DNA Fingerprinting
Student Guide

Background

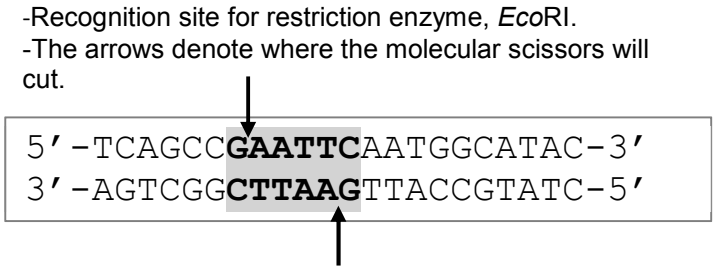
Forensic science is the application of scientific methods to collect, preserve and analyse data in order to investigate past events. Forensic science is used in many diverse areas of science such as antrhropology, archeology, pathology and botany to name a few. For example forensic science techniques are used to investigate remains in an archeological site or to determine ancestry. Forensic science techniques are commonly used in criminal, civil, public health, and conservation investigations.

DNA fingerprinting or profiling is a branch of forensic science that focuses on the use of DNA material for investigation. Deoxyribonucleic acid (DNA) is a double stranded molecule made of four types of nucleotide bases (A,T,C,G). Bases on the two strands are always paired up, A paired to T, and C paired to G. The human genome consists of approximately 3 billion base pairs on 23 pairs of chromosomes. Only about 0.1% of the 3 billion base sequence is different among the genome of humans. Except for identical twins, no two individuals have the exact same DNA sequence. Most variations do not affect the functions of the genes and occur in non-coding regions. The variations have occurred through mutations, single nucleotide polymorphisms or copy number variations of short DNA segment repeats. Forensic scientists use these variable regions of the genome to generate a DNA profile unique for each individual. In criminal cases, the DNA is often collected from the crime scene in form of blood, tissue, hair or other body products. The profile of the collected DNA is compared to the profile of DNA from individual suspects to find a match. DNA profiling also known as DNA fingerprinting is a powerful tool in forensic investigations.

Restriction endonucleases

Many of the revolutionary changes that have occurred in the field of biotechnology can be attributed directly to the ability to manipulate DNA in defined ways. The principal tools for this recombinant DNA technology are enzymes that can cut, mend, wind, unwind, transcribe, and replicate DNA. Restriction enzymes are the "chemical scissors" of the molecular biologist; these enzymes are powerful tools used to cut DNA at specific sites. Restriction enzymes were first discovered by a swiss scientist named Werner Arber who received the nobel prize in physiology and medicine in 1978 (Arber and Linn 1969). Arber noticed that bacteria cells produce restriction enzymes to protect themselves from invading bacteriophages. Similar to viruses, bacteriophages enter bacteria cells and propagate inside. Restriction enzymes are endonucleases produced by the bacteria to cut the bacteriophage DNA into pieces and destroy it. Since Arber's first discovery, scientist have discovered approximately 3,000 different restriction enzymes in different bacteria. Restriction enzymes may cut double stranded DNA at non-specific and specific nucleotide sequence. The most useful type for molecular biologists cut at specific sequences. Most (but not all) restriction enzyme recognition sites that are 4-6 nucleotides long and are palindromic meaning that the sequence reads the same on both strands of DNA 5' to 3'. For example the recognition sequence for the EcoRI enzyme is 5'GAATTC 3' on one strand and 3'CTTAAG5' on the complementary strand. One of the first applications of restriction enzymes was demonstrated by a biochemist named Daniel Nathan and his graduate student in 1971 (Danna and Nathans 1971). In order to study the structure of a cancer promoting virus, Nathan and his student used restriction enzymes to cut the viral DNA. They then used the gel electrophoresis technique to separate the resulting fragments by their size. Another powerful application of restriction enzymes is their use in the recombinant DNA technology where restriction enzymes are used to cut DNA at specific sites and the resulting DNA fragments from different sources are recombined to produce a new DNA.

