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# Using DNA Fingerprinting to Identify Genetic Relatedness for a Family

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## ABSTRACT

The two main uses of DNA fingerprinting today are for paternity tests and as evidence in a crime. DNA finger printing is a technique used to determine biological relatedness. In this study I obtained DNA from a family consisting of a mother, a father, and two sisters. DNA was also obtained from a possible half-brother, and a random sample of DNA from someone not related. DNA was extracted from saliva samples provided from each of the six participants. The DNA was then amplified through PCR using 10 of the CODIS loci. Gel electrophoresis was used to investigate variability in short tandem repeat regions. The different band patterns from each individual is what makes your DNA fingerprint unique to you. Based off of the bands that didn't appear after analyzing the gel sample under UV light, the experiment was inconclusive. A list of possible explanations for the lack of results are explained in the discussion.

Keywords: *DNA fingerprinting, STR, PCR*

## INTRODUCTION

DNA fingerprinting, also known as genetic fingerprinting is a series of techniques in trying to determine genetic relatedness or identification, with a DNA sample (Chankraborty and Schull, 1976). It is one of the most widely used techniques in forensic sciences (Chakraborty, 1976). It is a chemical test that shows the relationships between all living things (Alford 1994).

The history of DNA fingerprinting began September 10th, 1984, Alec Jeffreys wanted to know if it was possible to tell if people were related using their DNA (Phillips, 2019). They used VNTR's (now we use STR's) Variable number tandem repeats are regions of the human genome and occur in non-coding regions that vary across individuals in the number of short DNA repeats. VNTR's are 10-60 base pairs long, while STR's are 2-6 base pairs long (Lakna, 2018). Short tandem repeats are a tandem repeat where two or more base pairs are repeated a number of times in a head to tail manner (Alford, 1994). PCR could be used to find the number of repeats at a locus, using primers that anneal the STR (Hale, 2005). STR's are one of the most common types of genetic polymorphisms in forensic genetics today (Lakna, 2018). The technique for DNA fingerprinting takes place in a few steps. The collection of DNA, obtaining blood, saliva, semen, hair, skeletal remains, etc (Ambardekar 2018). Extraction and amplification of the DNA at different known STR loci throughout the genome (Gupta, 1994).. Then the use of gel electrophoresis, which involves making the 3% gel then running 10 µl of a DNA marker, a loading dye, and the replicated DNA from the participants. Depending on the percentage of the gel it will take about two hours for the gel to run at around 130 volts. After the two hours, the gel will be agitated in the stain before being refrigerated, then to the analyzation of band patterns over UV light, which will ideally show in the gel.

Not a day goes by where a criminal case isn't solved with the help of molecular genetics. It is seen on the news all over the world all the time. Methods using DNA are being used in different applications and fields, ranging anywhere from medicine, to conservation, to criminal justice. In any case scenario where DNA is left at the crime scene, it can be traced back to someone (Phillips, 2019). Also, in the case of paternity, whether a child wants to know who their parents are, or whether a parent wants to know if a child is there's (Chankraborty and Schull, 1976). DNA fingerprinting can also be used for medicinal purposes. In the future DNA fingerprinting will probably be used in more aspects of science as well (Hammond, 1991). DNA fingerprinting has been used to identify good genetic matches for organs and bone marrow donations. It can also be used to be sure that a tissue sample has been correctly labeled with the patient's name. (Phillips 2019). Agriculture is another common use of DNA fingerprinting where genetically modified plant can be identified, and find the pedigree in animals such as purebred dogs or prizewinning racehorses (Phillips 2019)

In this research project, the goal is to properly use DNA fingerprint testing, to determine if a potential brother, is biologically half siblings with both sisters. In this experiment the DNA used will come from brother, sister 1, sister 2, mother, and father, their identities will be concealed for personal reasons.

## MATERIALS AND METHODS

Collection and Storage of DNA Samples

DNA samples were collected from the mother, father, 2 sisters, the test subject, and a random person unrelated to the family. The subjects (who have all read and gave consent to using their DNA) received a tube with a lid, Para film wax strips (to seal the test

tube), a zip lock baggie, clear instructions, permission slip that needs to be signed, and the mailing address for the lab that they will send their DNA back. DNA provided through saliva samples.

