Name _____ **Instructor** _____ **Lab Section**_____

Objectives: To gain a better understanding of:
- Fundamental Biotechnology Techniques
- DNA profiling technique
- Gel Electrophoresis Technique

Background material may be found in
- Chapter: 10.2
- Chapter: 12.11-12.16

Biology: Concepts and Connections, 8th ed.

Biotechnology is a term that applies to a range of biological techniques that use molecules and cells to make medicines, foods and other products that are useful to humans. In 1953, James Watson and Francis Crick, with help from Rosalind Franklin and other scientists, published their discovery of the DNA (deoxyribonucleic acid) double helix and the first accurate description of the fundamental structure of DNA. In less than 50 years, science leapt from the discovery that DNA contains genetic information to the ability to effectively manipulate the size or behavior of laboratory animals by altering their genetic makeup. Scientists and doctors are now applying human gene therapies in hopes of curing cancer and treating AIDS. The criminal justice system is now using DNA profiling to identify criminals in cases of rape and murder. It is the universality of the genetic code in organisms as diverse as viruses, bacteria, plants, and animals that provide some of the most convincing evidence supporting the theory of evolution. The universal nature of DNA has also allowed information obtained from studies of organisms such as yeast and fruit flies to be applied to humans. In the following exercises you will employ some of the most basic molecular techniques used in laboratories around the world.

DNA PROFILING

DNA profiling (previously referred to as DNA fingerprinting) is a technique used to analyze DNA from human tissue samples for use in criminal forensics, missing persons identification, and even paternity testing. Sources of DNA from a crime scene typically include blood, hair (with a follicle containing cells), skin, semen and saliva (containing cheek cells). Even a small amount of tissue can yield enough DNA to generate a profile. DNA profiling evidence can be used to establish that a suspect was present at a crime scene or in some cases to exonerate a suspect.

The DNA profiling technique is based on the fact that there are **hypervariable** regions (or loci) in the non-coding areas of the human genome which vary greatly between individuals. In other words, no two individuals have the same nucleotide sequences in all of the hypervariable regions in the genome (except identical twins!), so a person's DNA profile will be unique to them. So by comparing the sequences at selected hypervariable regions it can be determined whether a suspect's DNA matches that from a tissue sample at a crime scene, for instance. And a biological parent (or sibling) would have 50% matching sequences to an individual, which can be assessed for missing persons identification or paternity testing.

The current standard DNA profiling technique uses 22 specific **STR** (**short tandem repeat) loci**, hypervariable regions in the human genome which have sufficient variation in the population to provide useful evidence in a court of law. In other words, the sequences found in an individual's DNA at these particular chromosome locations (loci) vary enough from others in the population that when the full set of 22 sequences (STRs) are compared to an "unknown sample", scientists can confidently determine if it is a "match" to the particular individual, or to a close relative. The Department of Justice (DOJ) forensics labs use this technique with 22 known STR loci, for which the FBI has determined the population frequencies. In addition, the DOJ maintains databases such as CODIS (Combined DNA Index System), of DNA profiles from convicted felons (and arrestees), which can be searched when there are no known suspects. Another section of CODIS contains profiles from family members of missing persons and unidentified remains.

STRs (short tandem repeats) are commonly repeated sequences of 4-8 base pairs in length, such as "GATA GATA GATA GATA GATA". This STR has 5 copies of the "GATA" sequence, which might occur at a 30% frequency in the population at a very specific region of the human genome. Since we all have two of each type of chromosome, one from each parent, an individual could have the above 5-repeat on one chromosome, and let's say a 6-repeat (40% frequency) of GATA, on the other chromosome. This STR locus would thus read as a (5,6) after analysis.
All 22 loci are determined similarly, giving an individual DNA sample (from a person, a crime scene, or a deceased body, for instance) 22 pairs of loci data which collectively would make a unique identification: a DNA profile. For instance the partial sample below would so far read: STR1 (5,6); STR2 (8,6).

