NILAMANI MAHAVIDYALAYA

RUPSA, BALASORE

A

PROJECT ON

"DNA FINGERPRINTING"

SUBMITTED TO

FAKIR MOHAN UNIVERSITY

IN FULFILMENT OF THE REQUIRMENTS FOR DEGREE OF

BACHELOR OF SCIENCE

GUIDED BY :-

Mrs. KALPANA MANJARI PATRA
(H.O.D. OF DEPT. OF ZOOLOGY)
Mr. SATYENDRA KU. PAL
(LECT. IN ZOOLOGY)

SUBMITTED BY :-

1. Saipriya Pani (Roll No : 5908B19020)
2. Sk Ruhullah (Roll No : 5908B19023)
3. Smruti Prangyan Jena (Roll No : 5908B19024)
4. Somdev Adhya (Roll No : 5908B19025)
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DECLARATION

We Saipriya Pani (Roll No : 5908B19020), Sk Ruhullah (Roll No : 5908B19023), Smruti Prangyan Jena (Roll No : 5908B19024), Somdev Adhya (Roll No : 5908B19025), Susanta Kumar Barik (Roll No : 5908B19027), Tanmay Kumar Das (Roll No : 5908B19028) do hereby certify that the project report entitled **"DNA FINGERPRINTING"**, being submitted to NILAMANI MAHAVIDYALAYA RUPSA, Balasore, Odisha for the award of Bachelor of Science is an original piece of work done by us and the same has not been submitted else where for any other academic degree or diploma to this college or any other college/university.

(Name of the Student)

Saipriya Pani

Sk Ruhullah

Smruti Prangyan Jena

Somdev Adhya

Susanta Kumar Barik

Tanmay Kumar Das

FAKIR MOHAN UNIVERSITY

F.M UNIVERSITY, BALASORE

CERTIFICATE

This is to certify that the project report entitled **"DNA FINGERPRINTING"** Submitted by Saipriya Pani, Sk Ruhullah, Smruti Prangyan Jena, Somdev Adhya, Susanta Kumar Barik, Tanmay Kumar Das for the award of the degree of Bachelor of Science from NILAMANI MAHAVIDYALAYA RUPSA, Balasore, Odisha, India, is a bonafied record of work carried out by them under my guidance. Neither this project report nor any part of it has been submitted for any degree or academice award elsewhere.

12.06.21

Satyendra Kumar Pal
(Signature of Guide) 10.6.21

FAKIR MOHAN UNIVERSITY

F.M. UNIVERSITY, BALASORE
ACKNOWLEDGEMENT

We sincerely express our heartfelt gratitude and indebtedness to our Supervisor **Mr. SATYENDRA KU. PAL** (Lect. in Zoology) & **Mrs. KALPANAMANJARI PATRA** (Head of the Department of Zoology), **NILAMANIMAHAVIDYALAYA RUPSA, Balasore, Odisha** for their valuable guidance,enlightened suggestions, constant encouragement, critical comments, creativesupport, deep involvement and immense cooperation throughout the research work.

We acknowledge with thanks the Head of the Department of Zoology **NILAMANI MAHAVIDYALAYA RUPSA, Balasore** for providing us the facilities tocarry out our research work. We express our heartfelt gratitude and sincere thanks toall our teachers of the U.G. Department of Zoology, **NILAMANI MAHAVIDYALAYA, RUPSA, Balasore** for their helping views, scientific and constructive comments incourse of completion of our research work. We are thankful to all the nonteachingstaff members of the U.G. Department of Zoology for their immense help andcooperation in smooth conduct of the research work.

We are indebted to our parents who have been a constant source of inspirationin our academic and research pursuit. We have the pleasure in placing on record ourdeep sense of love and gratefulness to our family members for their persistent moralsupport and cooperation. Their care and affection encouraged us to carry out this work successfully.

We are indebted to the almighty God for everything he had done for us till nowand also for all those that he will do for us in future. We have the pleasure in placingon record our deep sense of love and gratitude to our beloved God. Last but not the least; we are thankful to our friends, nears and dears for theirhelp at different stages of the work.

Saipriya Pani

Sk Ruhullah

Smruti Prangyan Jena

Somdev Adhya

Susanta Kumar Barik

Tanmay Kumar Das

CONTENT

CHAPTER –I

INTRODUCTION

DNA FINGERPRINTING

Introduction:-

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.

