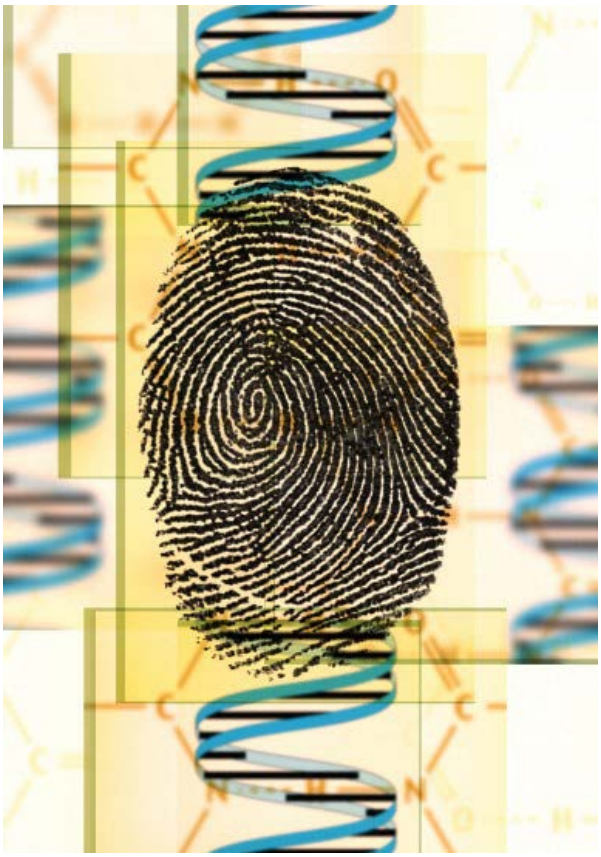


DNA FINGERPRINTING USING RESTRICTION ENZYMES

04/27/2018

Carlos E. Vasquez
General Biotechnology - BIOT 220

TABLE OF CONTENTS

I. ABSTRACT:

DNA fingerprinting is a genetic typing technique that allows the analysis of the genomic relatedness between samples, and the comparison of DNA patterns (Heras, 2016). DNA samples were collected from a crime scene and two suspects, using restriction enzymes (EcoRI, HindIII) the samples were cut and analyzed with agarose gel electrophoresis. A standard curve was generated and the molecular weight (size) of the fragments were calculated and analyzed.

II. INTRODUCTION:

1. Background:

DNA fingerprinting can be used to help identify individuals by revealing the differences in the DNA sequence among different people. Certain non-coding regions of DNA exhibit significant differences in base pair sequence among individuals. Such regions are called polymorphisms. Thus, you can compare the base pair sequence of a polymorphic region of DNA found at a crime scene with the base pair sequence of a polymorphic region of DNA obtained from the suspects. If the sequence of one of the suspects matches the sequence found at the crime scene, then you can be certain that the suspect was present at the crime scene. In order to carry-out this process four procedures must be followed.

1. Isolate genomic DNA from the crime scene and from the suspects.
2. Amplify a polymorphic region of the DNA using polymerase chain reaction (PCR).
3. Treat the DNA obtained from PCR with enzymes called restriction endonucleases.
4. Subject the fragments of DNA produced from the restriction endonucleases to agarose gel electrophoresis which will allow the visualization of the DNA fragments in order to determine their size.

Restriction endonucleases are enzymes that recognize a specific DNA sequence, called a restriction site, and cleave the DNA within or adjacent to that site. For example, the restriction endonuclease *Eco*RI, is isolated from the bacterium *E. coli*, recognizes the following sequence:

5’ GAATTC 3’
3’ CTTAAG 5’

The broken strand of DNA depicted here should line up such that the C and G are right across from each other as shown here:

5’ ----------G 3’ and 5’ AATTC---------- 3’
3’ ----------CTTAA 5’ 3’ G---------- 5’

The terminal produced by *Eco*RI, since they are complimentary at their single-stranded overhangs, are said to be cohesive or sticky.

A number of restriction enzymes have been isolated from a variety of microbial sources (Table 1.). Recognition sites for specific enzymes range in size from 4 to 13 bp, and, for most restriction enzymes used in gene cloning, are palindromes: sequences than read in the 5' -to- 3' direction on one strand are the same as those in the 5' -to- 3' direction on the opposite strand. Restriction enzyme activity is usually expressed in terms of units, in which one unit is the amount of enzyme that will cleave all specific sites in 1 µg of a particular DNA sample (usually λ DNA) in one hour at 37°C (Stephenson, 2010).

