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A COMPARISON OF BUCCAL SWABS FOR THE PURPOSE OF RESTRICTION FRAGMENT LENGTH POLYMORPHISM DNA ANALYSIS

By

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ABSTRACT

A COMPARISON OF BUCCAL SWABS FOR THE PURPOSE OF RESTRICTION FRAGMENT LENGTH POLYMORPHISM DNA ANALYSIS

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Restriction fragment length polymorphism (RFLP) DNA analysis is used in the field of criminal justice for characterizing biologic evidence. The standard method of collecting samples is by a blood draw. Buccal swabs are a relatively new collection method. These swabs collect buccal cells that are found in the mouth and that contain DNA. There are important advantages to using buccal swabs instead of blood which would make them a safer, less expensive, more convenient, and more comfortable method of obtaining samples for RFLP DNA typing. This study involves a comparison of six different types of buccal swabs to each other and to blood from each of ten people. Lifecodes RFLP DNA typing protocol was performed on all samples. DNA results produced by the buccal swabs were equivalent to those found with the traditional blood samples. The quality and quantity of DNA obtained with each of the six different types of swabs was also compared. The goal of this study was to find the type of swab that produced the most high molecular weight DNA to be tested by RFLP methods. The nylon brushes produced the best DNA results, were the easiest to work with, and were the most comfortable to use.

Each small task of everyday life is part of the total harmony of the universe.

St. Teresa of Lisieux

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INTRODUCTION

Restriction fragment length polymorphism (RFLP) DNA typing is used in the field of criminal justice to analyze biological evidence. It may be used in criminal cases that involve biologic samples from victims and suspects. It also may be used in civil cases involving paternity disputes or immigration cases. The reason for using RFLP DNA typing for these purposes is because of its' very powerful discriminating ability. Due to the nature of RFLP DNA testing, results can be used to help identify individuals based on probabilities of inclusion or exclusion.

There are specimen requirements that must be met to successfully perform RFLP DNA testing. At least 50ng of high molecular weight (undegraded) DNA is needed. The sample must be free of contamination and degradation that may cause poor results to be obtained. Contamination with bacterial DNA, animal DNA, or extraneous human DNA will cause a decrease in detection of the actual targeted DNA contained within the original sample, and may give ambiguous results. Degradation due to humidity, temperature, bacterial enzymes, and sunlight will cause the DNA in a sample to degrade and become lower in molecular weight. A degraded sample results in the loss of high molecular weight

DNA and is detected by a loss of signal in the high molecular weight bands of the DNA banding pattern. If the DNA is extremely degraded, no results at all will be obtained.

The standard procedure used to obtain samples for RFLP DNA typing is with peripheral blood draws. Blood, which contains nucleated white blood cells is only one possible source of DNA. DNA can be obtained from any nucleated cell which can be found in bone, hair roots, buccal cells (epithelial cells that line the inner cheeks of the mouth), skin, tissues, and sperm. Traditionally, blood has been the sample of choice. There are two major reasons for this. One, because blood is frequently found at crime scenes. Two, before the advent of DNA testing, blood was used for blood group typing and for red cell enzyme and protein analysis to help discriminate between blood stains. In recent years, an interest in using buccal cells as an alternative DNA source to blood has emerged. Past studies have indicated that buccal cells are a reliable source of abundant undegraded high molecular weight DNA.

The current interest in this DNA source may be attributed to the important advantages of using buccal cells as opposed to the traditional blood sample. Buccal swabs are a more cost-effective means of DNA sample collection, especially for high volume testing laboratories. Buccal swab collections reduce health risks to forensic scientists when handling samples, as compared to potentially

biohazardous liquid blood samples. Buccal swabs also decrease the health risks involved with shipping samples to laboratories for testing. Also, the special storage and handling conditions that blood samples require are eliminated. Buccal swabs also allow samples to be collected from persons who have medical and\or religious reasons against having blood drawn. Most importantly, buccal swabs are a less invasive method for sample collection and therefore less painful and traumatic to those involved in the testing. This is especially useful for obtaining specimens from infants and children for paternity testing where, quite often insufficient amounts of blood samples are obtained. The only possible disadvantage to using buccal swabs is that they can be more labor-intensive to process than a blood sample. Buccal cell collection is therefore, a superior method for RFLP DNA analysis due to its advantages in the areas of collection, transport, storage, and overall cost.