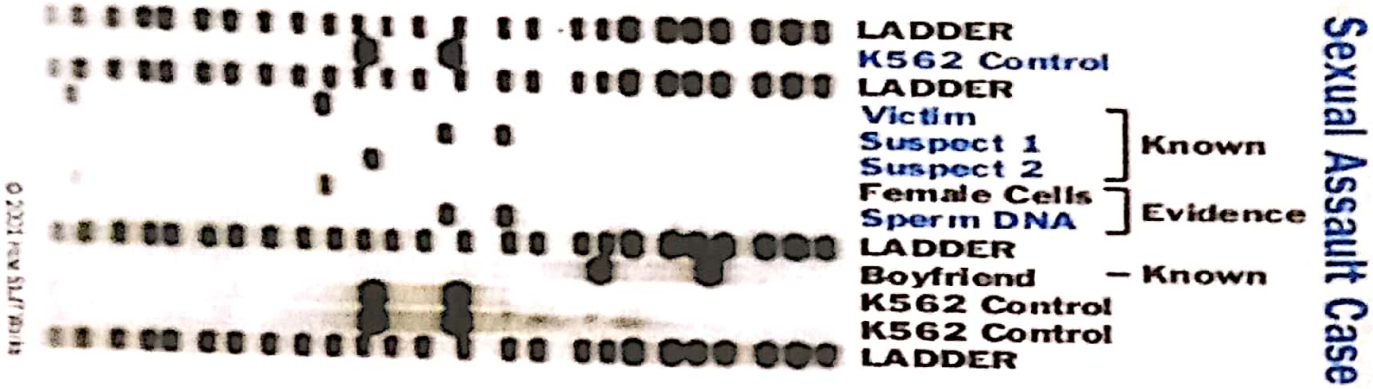
The concept of DNA fingerprinting.

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

o enzymes that break up cell membranes that are usually used in DNA extraction for epithelial issues. 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.

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 CATACAGAC, there might be three copies (CATACAGAC CATACAGAC CATACAGAC) 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.

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.

CHAPTER –II

STEPS OF DNA FINGER PRINTING

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.

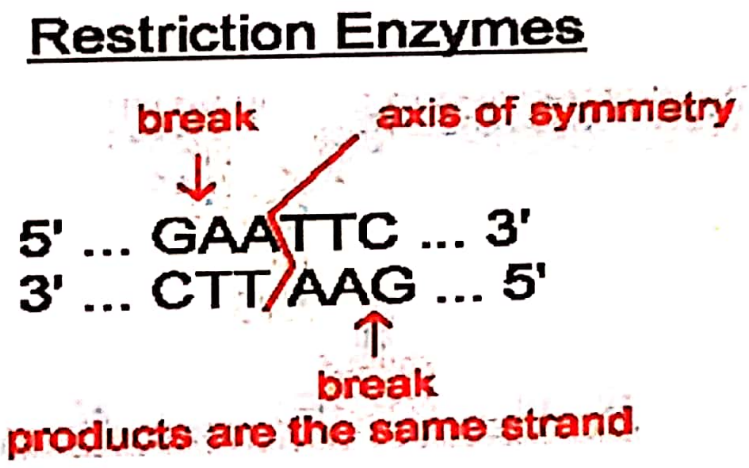
Extraction:-

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

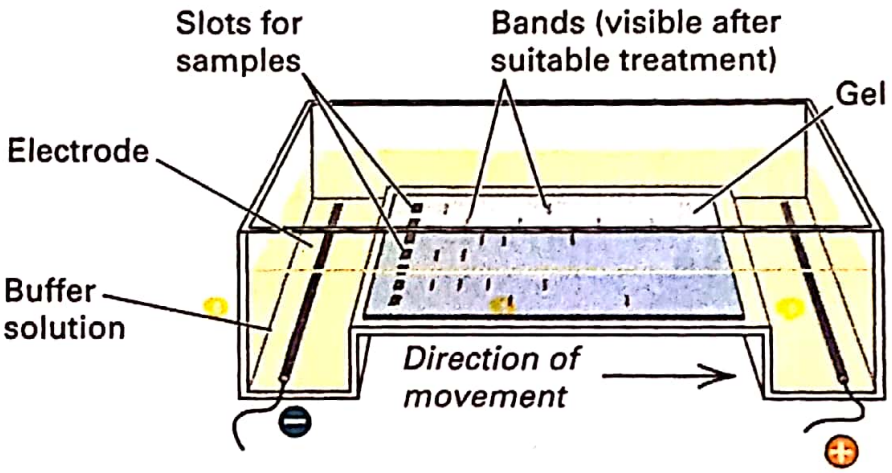
With some DNA analyses, polymerase chain reaction (PCR) can be used to amplify certain pieces of the DNA that contain the VNTRs. In STR profiles, restriction enzymes are unnecessary and PCR allows the amplification of the strands with the STR sequences.