Many restriction enzymes require $Mg^{2+}$for activity and recognize palindromic stretches of DNA, generally 4-8 base pairs in length. In DNA, a palindrome is a sequence of nucleotides in each of the two strands that is identical when either is read in the same polarity, i.e. 5' to 3' (Glick, & Patten, 2017). The probability that a given enzyme will cut a piece of DNA is directly proportional to the length of its recognition site. Statistically, an enzyme will average one cut for every 4^n base pairs, where *n* is the length of the recognition site. For instance, an enzyme that recognizes a four base pairs long sequence will cut DNA once every 256 base pairs, while an enzyme that recognizes a six base pairs long site such as *Eco*RI, will cut once every 4096 base pairs. For example, if *Eco*RI is used to digest human chromosomal DNA containing 3 billion base pairs and a plasmid containing 5000 base pairs, it will cut the chromosomal DNA over 700,000 times, but may only cut the plasmid once.

TABLE 1. Restriction Enzymes Recognition Sites

Restriction Enzyme	Genus	Species	Strain	Recognition Site
*Ava*I	*Anabaena*	*variablis*	n/a	C^YCGUG
*Bgl*I	*Bacillus*	*globigii*	n/a	GCCNNNN^NGGC
*Eco*RI	*Escherichia*	*coli*	RY 13	G^AATTC
*Hae*III	*Haemophilus*	*aegyptius*	n/a	GG^CC
*Hind*III	*Haemophilus*	*influenzae*	Rd	A^AGCTT
*Sac*I	*Streptomyces*	*achromogenes*	n/a	GAGCT^C

EDVOTEK. (2016). DNA Fingerprinting Using Restriction Enzymes[Pamphlet]

Therefore, the frequency with which a particular restriction site occurs in any DNA depends on its base composition and the length of the recognition site. For example, in mammalian genomes, a G base follows a C in a much lower frequency than would be expected by chance. A restriction endonuclease such as *Nru*I, which recognizes the sequence TCGCGA, cuts mammalian DNA less frequently than it does DNA from bacterial sources having a more random distribution of bases.

To analyze the mixture of DNA fragments the agarose gel electrophoresis technique is the more adequate. This technique separates DNA fragments according to size using an electrical field. While electrophoresis is a powerful separation technique, it is not without its technical limitations. For example, unexpected bands may be present in the undigested DNA sample. A sample containing both supercoiled and linear DNA will show two distinct bands when analyzed by electrophoresis. Furthermore, if two different fragments are similar in size, they will migrate together through the gel and may appear as a single band. If digestion produces a broad distribution of DNA sizes, the fragments may appear as a smear. If two restriction enzyme sites are close to one another, the fragment may not be visualized by standard agarose gel electrophoresis protocols (EDVOTEK, 2016).

Together with gel electrophoresis the staining method is also important at the moment of analyze the results. The most convenient method to visualize DNA in agarose gels is staining with the fluorescent dye ethidium bromide. The planar group intercalates between the stacked bases of DNA. Ultraviolet radiation at 302 nm and 366 nm is absorbed by the bound dye and the energy is re-emitted at 590 nm in the red-orange region of the visible spectrum. Because the fluorescence of the bound dye is about 20 times greater than that of the unbound one, small amounts of DNA can be detected in the presence of free ethidium bromide in the gel. The detection limit with this staining is about 2ng of DNA. The main disadvantage of ethidium bromide is its toxicity and powerful mutagenicity.

2. Objective:

2.1. The objective of this experiment is to understand the use of restriction enzymes as applied to RFLP-based DNA fingerprinting in forensic analysis. Additionally, agarose gel electrophoresis will be used to analyze the results, which also will provide a better understanding about this technique.

III. MATERIALS:

Experiments components included in Edvo-Kit #225 from Edvotek, The Biotechnology Education Company, with Lot #: 22511281702.