Current literature on buccal cells and DNA testing have involved comparative studies of buccal cells versus blood samples, and have shown similar DNA results. Very little is published, however, about different buccal cell collection devices and which is most optimal for quick, reliable, and reproducible results when used for RFLP DNA analysis. In previous literature there has been mention of different collection techniques for buccal cells, but a direct comparison of their consistency to yield sufficient high

molecular weight DNA has not yet been published. The present study includes six different types of buccal swabs, with their DNA results being compared to each other as well as to those of blood samples. The analyses are based on both the quality and quantity of DNA obtained. The ultimate goal of this research is to find which type of buccal cell collection method is best suited for the RFLP DNA analysis.

Chapter 1

HISTORY OF DNA PROFILING AND LITERATURE REVIEW

The history of DNA profiling, or restriction fragment length polymorphism DNA typing, began with the discovery of restriction enzymes in 1970. Arber, Smith, and Nathans accomplished this by finding a bacterial enzyme that cut DNA at certain sites. This finding led to the development of recombinant DNA technology, which involves combining different DNA molecules. The discovery of restriction enzymes laid the foundation for RFLP DNA analysis, which measures the difference between specifically cut DNA fragments that vary among individuals. In 1975, Edward Southern proposed the Southern Blot which involves transferring isolated DNA from an electrophoresis gel to a nylon membrane so that DNA hybridization could occur. Wyman and White in 1980 produced autoradiographs of hypervariable DNA, which produced many different bands among different people. In 1985, Alec Jeffreys discovered a different type of extremely variable polymorphisms within DNA. He realized how these hypervariable polymorphisms could be used to help identify individuals, and called it ''DNA fingerprinting''. This began the application of RFLP DNA typing for forensic

testing purposes. For a description of RFLP DNA testing see Chapter 3.

RFLP DNA testing in American casework began in 1986 by the Lifecodes Company. Cellmark Diagnostics began using RFLP in 1987 for pending cases. Also in 1987, the Tommy Lee Andrews case was the first ever criminal conviction based on DNA results. The FBI began using RFLP for casework in 1988. Virginia established the first state run crime laboratory to begin using RFLP on cases, beginning in 1989. Since then, crime laboratories in every state have been performing RFLP DNA testing for casework.

In 1986, another type of DNA testing was discovered by Kary Mullis, called Polymerase Chain Reaction (PCR). PCR allows for many copies of short DNA strands to be replicated. It begins with the amplification of a single DNA fragment by repetitive cycles of DNA synthesis, promoted by an enzyme called DNA polymerase. The amplified product is separated by gel electrophoresis and analyzed after DNA hybridization. It has advantages over RFLP in that it is especially useful for analyzing very small samples. It can also analyze samples that have been degraded and contain only low molecular weight DNA. There still are, however, important advantages to using the RFLP procedure if enough high molecular weight DNA is present in a sample. The areas of DNA which are variable among different individuals are called variable number of tandem repeats (VNTR). The VNTRs analyzed with the RFLP technique are much longer DNA

sequences than those analyzed with PCR. The length and hypervariableness of these regions allow for much greater polymorphism and therefore greater discrimination than with PCR systems.

After the development of RFLP DNA technology, came independent reviews of its accuracy and reliability. The first major examination was performed in 1990 by the Office of Technology Assessment, which is part of the United States Congress. It concluded that DNA evidence is reliable for use with forensic casework provided that appropriate quality control and quality assurance programs are utilized. Soon after came external proficiency testing programs and accrediting agencies to assist in quality assurance. The Technical Working Group on DNA Analysis Methods (TWGDAM), a large group of forensic scientists who develop DNA methods and guidelines, suggest quality assurance procedures which include external proficiency testing. The College of American Pathologists is another group that offers proficiency testing for RFLP DNA analysis. The American Society of Crime Laboratory Directors and the National Research Council have both developed specific recommendations regarding RFLP DNA testing. All of these programs and recommendations exist to ensure quality results and to promote general acceptance of RFLP DNA testing as sound scientific technology.