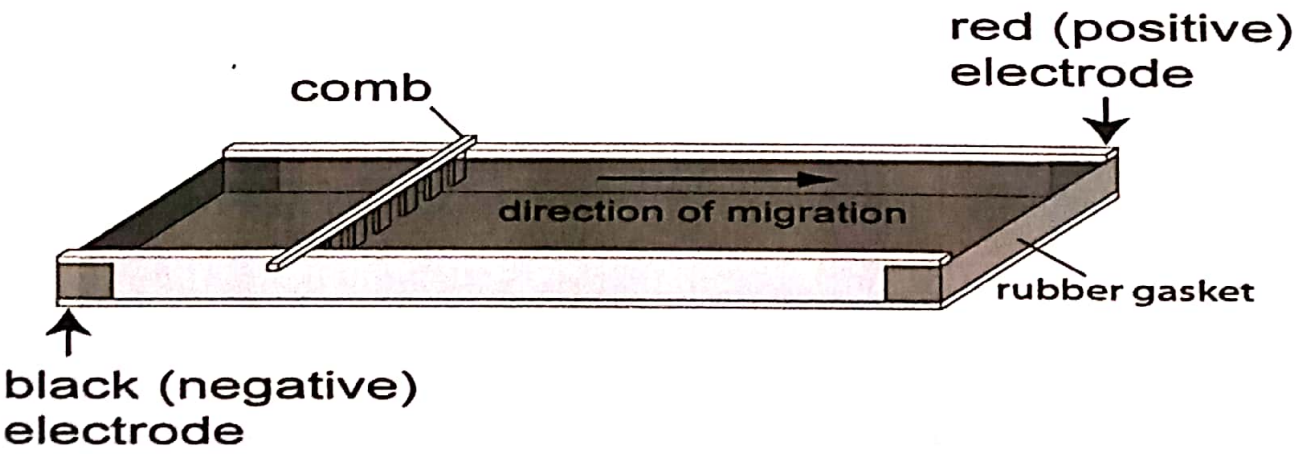
Electrophoresis

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

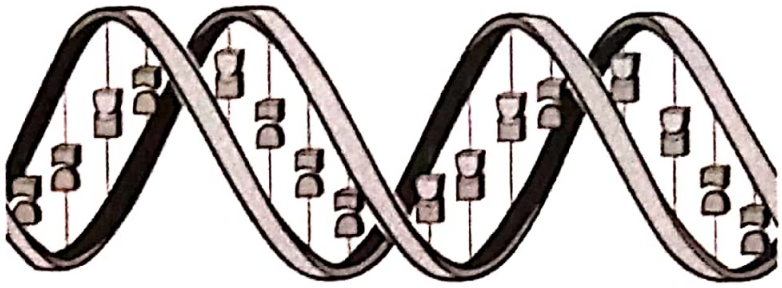


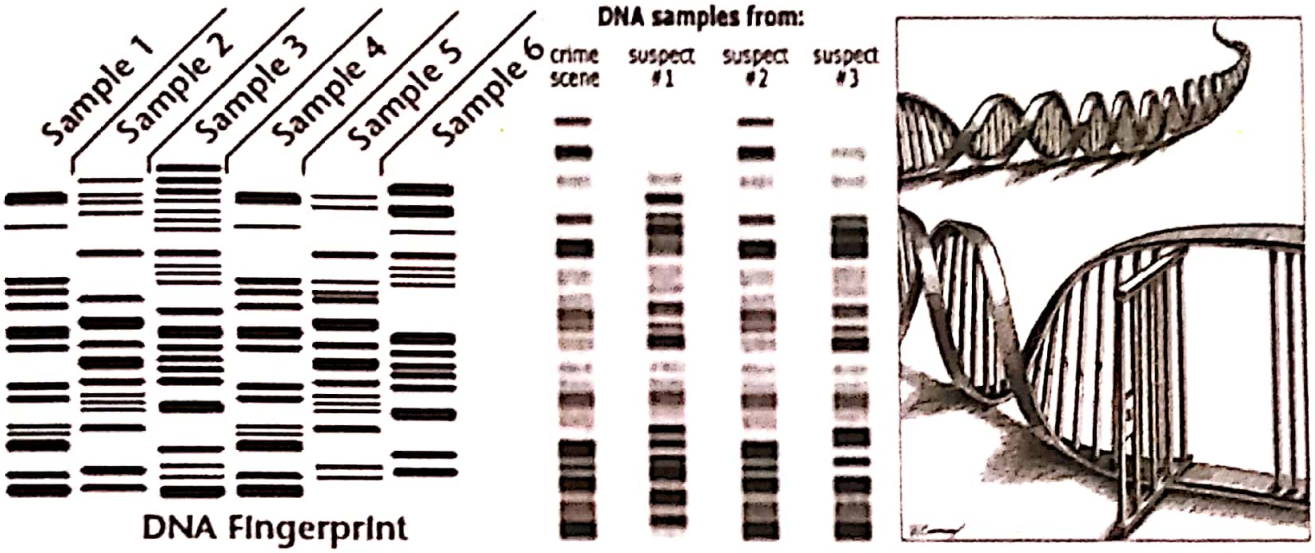


CHAPTER –III

FORENSIC DNA FINGER PRINTING

Forensic DNA Fingerprinting:-





CHAPTER –IV

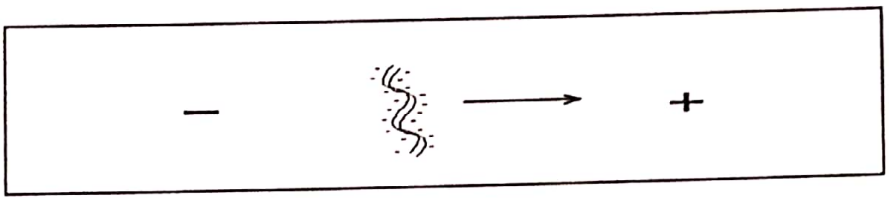
DNA GEL ELECTROPHORESIS

DNA Gel Electrophoresis

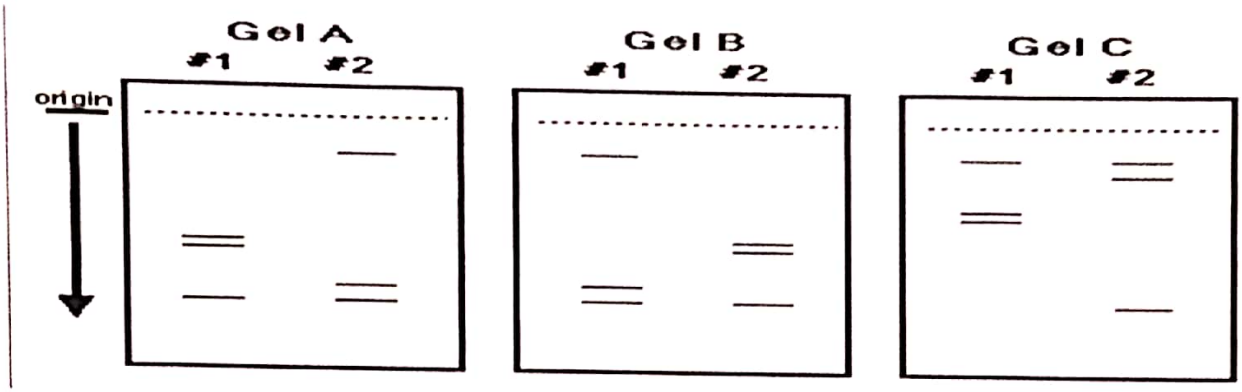
Agarose gel electrophoresis is an easy way to separate DNA fragments by their sizes and visualize them. It is a common diagnostic procedure used in molecular biological labs.