Figure 1: Molecular cutting of DNA



Restriction Fragment Length Polymorphism (RFLP)
RFLP refers to the difference in the lengths of DNA fragments produced after digestion by restriction enzymes. RFLPs are often used to study variations among the genome of different individuals. DNA is cut into fragments by restriction enzymes. The fragments are then separated by gel electrophoresis. Nucleotide sequence differences among individuals can create or eliminate restriction enzyme recognition sequences at specific sites therefore creating a unique fragment patten for each individual.

There are different ways to perform RFLP analysis depending on the size and complexity of the organism's genome.
The simplest way, on a small genome, is to obtain purified DNA and digest with different restriction enzymes. The enzymes give an different pattern or polymorphism when run on a gel electrophoresis. This is the type of RFLP we will perform in this experiement.

Another type, uses a technique called polymerase chain reaction (PCR) to amplify a specific portion of the DNA with known polymorphism. Amplification of DNA is needed to have larger amounts of the DNA available for further manipulations and easy visualization of the DNA fragments. The amount of DNA recovered from a study site is often too small to visualize. The resulting fragments are separated by gel electrophoresis. The polymorphism between the DNA fragment patterns observed on the gel are then studied. Another technique combines PCR with restriction enzyme digestion.

For large genomes, the separated DNA fragments are transferred from the gel to a membrane (a technique known as southern blotting). Small single stranded pieces of DNA known as probes are designed to bind to specific sequences of DNA (Narayanan 1991). The probe is labeled with radioactivity or fluorecense therefore marking the DNA fragments it binds to and making them visible. A polymorphism between the size of the fragments that are recognized by the probe is then analysed.

In forensic DNA technology, analysis of the pattern of DNA fragments, loosely called a DNA fingerprint, enables us to discriminate between suspects accused of a crime. The variation in DNA from one person to the next is so great that the probability of two people sharing the same DNA fingerprint pattern is essentially zero. Unlike conventional fingerprints, which are often difficult to gather at a crime scene or under other circumstances, a DNA fingerprint can be made from a very small sample of blood, skin or semen -- or even a single hair!

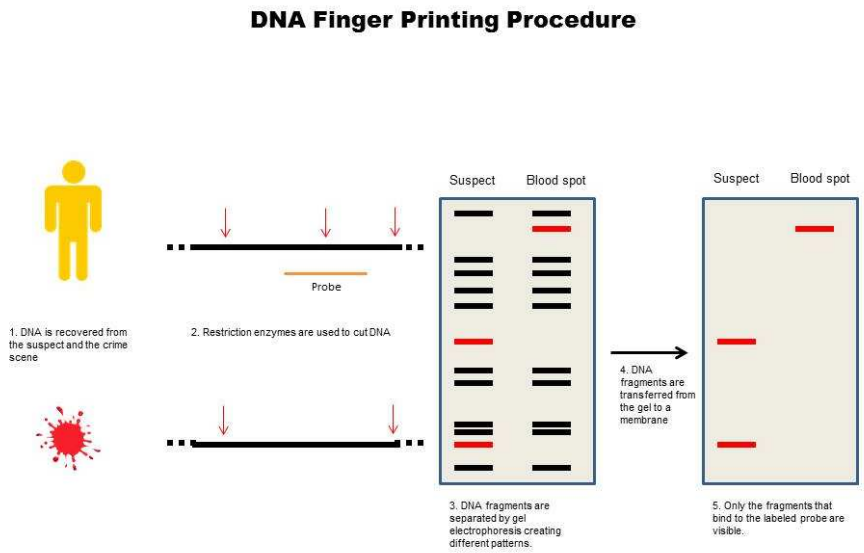


Figure 2: RFLP procedure
DNA from the crime scene and the suspect are cut with restriction enzymes. Because of the nucleotide polymorphism, the numbers and sizes of the DNA fragments are different among individuals. The fragments are separated by gel electrophoresis. The DNA is then transferred to a membrane, incubated with a marker or probe that binds specifically to the polymorphic region. The DNA fragments that bind to the probe are visualized and their patterns are compared. In this example does the blood come from the suspect?

