own guild.

Crime Lab Data

Crime Lab Investigator	Ricky Stone	Lab Technician	Edna Smith
List of Evidence	Plastic bag with Chocolate Cake	List of Procedures Used	DNA Extraction

Activity No. 12: "Who Ate the chocolate cake!"

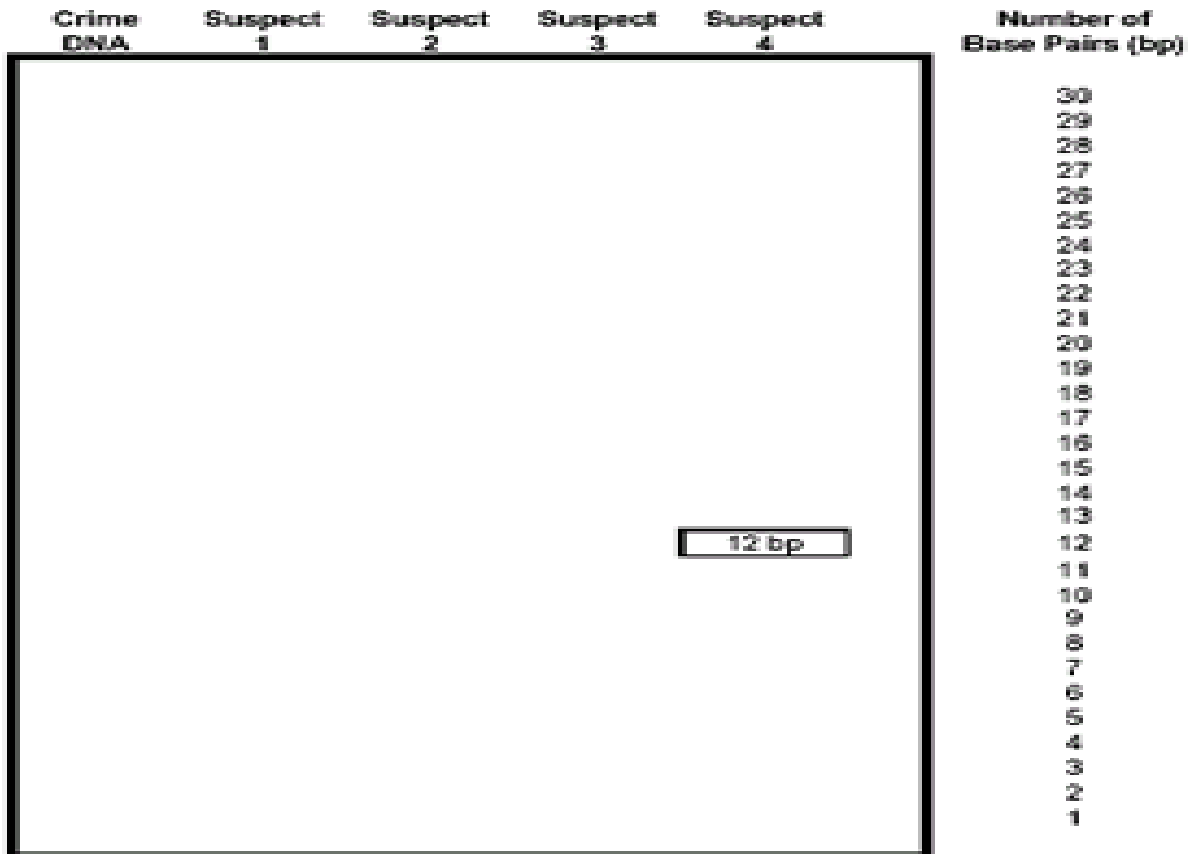
Who Ate the chocolate cake!

Narrative: After receiving the package with the plastic bag marked Crime Scene, the DNA was extracted. Because the sample was so small, the DNA was amplified using the polymerase chain reaction. We isolated the DNA from the four suspects and compared them to the crime scene DNA using DNA restriction analysis.