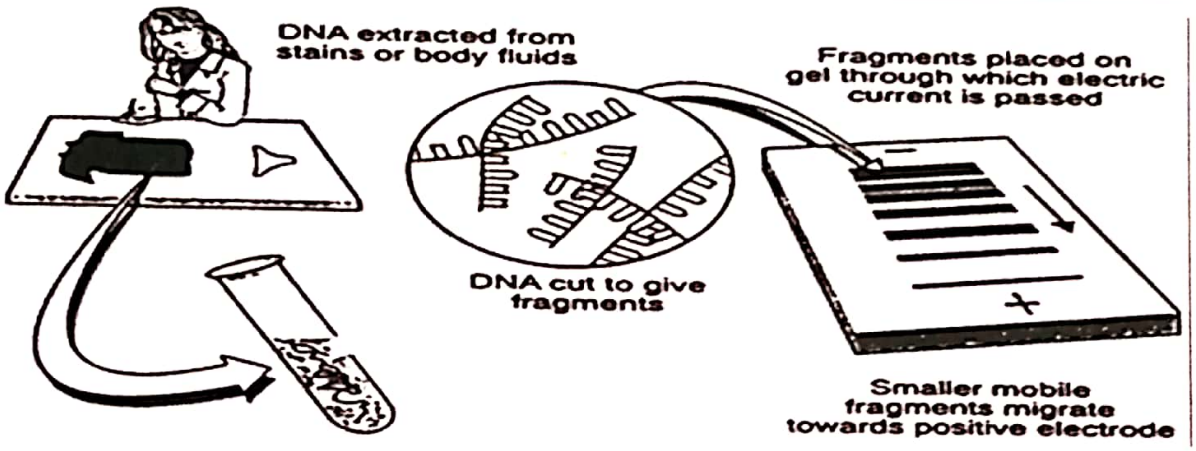
Electrophoresis:-

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

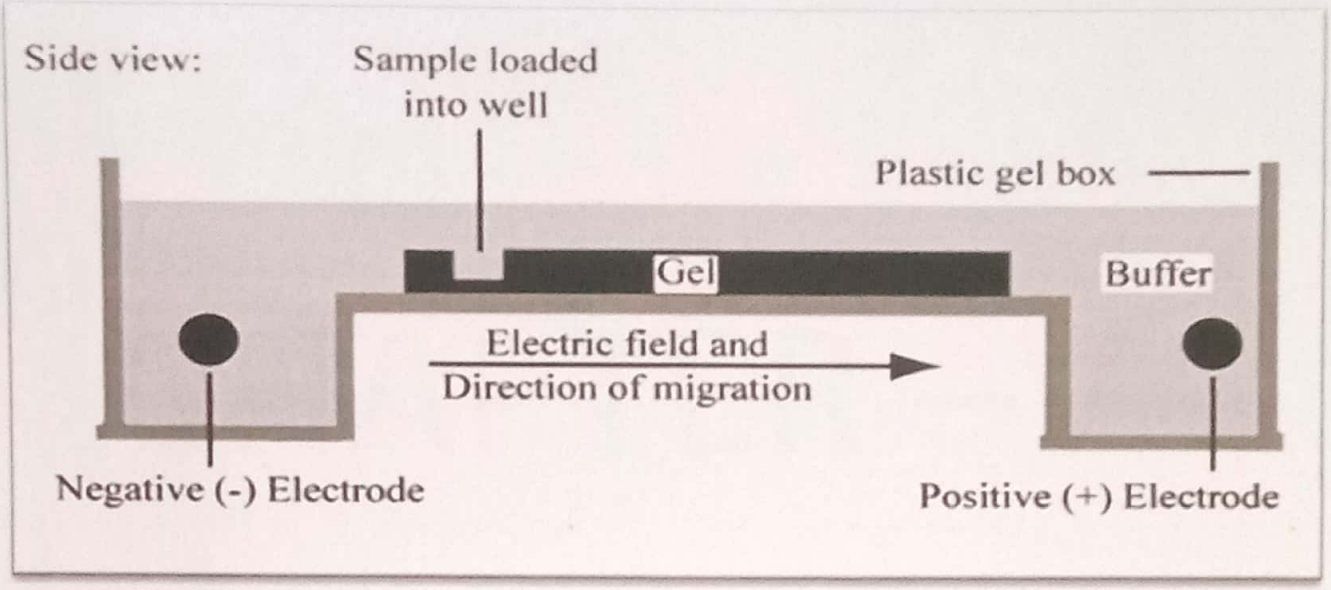
In order to analysis DNA fragments restriction enzymes are used. Restriction enzymes are proteins that cut long DNA pieces into smaller pieces or sections. Restriction enzymes cut DNA segments at particular sections or specific sites in order to make the DNA fragments smaller. Restriction enzymes act 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.

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

CHAPTER –V

JEWEL THIEF

DNA

FINGERPRINTING

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.

CHAPTER –VI