In recent years, there have been continued efforts to improve upon the original RFLP DNA technology. For example,

numerous restriction endonucleases, which are the restriction enzymes used in RFLP, were isolated in hopes of finding those more suited for forensic casework that involves degraded samples. The restriction enzyme of choice for forensic casework is presently Hae III because it produces smaller fragments and can be used with degraded samples. Pst I was found to be more appropriate for the undegraded samples obtained in paternity casework, and restricts larger sized fragments. Another area of improvement was the introduction of chemiluminescent probes for the hybridization steps in the RFLP procedure. Chemiluminescent probes are able to produce visual results without using radioactive substances, as the traditional P^{32} labeled probes. These new probes produce faster results, are safer to use, and are at least as sensitive as the radioactive methods. The isolation steps of the RFLP procedure have also been improved. There are alternatives now to the original organic solvents used to extract DNA. These aqueous systems precipitate cellular debris with high molarity salt solutions. The advantage is to eliminate biohazardous organic solvents from the process. New technology has also been developed to assist in analyzing the autoradiographs produced by RFLP. There are many computer-assisted devices that can be used to measure the position of sample fragments. What was once acheived only by manual methods can now be done faster, more accurately, and with the convenience of archiving large amounts of data.

Recently, the area of sample collection for RFLP DNA analysis has been evaluated for potential improvement. The numerous advantages of using buccal cells as opposed to blood samples has sparked interest and produced various comparative studies. The earliest of buccal cell collection studies began in the late 1980's. They explored the possibility that buccal cells could be used to isolate high molecular weight DNA, and could be used in RFLP and PCR DNA analysis. Additional studies in the early 1990's compared multiple types of buccal collection devices to see if they all were successful in obtaining DNA. It wasn't until 1993 that a study came about which compared multiple types of buccal swabs to each other and to blood draws. Most of these buccal swab comparative studies involve evaluating PCR DNA systems, not RFLP DNA methods. All of these studies have also employed organic extraction techniques, as opposed to aqueous extraction methods. To this date, there have been no studies which have included newer buccal swab collection devices such as felt swabs and both large and small pored foam swabs.

One of the earliest studies that involved buccal cells for the purpose of DNA testing was in 1988 by Lench, Stanier, and Williamson. It compared mouth washings for collecting buccal cells to both hair roots and blood draws, for their ability to isolate DNA. Organic extraction techniques were employed with all three sample types. PCR DNA analysis was performed and results compared. It was

concluded that buccal cells are excellent sources of DNA, and that they are much easier to process than hair follicles. It was also noted that ease of collection, non-invasiveness, and low cost, make buccal cell collection especially useful for large genetic screening projects.

One of the earliest buccal cell studies to utilize RFLP DNA testing was performed in 1989 by Tobal, Layton, and Mufti. It used vigorous mouthwashes to collect buccal cells for a genetic study. Testing was being done for a certain blood disease and the researchers wanted DNA from an unaffected site. Organic extraction procedures were performed, then RFLP DNA results compared. It was confirmed that buccal cells are capable of yielding abundant undegraded high molecular weight DNA that can be successfully used for RFLP DNA analysis.

One of the first comparisons of buccal cell collection devices came in 1990 by Mayall and Williams. Mouth washings for buccal cells were compared to scraping with a wooden spatula. It was found that five times more DNA could be isolated from washing. PCR DNA analysis was performed from which it was concluded that buccal cells produced reliable results. This also was the first study to report storage affects on buccal cells. The authors concluded that freezing buccal cells was the ideal method of storage because freezing and thawing repetitively did not reduce the quality of the DNA present.