1. 45 µl Crime scene DNA sample, pre-cut with Restriction Enzyme 1
2. 45 µl Crime scene DNA sample, pre-cut with Restriction Enzyme 2
3. 35 µl Suspect # 1 DNA sample
4. 35 µl Suspect # 2 DNA sample
5. 45 µl Enzyme reaction buffer
6. 120 µl Enzyme grade water

7. 120 µl Reconstitution buffer
8. 35 µl diluted Dryzymes™ Restriction Enzyme 1 (*Eco*RI)
9. 35 µl diluted Dryzymes™ Restriction Enzyme 2 (*Hin*dIII)
10. 85 µl DNA standard marker
11. 20 µl 10x gel loading solution
12. Ten 1.5 ml microcentrifuge tube with attached caps
13. 0.46 g Agarose
    Edvotek
    Edvo-Kit #225
    Lot #: D00086
14. 7.2 ml 50x concentrated electrophoresis buffer
    Edvotek
    Edvo-Kit #225
    Lot #: 05151700R
15. 10 µl Ethidium bromide 1% Solution
    FisherBiotech
    Biotech Grade
    RNase, DNase and Protease-Free
    Lot #: 035346
16. Horizontal gel electrophoresis apparatus
    Edvotek
    Electrophoresis Unit M6+
17. Power supply
    Fisher Scientific
    Model #: FB-300
    SN: 407N0987
18. Automatic micropipettes (5 - 50 µl) with tips
    Eppendorf Research Plus
    0.5-10 µl: J17400B
    10-100 µl: 212705A
    1-10 ml: J16179B
    epTIPS 10 µl
    epTIPS dualfilter 100 µl: D4112 (certified PCR clean & sterile)
    epTIPS 10 ml: D4111 (Physiocare concept)
19. Transfer pipettes
    Globe Scientific Inc.
    7ml transfer pipette graduated to 3 ml
    Non-sterile

Lot #: K102870-01

20. Water Bath
    Sheldon Manufacturing, Inc.
    Shel-Lab
    Model #: SWB7
    Serial #: 08051814

21. Balance
    AE ADAM
    Model #: PGL 303

22. Hot plate
    Corning Heating Plate
    Heat & Stir

23. Two 250 ml beaker
    Pyrex
    Model #: 1000

24. 100 ml graduated cylinder
    KIMBLE USA
    KIMAX
    TD
    Lot #: 20025-H

25. Hot globes

26. Deionized water

27. Ice buckets and ice

28. UV transilluminator (300 nm)
    Spectronics Corporation
    Spectroline
    Model #: TS-3123
    Serial #: 1553500

29. UV safety goggles

IV. METHODS:

DNA samples from two suspects were collected and analyzed using the restriction fragment length polymorphism (RFLP) technique to determine the variations of tandem repeats in DNA sequences (McDonald, 2012), against a sample collected in the crime scene. In order to accomplish this task, the DNA samples collected from the two suspects and the one from the crime scene were cut using restriction enzymes (*Eco*RI, *Hin*dIII) and analyzed using agarose gel electrophoresis. The agarose gel was stained with ethidium bromide and the resulting DNA bands from the standard marker were measured in centimeters to the nearest millimeter to generate a standard curve. Once the standard

curve was generated, using the line equation from the standard curve the molecular weight (size) of the fragments were obtained and recorded for analysis.

V. PROCEDURE:

1. Crime Scene Investigation - Restriction Enzyme Digestion

1.1. Label four 1.5 ml microcentrifuge tubes with the numbers 1,2,3, and 4.

1.2. Add 10 µl of Enzyme Reaction Buffer to each of the 4 reaction tubes.

1.3. Add 15 µl of suspect DNA sample #1 to reaction tube 1 and 2; 15 µl of suspect DNA sample #2 to reaction tube 3 and 4.

1.4. Add 15 µl of diluted Dryzymes™ Restriction Enzyme 1 (*Eco*RI) to reaction tube 1; 15 µl of diluted Dryzymes™ Restriction Enzyme 2 (*Hin*dIII) to reaction tube 2; 15 µl of diluted Dryzymes™ Restriction Enzyme 1 (*Eco*RI) to reaction tube 3; and 15 µl of diluted Dryzymes™ Restriction Enzyme 2 (*Hin*dIII) to reaction tube 4.

1.4.1. **IMPORTANT:** The final volume of each reaction tube will have 40 µl. To prevent contamination, be sure to use a fresh pipet tip before going into the enzyme, DNA, and buffer stocks. Keep the enzymes in ice when not in use.

1.5. Mix thoroughly the restriction digests by pipetting up and down or gently tapping the tubes.

1.6. Cap the tubes and incubate the samples at 37°C for 30 minutes.

1.7. After the incubation, add 5 µl of 10x gel loading solution to each reaction tube. Cap the tubes and mix by gently tapping the tubes and proceed to prepare the agarose gel for electrophoretic analysis.