CASE STUDY

CASE STUDIES-I

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 Bloodworth's DNA did not match the DNA evidence from the crime scene. He was pardoned after spending nine years in prison.

CASE STUDIES-II

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.

CHAPTER –VII

KOOL-AID 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 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 10µL into separate wells on** the gel. Load each Kool-Aid sample only once. The first line on the yellow pipet **tip is 10µL.**

TIPS:-

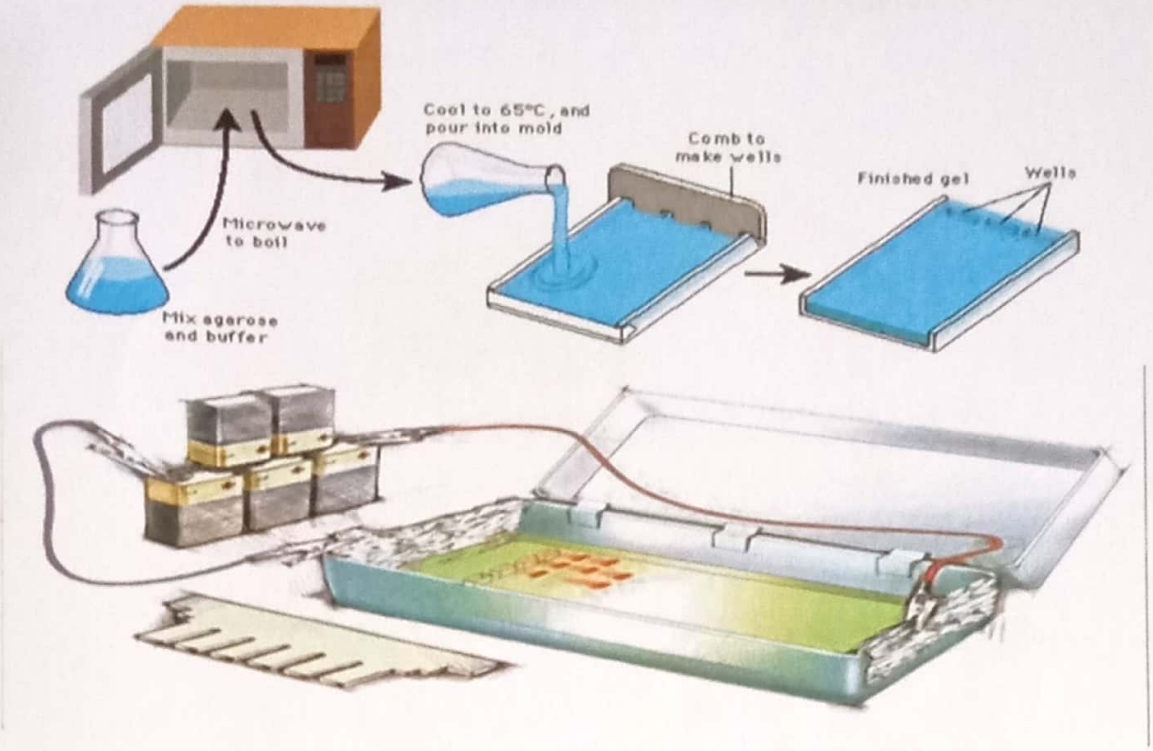
- Steady syringe over well using two hands

- 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.