In 1992, Walsh et. al., completed one of the more detailed studies that compared buccal cells to blood using RFLP DNA typing. Saliva, cotton swabs, and other saliva stained objects such as envelopes, cigarette butts, and cloth gags were evaluated as possible sources for DNA. Again, organic extraction procedures were utilized. It was found that the DNA banding patterns obtained by buccal swabs were similar to those produced by the same individual's blood sample. There were extremely weak or no results obtained with envelopes, cigarette butts, or gags, due to small amounts of saliva and therefore little DNA present on the objects. This study also evaluated storage affects on the stability of DNA in buccal cells. Aliquots of saliva containing buccal cells were stored at -20°C for two to three weeks. They produced near identical results to fresh saliva samples. Cotton swabs containing buccal cells that were stored at 4°C and 20°C under dry conditions for a week, produced reliable DNA results. It was only with warmer temperatures and humid conditions that evidence of DNA degradation was seen. It was therefore concluded, that buccal cells should be stored at 4°C or 20°C for short-term purposes and at -20°C for long-term storage.

A study performed in 1992 by Thomson, Brown, and Clague evaluated the use of hair roots and buccal cells as alternative DNA sources to blood for PCR DNA analysis. Buccal cells were collected using cotton swabs and extracted

using organic extraction. The purpose of this study was to show that buccal cells, hair roots, and blood all produce reliable PCR results. The results showed that the specificity and yield of PCR products were not different due to the sample type. Hair roots and buccal cells produced equivalent PCR results to the same individual's blood sample. This study emphasized, however, the numerous advantages of using buccal swabs. It was noted that blood requires special storage and handling especially when transporting, and requires extra time-consuming steps during the DNA extraction procedure. It was also noted that buccal cells are the sample of choice over hair roots for children under two years of age, because they most often do not have hair roots of suitable size.

In 1993 Richards et. al., compared buccal cells to blood using PCR testing for the purpose of diagnosing patients for cystic fibrosis. Buccal cells were collected using dacron swabs and cytology brushes. The authors stated that they both worked equally well to collect buccal cells. Organic extractions were employed in this study. PCR DNA results for both blood and buccal cell samples demonstrated 100% correlation. This study also looked at the effects of storage upon buccal cells. Both cytology brushes and dacron swabs containing buccal cells were stored at 4°C for different intervals of time up to one month, and were found to produce results similar to those of freshly collected

buccal cells. The same results were obtained with buccal cells stored at -20° C.

The study that compared the greatest number of buccal cell collection devices for RFLP DNA testing to date, was done in 1993 by Robert Bever. Cotton swabs, dacron swabs, cervical brushes, and toothbrushes were compared for their ability to produce RFLP results in comparison to blood samples. Organic extraction methods were utilized. The correlation of RFLP results for buccal swabs and blood samples was 0.9993. The cotton swab was said to be the specimen collection device of choice for buccal cells, due to its consistent high yield of DNA and nonabrasive texture. No quantitative comparison of RFLP results between the four collection devices were reported. Age and storage affects on buccal swabs were also investigated, and it was concluded that both had minimal implications on RFLP DNA results. Buccal swabs that had been stored for over six months at room temperature without chemical preservation had successful RFLP results. The advantages of using buccal swabs were stressed, especially those involving painless sample collection from infants and children. The conclusion stated that buccal swab collection is a very effective and advantageous technique for RFLP DNA analysis, particularly when used for collecting samples from children for paternity testing.

A more recent study performed in 1994 by Hagerman et. al., again compared buccal cells to blood for PCR DNA testing. This particular study used genetic testing for diagnosing ''fragile X'' syndrome. Buccal cells were collected from saliva samples and with cytology brushes. DNA extraction used organic methods. PCR results obtained for both buccal cells and blood were similar. The cytology brushes used to collect buccal cells were said to yield more DNA than the saliva samples.