STR1 ….GATA GATA GATA GATA GATA…. (5)
….GATA GATA GATA GATA GATA GATA…. (6)
STR2 ….CCAA CCAA CCAA CCAA CCAA CCAA CCAA CCAA…. (8)
….CCAA CCAA CCAA CCAA CCAA CCAA…. (6)

A sample of DNA that matches all 22 pairs of STRs with another sample could only come from the same person or identical twins, with a statistical confidence level of on the order of 1 in 500 million to 1 in a quadrillion, for example. This makes it extremely unlikely to be a match by chance. If even just one of the STR data pairs is not an identical match, it would be determined that they are not from the same individual.
For further reading on this topic, visit the National Institute of Justice:
https://www.nij.gov/topics/forensics/evidence/dna/pages/welcome.aspx

The Golden State Killer (GSK) in California was identified and located in 2018, forty-two years after his last known crime (in 1986), by using the DNA profile from the crime scene samples and comparing them to DNA profiles on an open source genealogy website (public DNA database). Although his DNA was not found on the site, it was a significant match to some individuals who were identified as distant cousins. Building the family tree backwards and then down again through 25 family lineages allowed researchers (experts in genealogy) to identify the most likely relatives to have been alive and in the correct age range and location during the GSK's 10-year crime spree. As of May 2018, the suspected GSK is in custody. He is suspected of committing ten murders and dozens of rapes throughout California (including 4 homicides in Goleta).

When specific STRs in a DNA sample are amplified, or copied, by a very common technique called **PCR (polymerase chain reaction),** these hyper-variable regions yield millions of copies of DNA fragments of various lengths. The DNA fragments can be fractionated (separated), on the basis of size, by **electrophoresis** through an agarose gel, as you will do today, or by capillary electrophoresis, more commonly used in forensics labs. Typically, prepared DNA is loaded on to a gel and an electric current is applied to the gel. DNA has a negative charge and therefore migrates to the positive pole (cathode) of the electric field. The speed of migration of DNA through an agarose gel depends on the size of the DNA fragment. Small pieces of DNA are able to move easily through the molecular pores of the agarose gel, and therefore move faster (farther) than larger pieces. Electrophoresis of a DNA sample containing various sized DNA fragments will yield a "ladder" of bands on the gel; each band representing a multitude of DNA fragments, all of the same length. Bands toward the top of the gel contain large fragments, while bands toward the bottom of the gel contain small fragments. **The banding pattern of DNA fragments is highly unique between individuals.** A comparison of banding patterns between suspects, victims and tissue found at the scene of the crime can often establish innocence or guilt. The following experiment is a simulation of DNA profiling associated with various crime scenes.

PROCEDURE

You will be asked to pour an agarose gel, load that gel with DNA samples derived from the "crime scene", size fractionate those samples by electrophoresis, stain the DNA, then view your results. You will also be given a crime scene that describes the origin of each sample and tells you the order in which the samples should be loaded. If you do good work then you should be able to determine "who done it" and who was innocent. A detailed description of the procedures is listed on the following pages.

AGAROSE GEL ELECTROPHORESIS PROCEDURE

- **POURING THE AGAROSE GEL**

1. Set up the electrophoresis gel box according to the directions provided by your instructor. Insert a comb into the end slots of the box as demonstrated by your instructor. Put the gel casting tray onto a paper towel in case the gel box leaks.

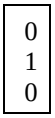
2. Melt agarose in the microwave, and then have your instructor add SYBR dye to the liquid in your flask.

3. Slowly pour the liquid agarose with SYBR evenly into the casting tray until the level is even with the top of the plastic screws, and try to avoid introducing air bubbles into the gel.

4. Allow the gel to solidify without moving or jarring the casting tray (this will take about 15 minutes).

- **PREPARING THE DNA SAMPLES FOR ELECTROPHORESIS**

1. From the ice trays on the side counter, obtain one tube each of DNA samples A-F

2. **Be sure your p20 micropipettor is set to 1 µL, which would read as**  **when set properly.**

Add 1 µL of loading dye to each tube. The loading dye will allow you to visualize the solution and more easily add it to the wells in the agarose gel.

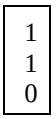
3. Spin all of the tubes in the microcentrifuge for 1-2 seconds in order to deliver the dye to the sample.