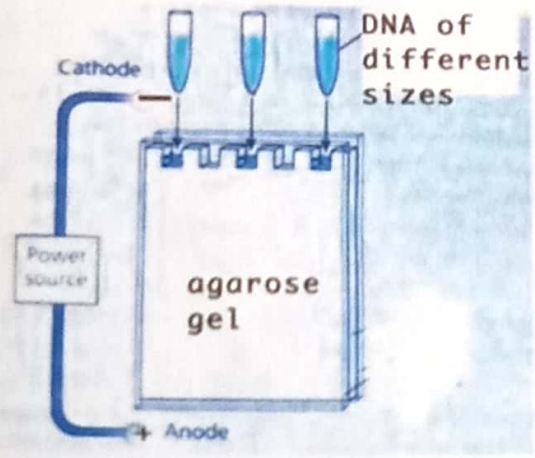
- **Be careful not to punch tip of the pipet through bottom of gel.**

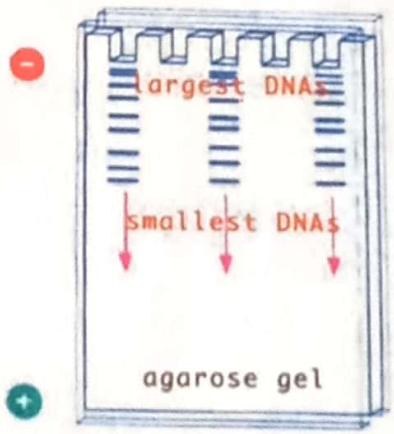
DNA Gel Electrophoresis Technique



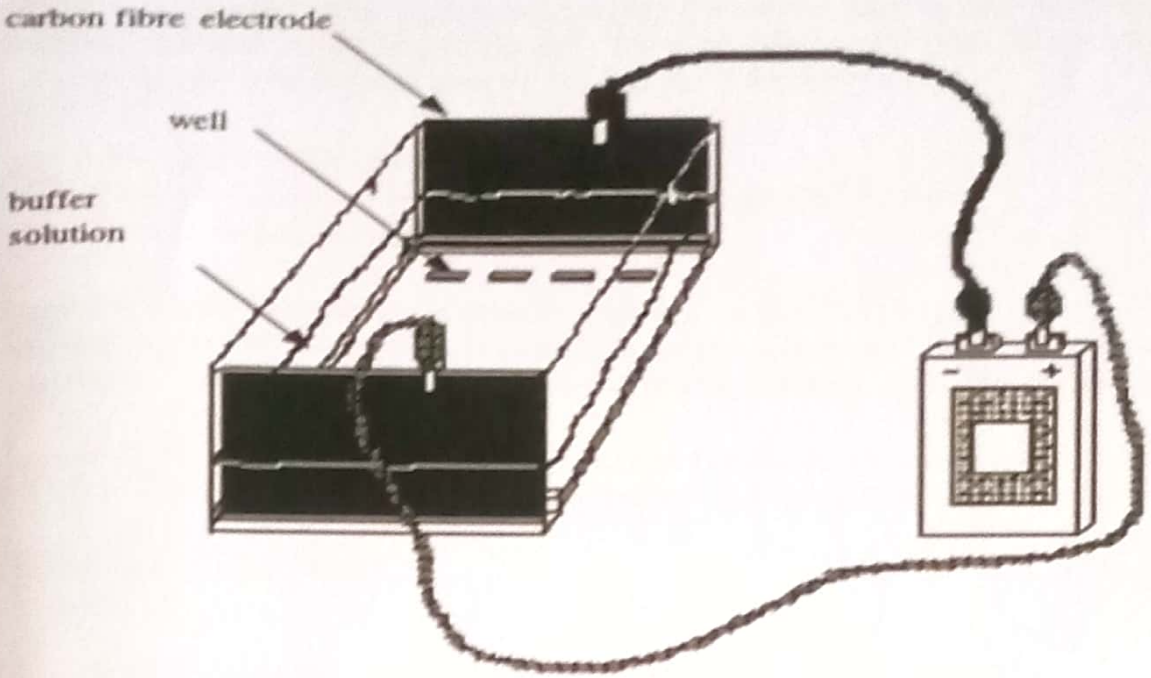
The term "gel" in this instance refers to the matrix used to contain, then separate the target molecules. In most cases, the gel is a cross linked polymer whose composition and porosity is chosen based on the specific weight and composition of the target to be analyzed.