A similar study was performed in 1994 by Swierczewski and Lockhart which compared buccal cells collected with cytology brushes to blood samples. This study was unique in that it utilized a combined PCR-RFLP method to determine DNA results. PCR was used to amplify DNA in the sample, and RFLP was used to analyze it. Organic extraction techniques were used on all samples. Similar yields of PCR products and RFLP results were found for both buccal cells and blood samples. It was concluded that buccal swabs are an excellent sampling method that is extremely useful for genetic typing of small samples, especially when coupled to the PCR-RFLP methodology.

Chapter 2

DNA AND RFLP

DNA stands for deoxyribonucleic acid, and is one type of macromolecule found in all nucleated cells. DNA is a long-chain polymer with repeating subunits called nucleotides. DNA nucleotides consist of three parts: a sugar (deoxyribose), a phosphate group, and a base. There are two groups of bases, purines and pyrimidines. The purine group contains adenine (A) and guanine (G). The pyridine group contains thymine (T) and cytosine (C). The bases are attached to one end of the sugar molecule and the phosphate group to the other end. When the nucleotides are linked together they form a polymer. The polymer always has a sugar-phosphate backbone, but the attached bases may be different. The phosphate group is the link between each sugar molecule. The bases are not only bound to the sugar molecules, they are also bound to each other by hydrogen bonds. DNA is double stranded and binding occurs across separate strands. One strand wraps around the other to form a double helix. The two strands are said to be complementary to each other because a ''T'' base is always paired with an ''A'' base on another strand, and a ''C'' base is always paired with a ''G'' base. The two strands run in

opposite but complimentary directions. This complimentary feature of opposite strands within double-stranded DNA is very important because it enable DNA to store and transfer genetic information. Genetic information is encoded by the order and sequence of bases within nucleotides that compose DNA strands.

There are three different classes of DNA, each with various characteristics and functions. The largest class consists of DNA with unique nucleotide sequences that are rarely repeated. This class makes up about 70% of the human genome. The second class consists of DNA with moderately repetitive sequences, and makes up about 20% of the human genome. The last 10% of the genome is made up of highly repetitive DNA that consists of millions of copies of short sequences. These sequences are usually less than 10 base-pairs and found within certain regions of the human genome.

In the first class, the DNA with the unique sequences represent the coding regions for genes. It's these coding regions that carry genetic information for the production of specific proteins which allow for metabolic processes to occur within an organism. The other two classes containing repetitious DNA make up what is known as hypervariable regions within the human genome. The repetitious sequences of DNA are non-coding forms and are not related to protein synthesis. These non-coding forms of DNA are considered functionless, but are still inherited into human genetic makeup just as coding DNA. Repetitious DNA varies in both

the sequence of nucleotides and the number of copies of each sequence. A tandem repeat is a sequence of bases repeated numerous times and attached end-to-end. Tandemly repeated sequences are one type of repetitious DNA and comprise approximately 10% of the total human genome. A small portion of the tandemly repeating sequences are regions of very short length. They are often called minisatellites and exhibits an extreme variability in the number of core sequences. Because of this, they are also called variable number of tandem repeats (VNTRs). The core sequences are short and usually only contain 9-64 base pairs. The overall length of the VNTR region depends on the number of times the core sequence is repeated, which is usually less than 100. VNTRs can be found in numerous loci throughout the genome. Single locus VNTRs are characterized by the repeating base sequence being unique to a single locus in the human genome. There are also VNTRs that can be found at many different loci and are called multi-locus VNTRs. VNTRs are highly polymorphic genetic markers and are extremely useful for characterizing DNA because of their discriminating power.

VNTRs within the human genome can be identified and analyzed by restriction fragment length polymorphisms. The RFLP DNA typing technique involves complimentary VNTR probes that are usually single locus specific. RFLP DNA typing systems allow for certain VNTR loci to be identified within an individual's genome. They are analyzed by measuring the variation in the length of restriction fragments that

contain the VNTRs. If a number of VNTR loci are analyzed, it can be shown that no two individuals possess the same alleles or exact length of VNTRs at all of them. Particular alleles found at a comprehensive set of VNTR loci are unique to an individual. A study of multiple VNTR loci lead to a ''DNA profile'' for each individual. Because all nucleated cells of the body contain DNA that can be analyzed for these VNTR loci, RFLP DNA typing is especially useful for forensic purposes.