- **LOADING AND RUNNING THE AGAROSE GEL**

1. Once the agarose gel has solidified, carefully pull the comb straight out of the gel. This will leave empty "slots" or wells in the gel.

2. Place the tray onto its platform in the gel box. **The wells must be located at the anode end (black lead; (-) end).**

3. Fill the electrophoresis box with electrophoresis buffer (1X TBE buffer) until the wells are covered (about 300 mL). The level of the buffer should be only a few millimeters above the surface of the gel.

4. Ask the instructor for your "crime scene". You will be assigned a crime scene that describes the nature of the crime and the origin of the DNA samples. The crime scene will dictate the order in which you load the DNA samples.
   Be sure your p20 micropipettor is set to 11 µL, which reads as  **.**

   Load 11 µL of each sample in the correct order, from left to right. You should only put one sample in each well. For ease in reading the results, do not skip wells between samples.

CRIME SCENE #1 : LOAD YOUR SAMPLES FROM LEFT TO RIGHT IN THIS ORDER B-F-C-A-E-D

CRIME SCENE #2 : LOAD YOUR SAMPLES FROM LEFT TO RIGHT IN THIS ORDER D-B-F-C-A-E

CRIME SCENE #3 : LOAD YOUR SAMPLES FROM LEFT TO RIGHT IN THIS ORDER A-D-F-B-E

5. Place the lid onto the gel box and connect the electrical leads to a channel of the power supply, (red to red and black to black).

6. Turn on the power supply and set it for about 100V.

7. Allow electrophoresis to run for **45-50 minutes**.

8. Turn off the power supply and disconnect the leads.

- **VIEWING AND DOCUMENTING THE GELS**

1. **Put on a pair of latex gloves** and carefully lift the casting tray out of the gel box.

2. Carefully slide the gel into its labeled weigh boat and bring your weigh boat with gel to your instructor at the back of the room. Your instructor will transfer your gel to the surface of the UV transilluminator. When the lid is closed, the transilluminator will pass UV light through the gel and the SYBR-coated DNA fragments will fluoresce, allowing you to see the DNA bands.

SYBR is a fluorescent dye that binds to and stains DNA. When a SYBR-stained gel is placed on the surface of a UV transilluminator, glowing "bands" of DNA can be seen.

3. **Observe your gel on the UV transilluminator.** Make sure that the lid on the illuminator is down to protect your eyes from UV rays. Do you see glowing bands?

4. **Making sure to wear latex gloves, your instructor will dispose of your gel** in the plastic box labeled **GEL WASTE ONLY**.

FORENSIC ANALYSIS OF DNA FINGERPRINTING DATA

CRIME SCENE #1

Harry Nukkels was murdered in his own home. Harry's 95-year-old mother, Agnes, was witness to the crime, but can't remember much. The usual suspects were rounded up. Grief-stricken though she was, Agnes agreed to inspect the lineup of suspects. Agnes identified two men as "looking familiar".
DNA samples were obtained from the following sources:

1. suspect #1 (sample)
2. suspect #2 (sample)
3. Harry Nukkels (sample)
4. Blood from the shirt of suspect #1 (sample)
5. Blood from the scene of the crime (sample)
6. Hair from the scene of the crime (sample)

The DNA samples were digested by the crime lab. Electrophoresis of DNA samples was performed by you and your classmates.

- Your instructor will distribute photocopies of images of the gels so that each group will receive a copy.
- Using your powers of observation and logic, analyze the banding patterns on the gel and determine "who done it". The DNA profiles shown on the gel represent the samples listed above, with numbers 1 through 6 appearing from left to right across the gel.

QUESTION

Crime Scene Analysis:

Which, if any, of the suspects is implicated in the crime? Provide a rationale for your answer.