Crime Scenario: The Case of the Missing Chocolate Zucchini Cake

It was the last day biology and Ms. Lopez brought chocolate zuchinni cake (which believe it or not, tastes just like brownies) for all the students to celebrate! She left it on her desk in the prep room. When the class was ready for the celebratory cake, the teacher found that the cake had vanished! Where had the cake gone? All the students denied eating any bit of the cake before it was time.

In search of the culprit, Ms. Lopez found some clues in the form of dark hair left on the table and Joanna, the crime scene technician, collected it for the class to extract the hair for DNA in CSI fashion. Ms. Lopez suggested that each of the suspects, who each arrived late to class, Jack, Tenzin, and Sienna, contribute a hair for DNA extraction. Can the biology students solve this mystery? Who does this hair belong to?

SUSPECTS	**Jack, age 14**	**Tenzin, age 14**	**Sienna, age 14**
PROBABLE CAUSE?	Jack was seen salivating over the cake before the morning class started. He loves healthy desserts.	Tenzin won a cake eating contest at the age of 6 where she downed a whole 8-inch cake in less than 5 minutes!	Sienna is a vegetarian. Any excuse to eat vegetables is a good enough excuse for her.
MOTIVE	Jack is a dedicated student and athlete (he has to rush off to practice before class ends) and he's worried that he won't get a taste of the cake before it's all gone. He had lamented this concern to Ms.Lopez.	The lone cake sitting on the table tempted Tenzin. It took her back to the good old days of cake eating contests. She nostalgically commented about her wonderful past to her friends and peers.	Sienna tries to get her 5 servings of vegetables everyday. This cake happened to have 2 cups of zuchinni, a great way to get that vegetable serving in!
ALIBI	As said, Jack is busy. He was seen running to his locker before class started to get his gym bag.	Tenzin was last seen obtaining lab materials for the class. She is a lab aide for Ms. Lopez and was obtaining microfuge tubes from the teacher next door.	Sienna was last seen asking Ms. Lopez about the ingredients of the cake. She did some mental math to determine that the zucchini cake has 2 whole servings of vegetables.

Have the students read the information out loud and ask them to assign the suspects with the names. Ask the students:

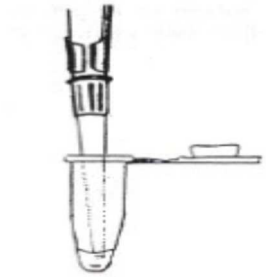
1. What is the crime scene DNA?
2. What is the source of each of the suspect DNA?

Laboratory Exercise

In this laboratory exercise you will perform a simulated and modified version of RFLP analysis. You will use a scenario your teachers gives you or make up your own scenario for your lab exercise. You will compare DNA finger print patterns from different individuals in order to solve the case.

Student Learning Objectives, after this lesson, students will

1. Be introduced to forensics science
2. Learn about restriction enzyme and their action (digesting sequences of DNA)
3. Run and analyze results of electrophoresis experiment
4. Using results from the results, give a conclusion on who they think the suspect is.

<table>
<tr><th colspan="2">Important Laboratory Practices</th></tr>
<tr><td>Add reagents to the bottom of the reaction tube, not to its side.
Add each additional reagent directly into previously added reagent.
Do not pipet up and down, as this introduces error.
Make sure contents are all settled into the bottom of the tube and not on the side or cap of tube. A quick spin may be needed to bring contents down.
  </td><td>Pipet slowly to prevent contaminating the pipette barrel.
Change pipette tips between each delivery.
Change tip every time the pipette is used even if it is the same reagent being delivered between tubes!
  </td></tr>
<tr><td>Keep reagents on ice. Restriction enzymes are proteins and will loose their activity if left at room temperature for too long.
  </td><td>Check the box next to each step as you complete it.
  </td></tr>
</table>