The RFLP DNA typing procedure is detailed and includes many steps. The first step involves isolating and purifying genomic DNA from the sample. A yield electrophoresis gel is used to determine the quantity and quality of genomic DNA extracted. The isolated DNA is then digested with specific restriction enzymes. The restriction enzymes cut the DNA at recognition sites which are specific sequences found at both ends of a VNTR. The restriction fragments vary in length, reflecting the variation found in VNTRs among individuals. A test electrophoresis gel is used to determine the completeness and specificity of the restriction enzyme digestion. Restriction fragments are then sorted by size using agarose gel electrophoresis. This separation is based on the molecular size of each fragment and the charge applied. The separated fragments in the agarose gel are denatured in an alkaline solution to make the double-stranded DNA come apart. The single-stranded DNA fragments are then transferred to a sturdy nylon membrane by a

capillary action procedure called Southern Blotting. The membrane is then baked in an oven and exposed to ultra-violet light to cause the DNA to become fixed on the membrane. The membrane bound fragments are then hybridized with DNA probes that are single-stranded and complimentary to the targeted VNTR sequence in the fragments. The DNA probes used are labeled with chemicals that will produce a chemiluminescent reaction in the presence of certain substances. The hybridized fragments are visually detected by the chemiluminescent reaction. Lumigraphs are produced by placing X-ray film over the membrane and the chemiluminescent reaction creates darken bands where the hybridized fragments are. The visualized bands reflect the fragment's position and size on the original membrane. It is the pattern of bands that result from this RFLP DNA typing procedure, which provides information useful for comparison.

Chapter 3

METHODOLOGY

This study involved ten individuals who volunteered to participate and in doing so, gave their informed consent to allow the testing performed on their donated samples. Based on the participant's informed consent, this study was approved by MSU's University Committee on Research Involving Human Subjects (UCRIHS). Half the participants were male and half were female, with a range of ages from 6-49. Each participant had his\her blood drawn (whole blood collected in EDTA tube with the preferred amount of 10ml from adults and 5ml from children). In addition, buccal cells were collected from each person with the six different types of buccal swabs being compared. The six types of buccal swabs are dacron-tipped swabs, felt-tipped swabs, nylon cytology brushes, large-pored (pink) foam swabs, small-pored (beige) foam swabs, and flat wooden spatulas. These six sets of samples were collected at least one day apart. There were four of each type of swab provided, so that buccal cells from each of the four quadrants of the inner cheeks (right side upper, right side lower, left side upper, left side lower) could be collected. This step was included to maximize the amount of sample obtained and also to maintain

the participants' comfort throughout the procedure. The exact swabbing location is not important because all of the soft tissue in the mouth contains the same type of buccal cells with the same amount of DNA present. Each study participant therefore donated a blood sample and twenty-four buccal swabs. The four quadrant swabbings for each of the six types of buccal swabs per participant, were combined into a single consolidated sample once processing had begun with the DNA isolation steps. Therefore, a total of seventy samples (ten blood samples and sixty buccal swabs) were analyzed with Lifecodes' RFLP DNA typing system. The correlation of allele measurements were determined for the blood sample and the six different buccal swabs from the same individual. Comparisons of the six different buccal cell collection devices were made regarding the quantity and quality of DNA isolated from each, as well as statistical correlation of allele measurements. Conclusions included these DNA result comparisons as well as comments from participants regarding preference towards any swab type, based on ease and comfort of collection.