Extraction & Amplification of DNA

To extract the DNA in each sample, a DNeasy Blood and Tissue Kit was used. The published protocol for the company QIAGEN, was used step by step. Cat No./ID: 69504. This required combining 20µl of proteinase K into a microcentrifuge tube with 250µl of the sample's saliva, then adding 200µl of buffer AL and mixing by vertexing. This mixture was then incubated at 56 degrees for 10 minutes. After 200 µl of ethanol was added and that combination was vortexed. Then the mixture was pipetted into a DNeasy Mini spin column placed inside a 2 ml collection tube. This was centrifuged at 800 rpm for 1 minute. The flow through and collection tube were discarded after. After, the spin column was placed in a new 2 ml collection tube along with 500 µl of buffer AW1 and was centrifuged for 1 minute at 8000 rpm. The flow through and collection tube were again discarded. After a new spin column was added, 500 µl of buffer AW2 was added and centrifuged for 3 minutes at 14,000 rpm. The flow through was again discarded. After the spin column was added to a new 2ml microcentrifuge tube but with a lid. Next to elute the DNA we added 200 µl of buffer AE to the center of the spin column membrane, incubated it for 1 minute at room temperature, then centrifuged it for 1 minute at 8000 rpms. We eluted the DNA with the 200 µl of buffer AE and centrifuged a total of three times. After DNA extraction, the DNA was amplified through PCR (Phillips,. The PCR will multiply copies of specific sequences of DNA. Primers (both forward and reverse) of ten of the CODIS alleles were used to amplify the different segments of DNA: D5S818, THO1, D132317, FGA, D7S820, TPOX, D1S80, D16S539, D8S1179, and D3S1358 (http://www.cybertory.org/resources/CODIS/index.html). There are three steps when it comes to polymerase chain reaction. Denaturing, Annealing, and extending (Hammond, 1991). Denaturing the DNA took place at 98 degrees, at this step the DNA is heated to separate the DNA into the two single stranded pieces. This took about one minute for each cycle, at 20-35 times. Annealing took place at 55 degrees; the temperature is decreased so the DNA can connect to the primers. This took 25-35 cycles and is usually run for 30-45 seconds. Extending the DNA took place at 72 degrees, so a new strand of DNA is made, this also took between 25- 35 cycles, taking place between 15-30 seconds (Hammond, 1994).

After a lack of amplification in the first 3 primers run through PCR, our method was modified by trying to increase the concentration of the participants template DNA through evaporation in a water bath.

Visualization of DNA Products

To visualize our PCR products, A 3% agarose gel was made. 10µl of loading dye was inserted into the first well, 10µl of a DNA marker was loaded into the second well. Then 10µl of the replicated DNA from the 6 participants are loaded into well 3 through 8. The gel was run at approximately 130 volts for about two hours, or until the 6X orange DNA loading buffer was at the bottom of the well. This marker is ideal for small fragments. Then was stained using gel green while being slightly agitated for one hour. Then refrigerated, the gel was then saved until it was ready to be seen under UV light. The band patterns are different for everyone, this is what makes your unique DNA fingerprint. Most procedures were found and followed from Cold Spring Harbor laboratory, "Using DNA barcodes to identify and classify living things."

## RESULTS

There was no amplification of the DNA at any of the loci analyzed (Fig. 1). This was shown by the lack of marks on the gel sample after gel electrophoresis and after being analyzed under the UV light. As a result, the experiment was inconclusive as we were unable to assess the patterns of STR lengths at any of the CODIS loci. I will discuss possible explanations for this in the discussion.

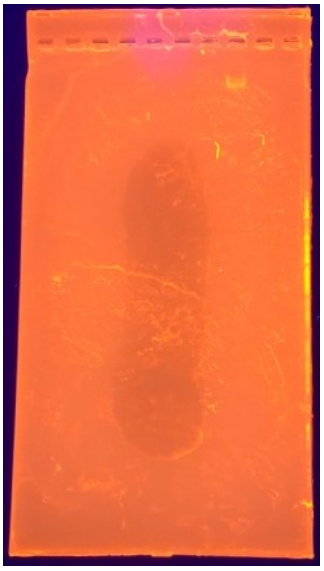

**Figure 1.** Agarose gel showing no amplification of CODIS allele.

## DISCUSSION

There are a number of possibilities that may have been the cause of not getting amplification of the DNA at any of the regions. A reason as to why band patterns didn't show up in the gel after electrophoresis could have been that the saliva that obtained didn't

have enough DNA in it. Saliva itself is not DNA but has DNA in it from the cells inside of the mouth. Even though the participants were asked to scrape the side of their cheeks with their teeth to get some of the cells, it was not guaranteed that they did, or they did a sufficient job doing it.

There is also the possibility that the techniques and methods used in the lab, were not strong enough to extract the DNA from the saliva. In the future, a blood sample would have been used to guarantee that DNA would have been present due to the abundance of white blood cells. As far as PCR, there are possibilities that there was a contamination anywhere from laboratory environment to reagents. There are also possibilities that either the denaturing, annealing, or extending steps were too short or too long. Other possibilities might include that too many cycles were used.

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