Lab Activity: Pipetting the Reactions

Step 1: Obtain 5 tubes for each team. Label the tubes with your initials or a mark that you can recognize. Also label the tubes as follow:

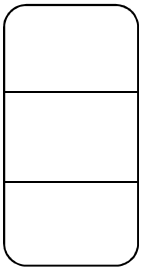
Tube label	What the tube stands for
U	Uncut control DNA
CS	Evidence or crime scene DNA
1	Suspect DNA 1
2	Suspect DNA 2
3	Suspect DNA 3

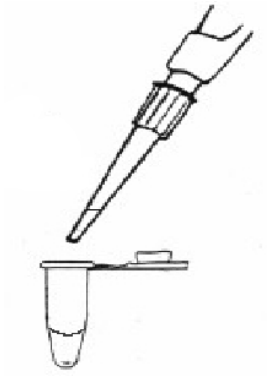
Step 2: Keep the working tubes in your rack, while the other reagents should be ON ICE.

Using a p20 add 12ul of 2X RB to each of your 5 tubes.
What should the dial on the P20 look like?

2X RB

12ul



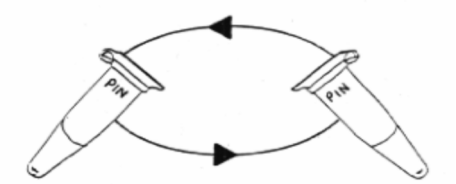
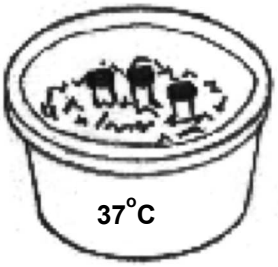
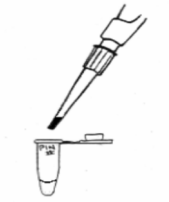
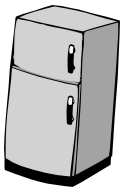


Step 3: Pipette reagents from the reagent stocks into your tubes. Make sure to check that you are pipetting the reagent(s) into the correct tube!

Cross out the reagents on the chart on the right as you add your reagents to keep track.

Repeat with a different set of reagents.

Tube→	U	CS	1	2	3
dH_2O	6ul				
"U"	4ul of "U"				
"CS"		4ul of "CS"			
Suspect 1 DNA			4ul of "S1"		
Suspect 2 DNA				4ul of "S2"	
Suspect 3 DNA					4ul of "S3"
Enzyme		6ul	6ul	6ul	6ul

Step 4: Close the caps of the tubes tightly. Mix the contents of the tubes by flicking lightly.	 ☐
Step 5: Balance the tubes in a microcentrifuge and bring the contents to the bottom of the tubes by spinning for 2-3 seconds.	 ☐
Step 6: Place your tubes in a 37°C water bath. **Incubation time:** Your teacher will tell you how long to incubate your samples.	 ☐
Step 7: Remove the tubes from the water bath. Add 2ul of loading buffer to each tube and mix by gently flicking the tubes. Note: The loading buffer stops the digests and is used for the gel electrophoresis step.	 ☐
Step 8: Keep the tubes on ice or store in the refrigerator until ready to load on a gel.	 ☐

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Electrophoresis

To determine the pattern of the DNA fingerprints and make a comparison, you will need to visualize the products of your restriction digest. This will be done using a process called **gel electrophoresis** in which electric current forces the migration of DNA fragments through a special gel material. Since DNA is negatively charged, it will migrate in an electric field towards the positive electrode. When electrophoresed through a gel, **shorter fragments of DNA move at a faster rate than longer ones** and will occur farther from the wells.

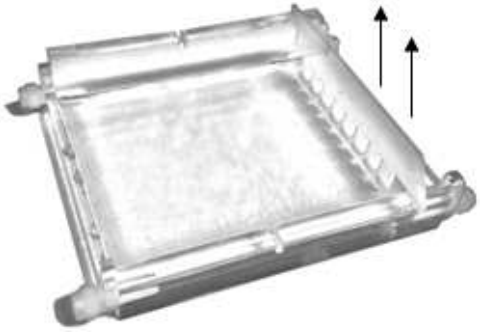
The gel material to be used for this experiment is called agarose, a gelatinous substance derived from a polysaccharide in red algae.