FORENSIC ANALYSIS OF DNA FINGERPRINTING DATA

CRIME SCENE #2

Lucy Lovelace and Stan Studley died in a sleazy low rent motel, the Shangrila, off highway 126. The cause of death in both cases was a close-range gunshot to the head. There were signs of a scuffle. The immediate evidence suggested a lover's quarrel gone bad. The cops figured that Stan, known to be a violent man, shot Lucy in a fit of rage then took his own life in despair. Lucy's husband, a powerful movie producer, claimed to know nothing of the love affair or the murder. He showed no sympathy for his slain wife, and in fact was heard muttering to himself, "she had it coming".
DNA samples were obtained from the following sources:

1. Lucy Lovelace (sample)
2. Stan Studley (sample)
3. The husband (sample)
4. Hair from the scene of the crime (sample)
5. Blood from the scene of the crime (sample)
6. Skin taken from under Lucy's fingernails (sample)

The DNA samples were digested by the crime lab. Electrophoresis of DNA samples was performed by you and your classmates.

- Your instructor will distribute photocopies of images of the gels so that each group will receive a copy.
- Using your powers of observation and logic, analyze the banding patterns on the gel and determine "who done it". The DNA profiles shown on the gel represent the samples listed above, with numbers 1 through 6 appearing from left to right across the gel.

QUESTION

Crime Scene Analysis:

Which, if any, of the suspects is implicated in the crime? Provide a rationale for your answer.

FORENSIC ANALYSIS OF DNA FINGERPRINTING DATA

CRIME SCENE #3

John Doe, a wealthy community college teacher, was murdered May 5th 1985. A student of Doe's, Jeffrey Wyner, was accused of the crime and convicted. Evidence for the conviction was slim, but compelling. The week before the crime, Wyner had argued with Mr. Doe about his poor grades. Also, Wyner had been seen going into Doe's house the day of the crime. Finally, a spot of blood was found next to the victim's body. Analysis of the proteins in the blood revealed a rare blood type, AB^-. John Doe's blood type was O+, but Jeffrey Wyner's blood type is AB^-. Wyner claimed to be innocent, but the jury thought otherwise. Wyner was sent to the state penitentiary to serve out a life sentence.
In 1999, Sam Snooper, a student at Santa Barbara Law School, was studying cases when he came upon Wyner's case. Sam thought the blood type evidence was weak, at best, and reasoned that DNA evidence could possibly overturn the case. The Santa Barbara County forensic lab still had the crime scene blood samples used in the Wyner case. They agreed to perform a DNA fingerprint on all the crime scene samples.
DNA samples were obtained from the following sources:

1. John Doe
2. Jeffrey Wyner
3. Mrs. Doe
4. Crime scene blood sample #1 (O+ blood type)
5. Crime scene blood sample #2 (AB^- blood type)

The DNA samples were digested by the crime lab. Electrophoresis of DNA samples was performed by you and your classmates.

- Your instructor will distribute photocopies of images of the gels so that each group will receive a copy.
- Using your powers of observation and logic, analyze the banding patterns on the gel and determine "who done it". The DNA profiles shown on the gel represent the samples listed above, with numbers 1 through 5 appearing from left to right across the gel.

QUESTION

Crime Scene Analysis:

Does the DNA evidence support or refute the evidence used to convict Jeffrey Wyner? Provide a rationale for your answer.

QUESTIONS

1. What are short tandem repeats (STRs), and *why* are they used in DNA profiling?

2. What is the role of polymerase chain reaction (PCR) in DNA profiling?

3. Why does DNA move through the agarose gel during electrophoresis?

4. How does the size of a DNA fragment affect the rate at which it travels through an agarose gel?

5. What is the role of SYBR stain in gel electrophoresis of DNA?

6. What are some of the sources of DNA that can be found at the scene of a crime?

Clean up instructions are on the NEXT PAGE.

Clean-up:

_____ Recycle used TBE buffer (bottles on counter by stove). Use funnels provided and set bottle in the sink when pouring.

_____ Rinse and dry gel box, lid, casting tray and comb and place on tray on student table.

_____ Turn off and unplug power supply. Do not remove leads from power supply.

_____ Return tube with loading dye and test tube rack to back counter.

_____ Place used micro centrifuge tubes labeled A-F in tip waste container on your table.

_____ Return all supplies to correct trays on the back counter.

LABORATORY NOTES