2. Agarose Gel Electrophoresis

2.1. Dilute concentrated (50x) buffer with distilled water to create 1x buffer.

2.1.1. Mix 1.2 ml of concentrated (50x) buffer with 58.8 ml of distilled water.

2.2. Mix 0.46 g of agarose powder with 1x buffer in a 250 ml beaker.

2.3. Place the mixture on a heating plate, add a magnetic stirrer. Set the heat at 7 and stirring at 2 or 3, and heat until the mixture just begins to boil and become transparent.

2.4. Let the mixture cool until its temperature is between 68 and 70°C.

2.5. While agarose is cooling, seal the ends of the gel casting tray with the rubber end caps. Place the well comb in the appropriate notch.

2.6. Pour the cooled agarose solution into the prepared gel casting tray. The gel should thoroughly solidify within 20 to 30 minutes. The gel will stiffen and become less transparent as it solidifies.

2.7. Carefully remove end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

2.8. Place the casting tray with agarose gel into the electrophoresis chamber. Pour 1x diluted chamber buffer into the electrophoresis chamber. Completely submerge the gel.

2.8.1. Mix 6 ml of concentrated (50x) buffer with 294 ml of distilled water.

2.9. Check that the gel is properly oriented before loading the samples.

2.10. Load the entire sample volumes into the wells as follow:

2.10.1. Well 1: DNA standard marker

2.10.2. Well 2: DNA from crime scene cut with Enzyme 1

2.10.3. Well 3: DNA from crime scene cut with Enzyme 2

2.10.4. Well 4: DNA from suspect 1 cut with Enzyme 1

2.10.5. Well 5: DNA from suspect 1 cut with Enzyme 2

2.10.6. Well 6: DNA from suspect 2 cut with Enzyme 1

2.10.7. Well 7: DNA from suspect 2 cut with Enzyme 2

2.11. Place safety cover on the electrophoresis apparatus. Remember, the samples will migrate toward the positive (red) electrode.

2.12. Connect the leads to the power source and perform electrophoresis at 150 Volts for 20 minutes.

2.13. After electrophoresis is complete, remove the gel and casting tray from the electrophoresis chamber and proceed to staining the agarose gel.

3. Staining Agarose Gel

3.1. Wearing gloves, prepare Ethidium Bromide solution of 0.01% (v/v).

3.2. Add 10 µL of Ethidium Bromide into a graduated cylinder and bring to volume using deionized water to 100 ml.

3.3. Carefully remove the agarose gel and casting tray from the electrophoresis chamber. Slide the gel of the casting tray onto a clean gel-staining tray.

3.4. Pour the Ethidium Bromide solution and let the gel stain for 30 minutes.

3.5. Wearing gloves, remove the gel from the gel-staining tray. Visualize the gel using a mid-range ultraviolet transilluminator (300 nm). DNA should appear as bright orange bands on a dark background.

3.5.1. **IMPORTANT:** Be sure to wear UV-protective eyewear.

4. Size determination of DNA Restriction Fragments

4.1. Measure and record distances using standard DNA fragments

4.1.1. Using the stained agarose gel, measure the distance traveled by each standard DNA fragment from the lower edge of the sample well to the lower end of each band. Record the distance in centimeters to the nearest millimeter. Repeat this for each DNA fragment in the standard.

4.2. Generate a standard curve

4.2.1. To create a standard curve on a semi-log paper, plot the distance of each standard DNA fragment migrated on the x-axis in millimeters versus its size on the y-axis in base pairs (bps). Remember to label the axes.

4.2.2. After all the points have been plotted, use a ruler to draw the “best fit line” possible through the points.

4.2.3. The line should have approximately equal numbers of points scattered on each side of the line. It is okay if the line runs through some points.

4.3. Determine the length of each unknown fragment

4.3.1. Locate the migration distance of the unknown fragment on the x-axis of your semi-log graph. Draw a vertical line extending from that point until it intersects the line of your standard curve.

4.3.2. From the point of intersection, draw a second line, this time horizontally, toward the y-axis. The value at which this line intersects the y-axis represents the approximate size of the fragment in base pairs. Record the results.

4.3.3. Repeat for each fragment in your unknown sample.

VI. RESULTS:

1. Summary:

Using the most intense fluorescent points seen on the stained agarose gel (Figure 3. Red Arrow) the bands were measured using a metric ruler and a standard curve was generated to find out the size of each band. The results (shown below) were compared against the ideal results from the experiment manual (Table 2).