When agarose granules are placed in a buffer solution and heated to boiling temperatures, they dissolve and the solution becomes clear. A comb is placed in the casting tray to provide a mold for the gel. The agarose is allowed to cool slightly and is then poured into the casting tray. Within about 15 minutes, the agarose solidifies into an opaque gel having the look and feel of coconut Jell-O™. The gel, in its casting tray, is placed in a buffer chamber connected to a power supply and running buffer is poured into the chamber until the gel is completely submerged. The comb can then be withdrawn to form the wells into which your PCR sample will be loaded.

Objectives – student should be able to:

1. Safely pour an agarose gel
2. Use pipetting skills to load samples into gel wells
3. Run electricity through the unit to facilitate electrophoresis
4. Explain the concept behind electrophoresis

☐ 6. Once the gel has cooled completely, carefully remove the comb by lifting it straight up. **Lower (or remove) the barriers at either end of the tray.**



DO NOT THROW AWAY THE COMB!! Please return it to your teacher.

☐ 7. Place the tray in its place within the gel box, and pour in enough buffer so that the liquid level is about a centimeter above the surface of the gel tray. Wells should be oriented towards the negative (black) side of the gel box.

Your gel box may look like either of these, or something slightly different.

The red cable has the positive charge, while the black cable has the negative charge. Your DNA sample has a negative charge, so it will be traveling through the gel towards the positive charge as electrophoresis takes place.

☐ 8. Retrieve your PCR tube and check that all liquid is resting at the bottom of the tube. If not, give a quick spin in a balanced centrifuge (place within an adapter if the hole is too large).

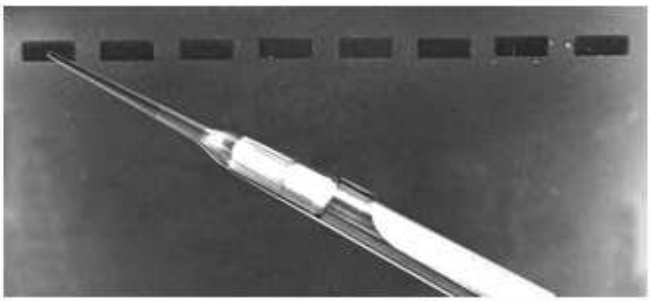
Be sure to keep your PCR tube **on ice** when not in use!

☐ 9. Add 5 uL of loading dye to the PCR tube. Flick gently to mix. If droplets move from the bottom of the tube, spin quickly in a balanced centrifuge to bring contents back down (using an adapter for the tiny tube).

☐ 10. On a piece of paper, draw out a map of the wells in the gel plate and write out what is going into each well (your sample, base pair ladder, another student's sample, positive/negative controls, etc).

11. The gel box should have enough buffer to be 1 centimeter above the gel tray **before** loading your samples. Following your written plan, carefully load **20 uL** of sample into each well. **Change tips between samples to avoid contamination.** Handle your pipette delicately, do not damage the gel by stabbing the sides or bottom of the well with the pipette tip, or your sample will not run properly. There should also be at least one base pair ladder loaded per gel in order to compare your sample – decide with your partner who will load it.

Your gel wells will look something like this, but they will be a bit harder to see. Look carefully before aiming your pipette tip.



Because the loading dye adds density, your 20 uL samples will be more dense than the buffer. They will sit nicely at the bottom of the well if loaded correctly.

12. When all samples are loaded, attach the electrodes from the gel box to the power supply, **red to red (+) and black to black (-)**. Have your teacher check your connections, then turn the power supply on and electrophorese your samples at 150 volts for 25-40 minutes.

Power supplies come in all different shapes and sizes. Your power supply may look like any of these, or slightly different...