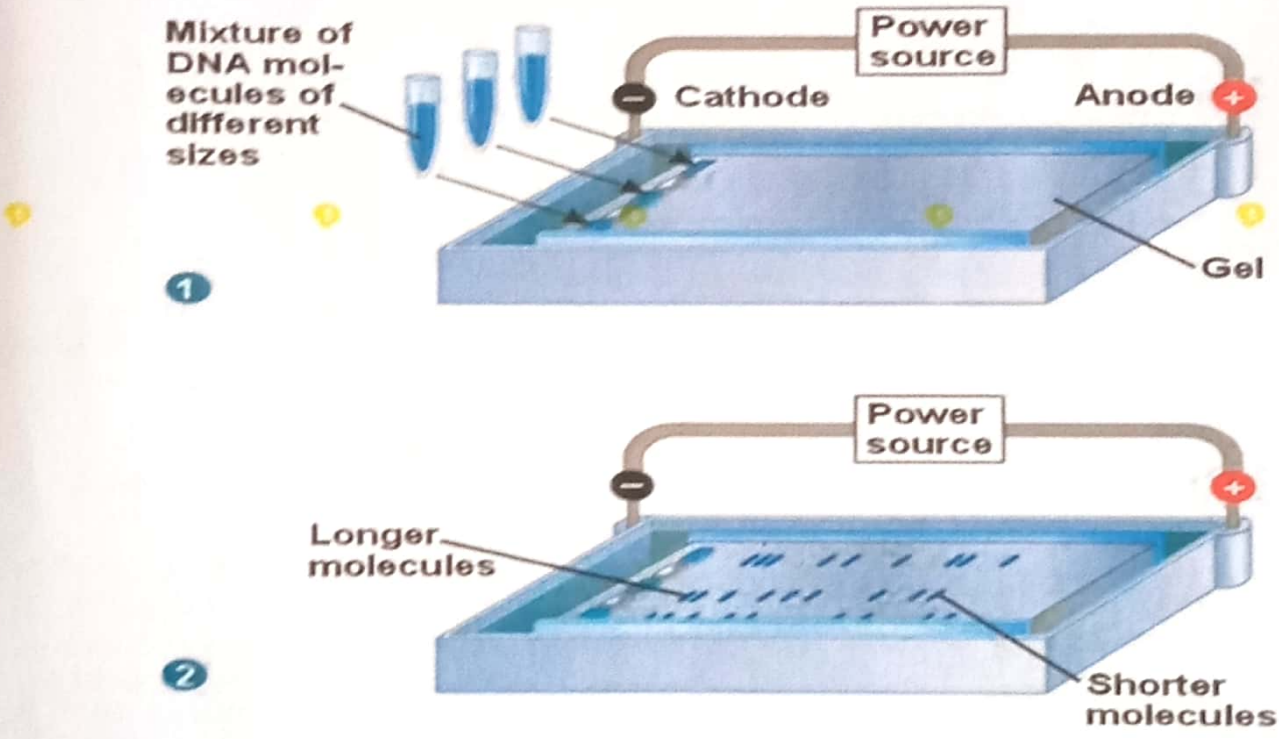
Agarose gels are easily cast and handled compared to other matrices, because the gel setting is a physical rather than chemical change. Samples are also easily recovered. After the experiment is finished, the resulting gel can be stored in a plastic bag in a refrigerator





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

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

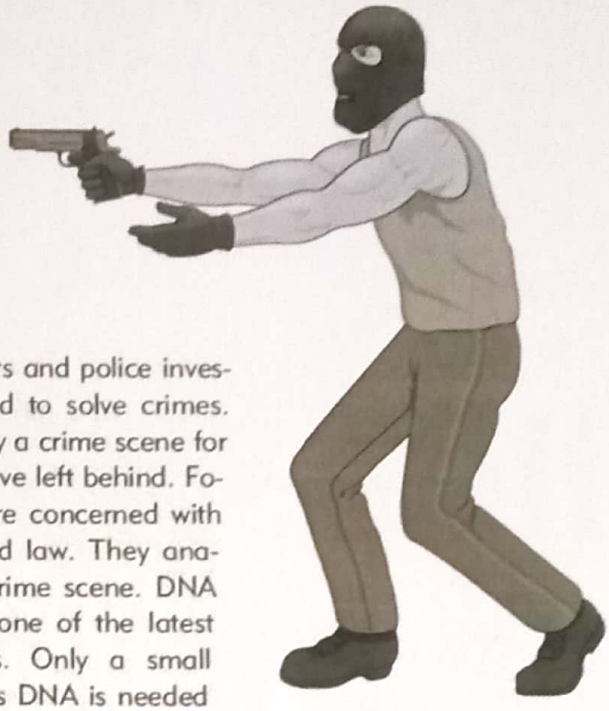
Preparing the agarose gel

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.

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.