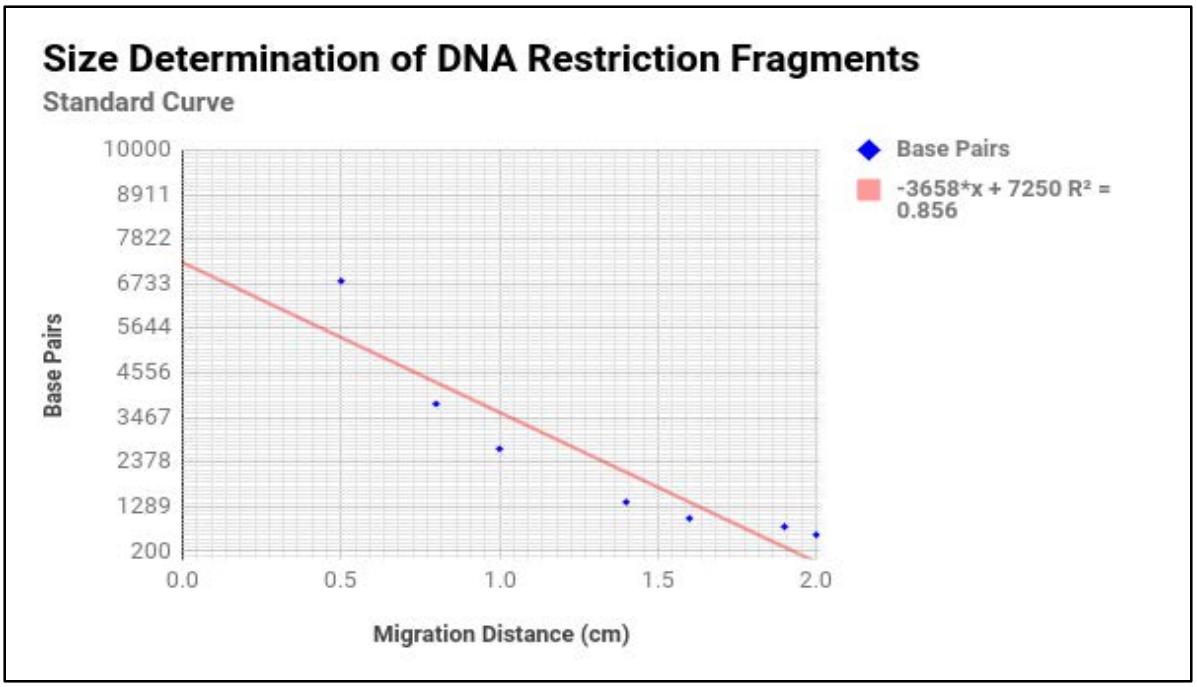
2. Data table and Calculations:

TABLE 2. Ideal Results vs. Actual Results Based on Standard Curve Calculations

Well	Tube	Sample	Molecular Weight from Ideal Results	Molecular Weight from Actual Results
1	Markers	DNA Standard Marker	6751, 3652, 2827, 1568, 825, 630	6800, 3800, 2700, 1400, 1000, 800, 600
2	CS1	DNA from crime scene cut with Enzyme 1 (*Eco*RI)	3000, 1280	3958, 2860
3	CS2	DNA from crime scene cut with Enzyme 2 (*Hin*dIII)	3650, 630	4324, 2129
4	1	DNA from Suspect 1 cut with *Eco*RI	3000, 1280	4324, 3226
5	2	DNA from Suspect 1 cut with *Hin*dIII	3000, 760, 650	4324, 3226, 2495
6	3	DNA from Suspect 2 cut with *Eco*RI	3000, 1280	4324, 3226
7	4	DNA from Suspect 2 cut with *Hin*dIII	3650, 630	4324, 2129

Actual results were obtained measuring in centimeters to the nearest millimeter the distance traveled by each standard DNA fragment from the lower edge of the sample well to the lower end of each band. From these results a Standard Curve was generated (Graph 1.) and the line equation was used to determine the molecular weight of the rest of the DNA fragments.

3. Graphics and relevant images:



Graph 1. Standard Curve. Generated using the migration distance of the standard markers from the actual results.

Figure 1. Agarose gel with samples after electrophoretic analysis and staining process.