But, among all power supplies, red connects with red and is positively charged. Black connects with black and is negatively charged.

Do not leave the room while a power supply is on. Be sure power supply is turned off and disconnected before removing gel box lid.

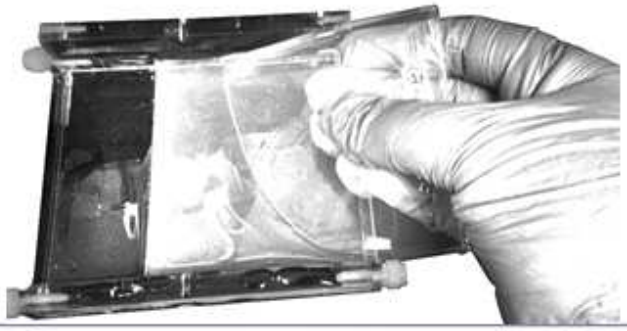
13. After electrophoresis, gels pre-cast with fluorescent dye will be ready to view and photograph using a UV light box.

Your UV box may or may not have a cover (like the one pictured) to protect your eyes and skin from the UV light. If your UV source does not have a safety cover, **protect your eyes and skin from the UV light using protective equipment** provided by your teacher.

☐ 14. Be sure that the power supply is turned off and disconnected. Then, gently remove the gel box lid. Put on gloves – the fluorescent stain pre-mixed into the agarose contains potentially carcinogenic materials – and remove the entire tray from the gel box. Place the tray on a clean paper towel. The used buffer in the box can be poured into **a hazardous materials collection** container when you are ready to clean up.

Running buffer can also be re-used – check with your teacher to see how it should be put away or disposed of for clean-up.

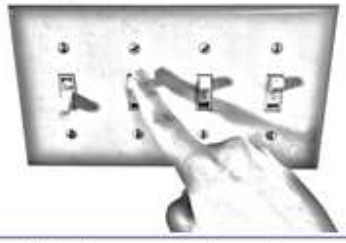
☐ 15. With your gloved hands, carefully slide the gel out of the gel tray (it should come out easily). Handle it gently so as not to break it. Place your gel on the UV light box for viewing.



Wear gloves! The fluorescent dye in the gel is potentially carcinogenic.

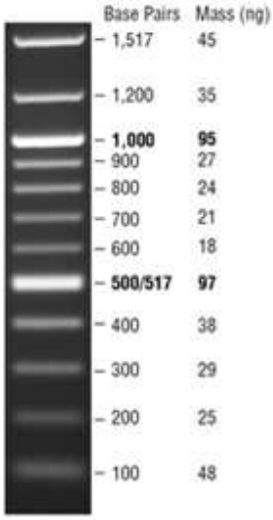
☐ 16. It helps to dim the room lights before taking a picture to reduce glare, or use a box with a hole in the top to cover the UV source to reduce glare, taking a picture through the hole. Have your partner take the picture and share it with you, or if you are taking the picture, remove your gloves first. **Don't contaminate your camera (or cell phone)!**

A dark room is better for gel pictures.



Always protect yourself from UV light – either using the plastic cover, or other personal protective equipment.

☐ 17. Record the base pair lengths at which you see bands for your samples. You can figure this out by comparing the sample with the lane containing your base pair ladder, and with the lane containing your positive (+) control.



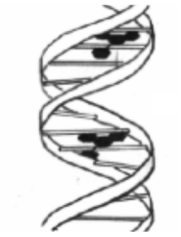
Because smaller pieces of DNA travel faster than larger pieces, the closer to the bottom of the gel the sample is, the smaller the number. The band at the very bottom of the ladder is 100 base pairs, then 200bp, and so on. You will notice that the 500bp and 1000bp bands are extra bright – this is to help you eyeball the number of base pairs easily.

Check your experiment protocol to see how many base pairs you can expect your sample to be.