The ten study participants were given written instructions for buccal cell collection. The swabs were sterile and kept in small sterile paper bags contained along with instructions in a larger plastic bag. All study participants were told to gargle with approximately 8oz. of tap water to rinse their mouth out before swabbing. Also swabbing was not to be done immediately after eating or

after brushing teeth. They were asked to swab the four different quadrants of the inner cheeks for each of the six types of swabs provided. Swabbing involved gently rubbing and rotating the swab over a given area of the cheek lining for approximately one minute per swab. After use, the participants were instructed to place the swabs into labeled, sterile plastic tubes without sealing them. The participants were asked to allow the used swabs to sit open and uncapped to air dry at least over night at room temperature. Of the six different types of buccal swabs provided, study participants were asked not to use more than one type of swab per twelve hours. It was preferred that they collect one type of swab per day, to minimize irritation. After all swabs were collected, they were checked for complete dryness. The swabs were then held for 3-7 days prior to testing, to reflect the average length of time involved from sample collection and transport to actual testing in the laboratory. It's important to note that the buccal swabs were self-collected by the study participants and there was no control present for the consistency in collecting the swabs. However, most of the study participants were laboratory employees at Sparrow Hospital and possessed a working knowledge of the importance for uniform sample collection for testing purposes, thereby reducing the variation in sample collection among the study participants.

Once the buccal swabs were received in the laboratory, the first step was to extract the collected buccal cells. The majority of the previous studies involved either rinsing the swabs in saline or incubating them in a cell lysing buffer. This study involved a total of five extraction procedures used with the different types of buccal swabs. The goal was to find a single method that could be used with any of the swabs and that maximized the number of buccal cells available to isolate DNA. The first extraction method utilized Lifecodes' Cell Lysis Buffer to rinse the buccal swabs, and attempts were made to ring out the collected buccal cells. The second extraction method used a ''master mix'' which contained Lifecodes' Protein Lysis Buffer and Proteinase K, (instead of just Cell Lysis Buffer) to do the same thing. The third extraction method involved removing the swab heads and rinsing them with saline washes in Gibco Spinease basket\tubes. The fourth method involved vigorously rinsing the swabs with saline in petri dishes and rubbing the swabs together to remove the buccal cells. The last method involved removing the swab heads and manually manipulating the cells off in saline with tweezers. Not all of these extraction procedures were used with every type of buccal swab due to poor recovery of cells. Some of these extraction methods were only attempted with one or two different swabs and eliminated based on their poor results. The efficiency of cell recovery was determined after the isolation and yield electrophoresis gel steps of the RFLP

DNA typing procedure were performed. Table 1 shows which extraction procedures were used with the various types of swabs. Appendix A details the steps involved with each of the extraction methods.

After buccal cell extraction and DNA isolation with all six types of buccal swabs, three were eliminated due to poor results obtained with the yield electrophoresis gels. The remaining three types of swabs continued the RFLP procedure with DNA restriction, electrophoretic separation, Southern blotting, DNA hybridization, and lumigraph analysis. Appendix B outlines the RFLP procedure used in greater detail.

As part of the RFLP procedure, the lumigraphs produced were analyzed using the Lifeprint Sizing Program. Allele measurements were made for all sample bands including those for blood and buccal swabs. The difference in band sizes for each of the swabs as compared to the blood samples were calculated. This is important because two separate membranes with the same samples and probes must produce band sizing results within 2% of each other to be considered acceptable. This 2% difference represents the ''match window'' which is established by individual laboratories, and allows replicated samples run on separate gels to be declared a ''match''. The difference and percent difference of the band sizes for each of the swabs and their corresponding blood samples were compared for accuracy and reproduciblity. Appendix D contains instructions for using the Lifeprint Sizing Program to analyze lumigraphs.

Table 1-Buccal Cell Extraction Methods

Dacron Swabs

1. Saline Rinse Method
2. Gibco Spinease Basket\Tube Method
*3. Manual Buccal Cell Extraction Method

Felt Swabs

1. Gibco Spinease Basket\Tube Method
2. Saline Rinse Method
3. Master Mix (Protein Lysis Buffer & Pro-K) Method

Wooden Spatulas

1. Master Mix (Protein Lysis Buffer & Pro-K) Method

Large-Pored (Pink) Foam Swabs

1. Cell Lysis Buffer Rinse Method
2. Master Mix (Protein Lysis Buffer & Pro-K) Method
*3. Saline Rinse Method

Small-Pored (Beige) Foam Swabs

*1. Manual Buccal Cell Extraction Method

Nylon Brushes

1. Master Mix (Protein Lysis Buffer & Pro-K) Method
*2. Saline Rinse Method

*Procedures which produced successful yield electrophoresis gel results.