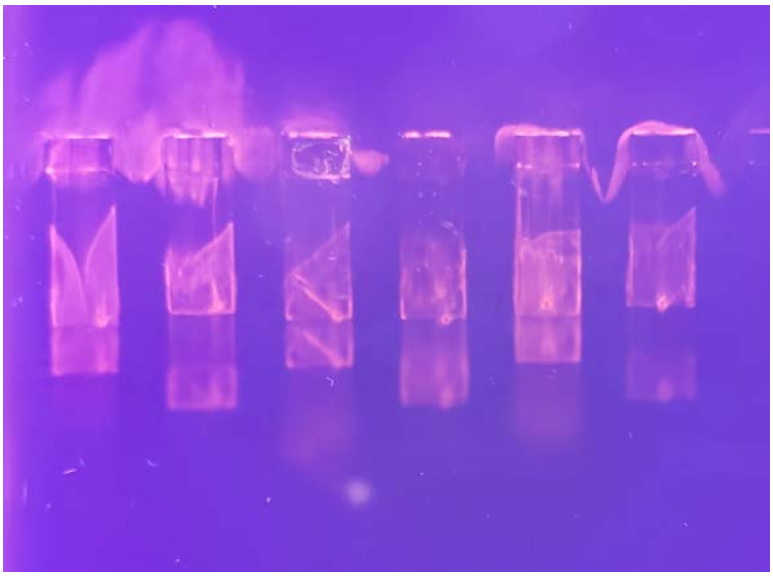


Figure 2. Amplification from figure 1, in order to appreciate in more detail the results of the electrophoresis after the use of restriction enzymes in the samples obtained from the crime scene.

Figure 3. Analysis of results based in dots (red arrow) on fragments.
Well 1: 2 fragments (black & grey)
Well 2: 2 fragments (blue & white)
Well 3: 2 fragments (black & grey)
Well 4: 3 fragments; 1 large fragment (black/blue) and 2 small fragments (green)
Well 5: 2 fragments (black & grey)
Well 6: 2 fragments (blue & white)

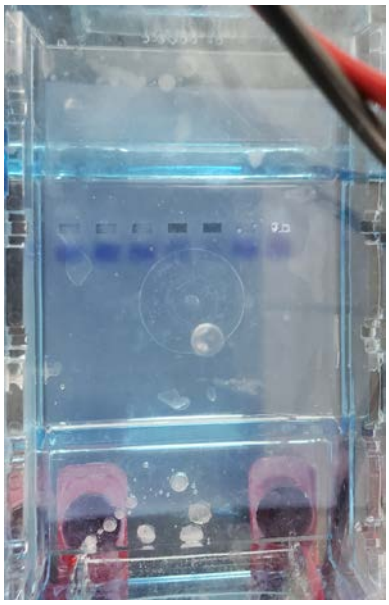


Figure 4. Agarose gel during electrophoresis process. Note the tracking dye (blue).

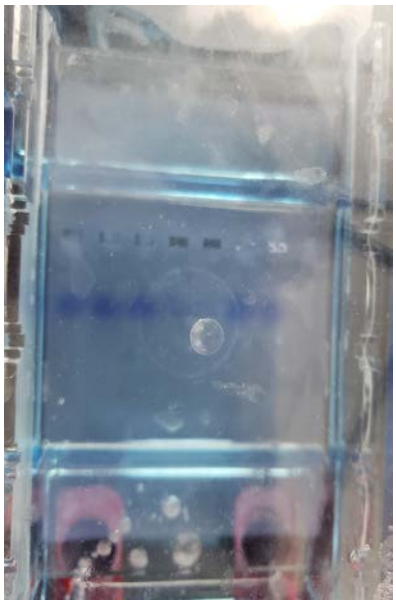


Figure 5. Agarose gel during electrophoresis process. Note the tracking dye (blue).

Figure 6. Agarose gel after electrophoresis in staining tray. Note the tracking dye (blue).

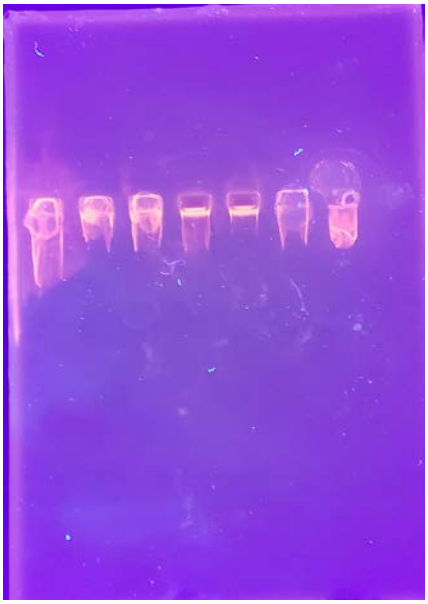


Figure 7. Agarose gel with samples after electrophoretic analysis and staining process from a second attempt performed a week later. Notice the lack of separation between DNA bands.