Staining and Photographing Agarose Gels

If you are using EtBr (Gel Red and Gel Green works the same way)
The DNA fragments separated on your agarose gel are invisible to the naked eye. If you look at your gel in normal room light, you will not be able to see the DNA fragments. In order to "see" them, we must stain the gel with a fluorescent dye called ethidium bromide (EtBr). Molecules of ethidium bromide are flat and can intercalate, or insert, between adjacent base pairs of double stranded DNA (Figure 3). When this interaction occurs, they take on a more ordered and regular configuration causing them to fluoresce under ultraviolet light (UV). Exposing the gel to UV light after staining, allows you to see bright, pinkish-orange bands where there is DNA (figure 4).

Ethidium bromide
molecules intercalated between
DNA base pairs.



DNA Finger Printing Results – what you might see on your gel…
Below is the pattern of DNA fragments you might see for each lane.

Figure 5. Representation of an agarose gel results.
Lane1 - Uncut DNA (Control)
Lane 2 - Crime scene or Evidence DNA
Lane 3 - DNA from source 1
Lane 4 - DNA from source 2
Lane 5 - DNA from source 3

Name:_____

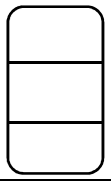
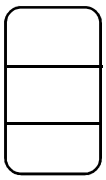
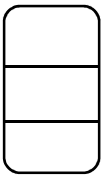
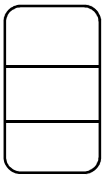
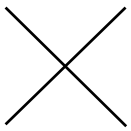
Date: _____Period_____

Student Worksheet - DNA fingerprint lab data table

This table outlines the reagents that you will be adding to each restriction enzyme reaction tube during the DNA fingerprint lab and the P-20 pipette settings.

1. Show what the dial will read to measure each of the volumes of liquid under the column P-20.

2. Calculate total volume in each tube by filling in the blanks in the row "Total Volume".

	P-20 Setting	**U**	**Crime scene/ Evidence**	**1**	**2**	**3**
2X Restriction Buffer		12ul	12ul	12ul	12ul	12ul
DNA		4ul "U"	4ul "CS/E"	4ul DNA Suspect 1	4ul DNA Suspect 2	4ul DNA Suspect 3
Enzyme		N/A	6ul	6ul	6ul	6ul
Sterile H2O		6ul	N/A	N/A	N/A	N/A
Total Volume		**_____ul**	**_____ul**	**_____ul**	**_____ul**	**_____ul**

3. What is DNA fingerprinting? Give 2 examples of how this method can be used.

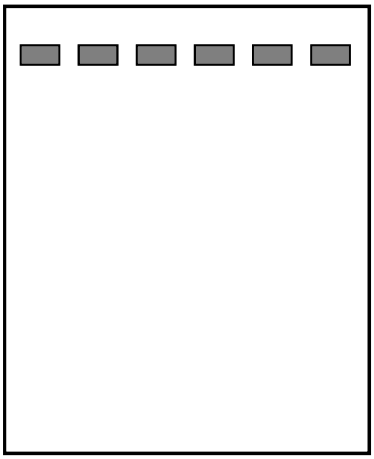
4. What other types of forensics is used in the past? How does DNA fingerprinting compared in terms of reliability and statistical significance?

Gel Analysis Questions

5. Based on the available scenario, can you predict culprit of the crime? Yes or No. Please explain.

Results:

6. Examine the photograph of your gel. Study the patterns of DNA fragments on your gel. Copy your banded pattern results from your picture to the figure below. Make sure to label your lanes appropriately.



7. Does your gel show any signs of improper laboratory technique? (i.e. blurry or smudged bands, crooked band fronts, multiple matching banding patterns of several lanes, multiple bands in the "U" lane, etc.) If yes, describe the possible errors in technique that was made.

8. Explain your results. Do you have a matching DNA?

Suspect DNA	Ruled out? Y/N	Possible match Y/N	Reasons
# 1			
# 2			
# 3			

9. Discuss some of the social or ethical issues that are raised by the application of the DNA fingerprinting technique. What are some ways this method may not be trusted?

10. In what other example would you like to see this technique used?