Results: See attached DNA Results

DNA Evidence Evaluation

1. Turn your paper strips (DNA sequences) so that the side with the bases is facing you. The restriction enzyme cuts at every point it finds C C G G, always cutting between the C and the G. Label the back of the slips with the suspect number so that you don't get them confused after cutting. Use scissors to cut the DNA sequence at the C C G G points.
2. Count the number of base pairs (bp) in each piece of DNA that you created. Record the base pair number on the back side of the DNA fragment.
3. Make an enlarged chart like the one shown. Your teacher will give you paper for this. Use a ruler to ensure that the lengths are uniform.
4. Tape your DNA fragments to the chart, using the base pair numbers as a guideline for fragment placement.
5. Compare the crime scene DNA to the suspects and indicate on your chart, which suspect is guilty of eating the German Chocolate cake.



Who Ate the chocolate cake!

Crime DNA Crime DNA Crime DNA Crime DNA Crime DNA Crime DNA Crime DNA Crime DNA Crime DNA Crime DNA Crime DNA Crime DNA Crime DNA
 GTCGACCGGTGACCGTGCGTACACAGTGCTCCGGATAGCTGATAGCTCCGGTG
 CAGCTGGCCACTGGCACGCATGTGTCACGAGGCCTATCGACTATCGAGGCCAC

Suspect 1 DNA Suspect 1 DNA Suspect 1 DNA Suspect 1 DNA Suspect 1 DNA Suspect 1 DNA Suspect 1 DNA Suspect 1 DNA Suspect 1 DNA Suspect 1 DNA Suspect 1 DNA Suspect 1 DNA
 GTCCCAGCCGGACCGTACCGGTAGATCAGCCGGTAGATTGATAGCGTGATGTG
 CAGGGTCGGCCTGGCATGGCCATCTAGTCGGCCATCTAACTATCGCACTACAC

Suspect 2 DNA Suspect 2 DNA Suspect 2 DNA Suspect 2 DNA Suspect 2 DNA Suspect 2 DNA Suspect 2 DNA Suspect 2 DNA Suspect 2 DNA Suspect 2 DNA Suspect 2 DNA
 GTCTACGTAATCGTAGCCATCCGGACAGTGTGCACGATCGTACATGCTACGTG
 CAGATGCATTAGCATCGGTAGGCCTGTCACACGTGCTAGCATGTACGATGCAC

Suspect 3 DNA Suspect 3 DNA Suspect 3 DNA Suspect 3 DNA Suspect 3 DNA Suspect 3 DNA Suspect 3 DNA Suspect 3 DNA Suspect 3 DNA Suspect 3 DNA Suspect 3 DNA
 GTCGACCGGTGACCGTGCGTACACAGTGCTCCGGATAGCTGATAGCTCCGGTG
 CAGCTGGCCACTGGCACGCATGTGTCACGAGGCCTATCGACTATCGAGGCCAC

Suspect 4 DNA Suspect 4 DNA Suspect 4 DNA Suspect 4 DNA Suspect 4 DNA Suspect 4 DNA Suspect 4 DNA Suspect 4 DNA Suspect 4 DNA Suspect 4 DNA Suspect 4 DNA
 GTCTCCATCCGGACTACCATAACATCTGGTGTACCCGGTGATATCGTCCGGGTG
 CAGAGGTAGGCCTGATGGTATGTAGACCACATGGGCCACTATAGCAGGCCAC

Thinking Critically

1. On your chart, label the positive (+) and the negative (-) ends. Circle the suspect's DNA who matches the DNA at the crime scene and write the name of the suspect.
2. For each of the following tasks performed in the activity, describe what they are actually simulating.
 - a. Cutting the DNA into fragments: _____
 - b. Taping the DNA onto the large paper: _____
3. For each word below, describe how it relates to DNA Fingerprinting:
 - Polymerase Chain Reaction _____
 - Gel Electrophoresis _____
 - Restriction Enzyme _____