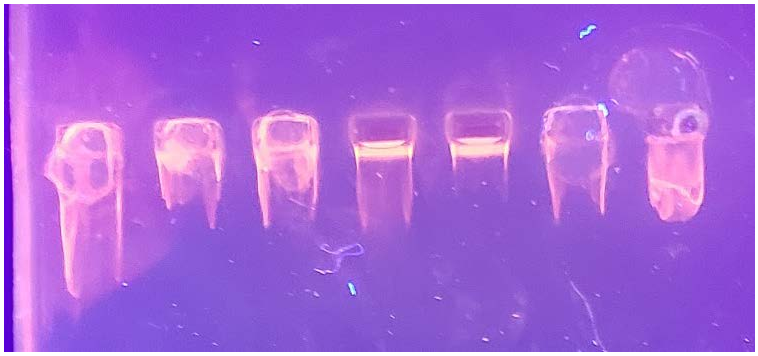


Figure 8. Second attempt performed after a week of the t one above. In this amplified image it is noticeable the lack of separation between DNA bands.

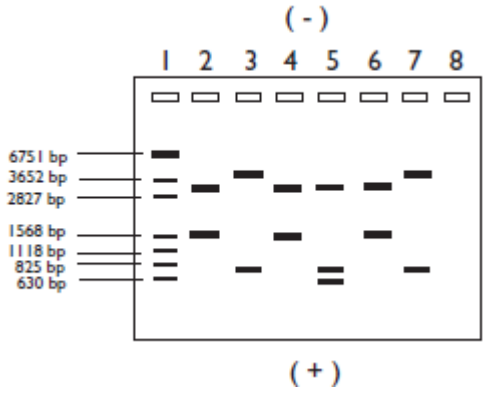


Figure 9. Idealized schematic shows relative positions of DNA fragments.

	DNA from Crime Scene		DNA from Suspect 1		DNA from Suspect 2	
Bp	CS 1 (EcoRI)	CS 2 (HindIII)	3 (EcoRI)	4 (HindIII)	5 (EcoRI)	6 (HindIII)
4324						
3958						
3226						
2861						
2495						
2129						

Figure 10. Actual results schematic shows relative positions of DNA fragments. Notice that only the fragments cut with *Hin*dIII between the crime scene and DNA from Suspect 2 are the only fragments that match.

VI. DISCUSSION:

The analysis of DNA gel fingerprinting images is a widely studied problem (Heras, et al., 2016; McDonald, 2012). In this experiment in order to interpret the results a standard curve was made to determine the fragment sizes. Even so, may be because of faulty restriction enzymes the results are not what was expected according to the experiment manual. Table 2 shows the difference between the molecular weight based on the ideal results (EDVOTEK, 2016) and the molecular weight from the actual results which were obtained by measuring in centimeters to the nearest millimeter the bands of DNA. From these measurements a standard curve was generated (Graph 1) and using the formula from the "best fit line" the measurements in cm from each non-standard band was calculated. In the case of the DNA samples from the crime scene cut with the restriction enzymes EcoRI (3000, 1280 bp) and HindIII (3650, 630) in correlation with the DNA from suspect 2 the DNA fragments are the same, pointing suspect 2 as the possible culprit. On the other hand, the actual results show a more doubtful outcome since the only matching results are between the DNA from the crime scene cut with HindIII (4324, 2129 bp) and the suspect 2 which also has the same number of DNA bands with the same molecular weight.

Figure 1 and 2 show the results after the electrophoretic analysis and the bromoethane staining process. To examine the results, the more fluorescent intense dots (Figure 3; red arrow) in each DNA fragment was considered and measure accordingly. Some intense fluorescent dots were excluded since they are between the lanes of the agarose gel and they were considered as noise (Heras, Domínguez, Mata, & Pascual, 2016). The DNA fragments considered in this experiment are marked with boxes of different color in order to differentiate each band (Figure 3). Figure 9 and 10 exhibit and schematic analysis between the idealized results and the actual results respectively, with the size of the band in base pairs previously calculated using the standard curve (Figure 10 & Graph 1.).