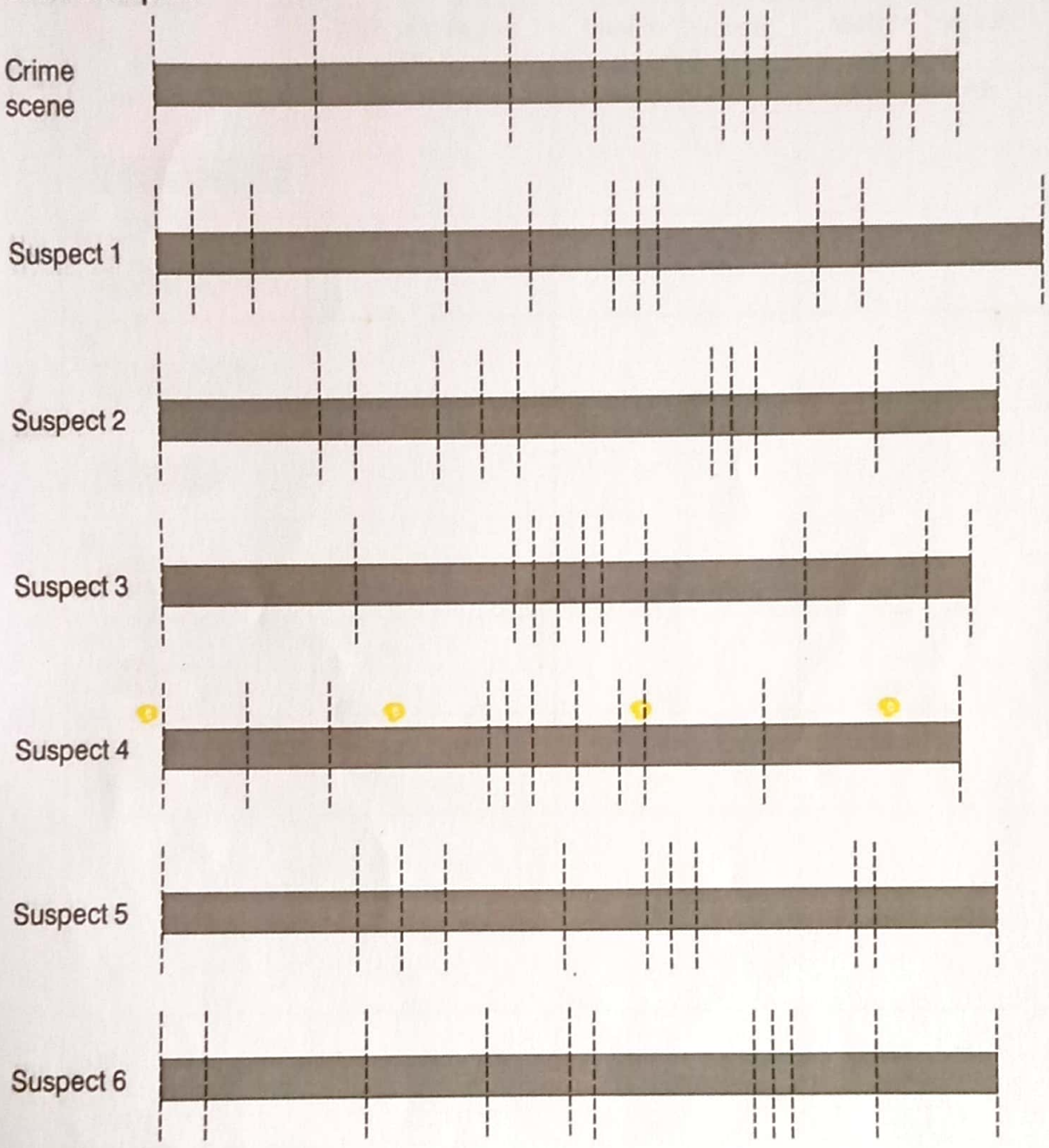
CHAPTER –VIII

MODELS OF DNA SAMPLE

Models of DNA Samples

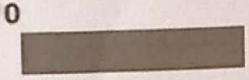
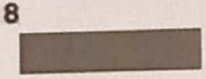
Dotted lines represent locations where restriction enzymes will cut these pieces of DNA.

DNA Samples



ELECTROPHORESIS

NEGATIVE ELECTRODE

Sample Sizes	Well to Deposit DNA Suspect #1	Well to Deposit DNA Suspect #2	Well to Deposit DNA 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

CHAPTER –IX

CONCLUSION

CONCLUSION

Twenty years after the development of DNA fingerprinting, DNA analysis remains the key to linking suspects to biological evidence and to identifying individuals in crimes and disasters. Another important use is the establishment of paternity in custody and child support litigation.

DNA fingerprinting is a laboratory technique used to determine the probable identity of a person based on the nucleotide sequences of certain regions of human DNA that are unique to individuals.

DNA fingerprinting plays an important role in medicine. It is used to match the tissue of organ or marrow donors with transplant patients, to identify hereditary health conditions, and to help find cures for those conditions.

DNA fingerprinting, one of the great discoveries of the late 20th century, has revolutionized forensic investigations. This review briefly recapitulates 30 years of progress in forensic DNA analysis which helps to convict criminals, exonerate the wrongly accused, and identify victims of crime, disasters, and war.

In the future, we may go much further than just comparing evidence from a crime scene to a known suspect. Instead, we may use DNA from crime scenes to create descriptions of potential suspects or unidentified victims from scratch via a method called DNA phenotyping.

CHAPTER –X

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