A second experiment using the same reagents was performed a week later with the hope to obtain a better result, this time the only variant was the time and the voltage used in the electrophoretic analysis (60 min at 100 Volts). The results are shown in figure 7 and 8, proving once again that probably the restriction enzymes were defective, since the DNA samples did not move from the wells despite the fact that the DNA tracking dye traveled more than the 3.5 cm (Figures 4, 5 & 6) recommended by the experiment manual.

In this assay the DNA fingerprint technique used to detect the bands was performed without the use of new technological tools or software that is currently used to analyze the DNA gel fingerprint images. Al the measurement was made manually and the detection of the molecular weight was calculated using a standard curve. Nowadays, there are multiple DNA fingerprinting techniques, and the choice of which of them we must use depends on their applications (medical, diagnosis, forensic science, and many others). The evaluation criteria to analyze these gel images can now be divided in five steps with the use of the right software: image processing, lane detection, band detection, normalization and fingerprint comparison (Heras, et al., 2016). One of the biggest advantages of these new tools (Manabe, et al., 2017) is the automation of the process although there is no best tool for all the possible scenarios and in the end the task of interpreting the results is performed by a skill professional.

VII. CONCLUSIONS:

In reality, techniques such as DNA fingerprinting cannot always be utilized to provide conclusive evidence for an investigation. For example, the initial integrity of a DNA sample can affect the quality of its subsequent DNA fingerprint. DNA quality can be negatively altered by conditions such as heat, humidity, and ultraviolet light, all of which can damage DNA by inducing random breaks in the DNA double helix (Laura Palmer, 2010). Thus, samples that suffer prolonged exposure to the environment may not be suitable for DNA fingerprinting. It is critical to acknowledge that there is always the possibility of error due to mistakes by crime lab technicians during DNA sample processing.

DNA profiling is still a lengthy, expensive, and complicated process. It would be helpful to have new technologies that could quicken the process, reduce the costs, and make it easier to do DNA testing

at the crime scene. Other improvement could be the expansion of DNA testing to more applications such as linking ink samples with synthetic DNA fragments in order to determine the origin on a written document (McDonald, 2012). A promising new technology is the "laboratory on a chip" or a portable DNA testing device, this would not only allow for quicker DNA profiling, but also will provide portability to analyze samples directly on crime scenes. To conclude it is important to mention that DNA profiling is not only used for criminal case work, but it has expanded to encompass paternity testing, disaster victim identification, monitoring bone marrow transplants, detecting fetal cells in a mother's blood, tracing human history, and many other areas. The future of DNA profiling looks promising and expansive with the development of new tools and better techniques.

VIII. REFERENCES:

1. EDVOTEK. (2016). *DNA Fingerprinting Using Restriction Enzymes*[Pamphlet]. Washington, DC: EDVOTEK The Biotechnology Education Company. Edvo-Kit # 225.

2. Glick, B. R., & Patten, C. L. (2017). Chapter 2/Preparation of DNA cloning. In Molecular Biotechnology; Principles and Applications of Recombinant DNA (Fifth ed., pp. 11-16). Washington, DC: ASM Press.

3. Heras, J., Domínguez, C., Mata, E., Pascual, V., Lozano, C., Torres, C., & Zarazaga, M. (2016). A survey of tools for analysing DNA fingerprints. Briefings In Bioinformatics, 17(6), 903-911. doi:10.1093/bib/bbv016

4. Heras, J., Domínguez, C., Mata, E., & Pascual, V. (2016). Surveying and benchmarking techniques to analyse DNA gel fingerprint images. Briefings In Bioinformatics, 17(6), 912-925. doi:10.1093/bib/bbv102

5. Laura K. Palmer, a. (2010). Using Harry Potter to Introduce Students to DNA Fingerprinting & Forensic Science. The American Biology Teacher, (4), 241. doi:10.1525/abt.2010.72.4.8

6. Manabe, S., Morimoto, C., Hamano, Y., Fujimoto, S., & Tamaki, K. (2017). Development and validation of open-source software for DNA mixture interpretation based on a quantitative continuous model. Plos One, 12(11), e0188183. doi:10.1371/journal.pone.0188183

7. McDonald, J., & Lehman, D. C. (2012). Forensic DNA analysis. Clinical Laboratory Science, 25(2), 109-113.

8. Stephenson, F. H., Ph. D. (2010). Chapter 10 / Restriction Endonucleases. In *Calculations for Molecular Biology and Biotechnology - A Guide to Mathematics in the Laboratory*(Second ed., Vol. 1, pp. 313-318). Burlington, MA: Academic Press.