Chapter 4

FINDINGS

The first set of results were obtained after running a yield electrophoresis gel. Initially the yield gel provided information that would determine which of the extraction methods were best for removing the collected buccal cells off of the swabs. Of the five different extraction methods used, the one that yielded the greatest amount of high molecular weight DNA was the one which used saline to rinse and rub off the buccal cells. The other method which was similar in that it also used saline but due to the nature of the swab, tweezers were used to remove the cells, proved to also yield acceptable amounts of high molecular weight DNA. This particular method however, was more time consuming and labor intensive than the first one mentioned. Because both of these methods were able to produce enough high molecular weight DNA to continue on to the restriction steps, they were both used. The second purpose of analyzing yield electrophoresis gel results, was to determine which of the swabs were most efficient for obtaining the most high molecular weight DNA when using these two buccal cell extraction methods. Three of the six original types of buccal swabs were eliminated after the isolation steps, due

to their poor yield gel results. The wooden spatulas, dacron swabs, and felt swabs were the three eliminated. They provided either no results or very faint results after the DNA quality and quantity were assessed by yield electrophoresis gel analysis. The minimum amount of DNA, as estimated from the yield gel, which is necessary to continue on to the restriction and hybridization steps, is 25ng\ul. This DNA must be undegraded and of high molecular weight. Yield electrophoresis gel results were also obtained for the ten blood samples. They all produced adequate amounts of high molecular weight DNA. It must also be noted that the calibration standards used which ranged from 50ng to 300ng, all produced yield gel results that accurately reflected their known amounts of DNA. The calibration control ran on the yield electrophoresis gel also produced accurate results, by representing the 50ng of DNA that the control contained. Figure 1 contains the photographs made of the yield electrophoresis gels.

The next set of results were obtained after the restriction digestion by running a test electrophoresis gel. This determines if complete digestion occurred. Because of the Pst I test gel standards which were run, estimates of the amount of restricted DNA contained in the samples were also made. All of the blood samples were run first. They were all completely digested but they all appeared to contain greater than 500ng of DNA, so they all were diluted. The large-pored (pink) foam swabs, small-pored (beige) foam

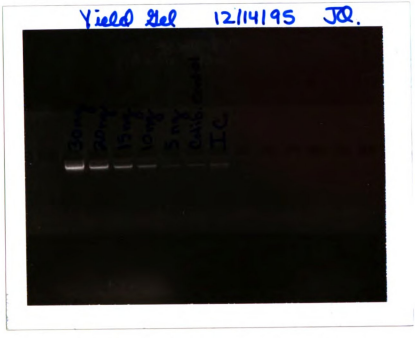

Figure 1.1-Yield Gel Photo (Yield Gel Standards)

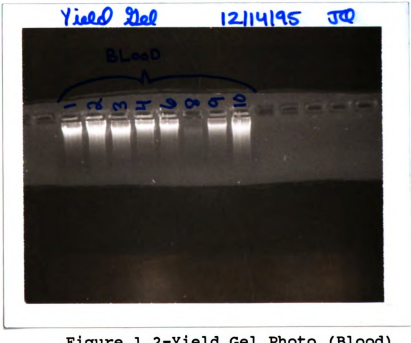

Figure 1.2-Yield Gel Photo (Blood)

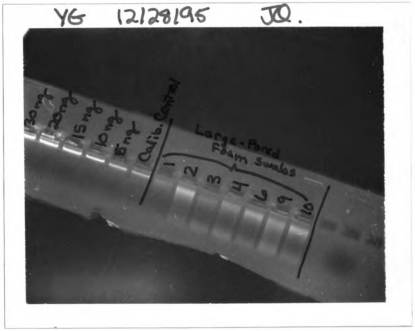

Figure 1.3-Yield Gel Photo (Large-Pored Foam Swabs)

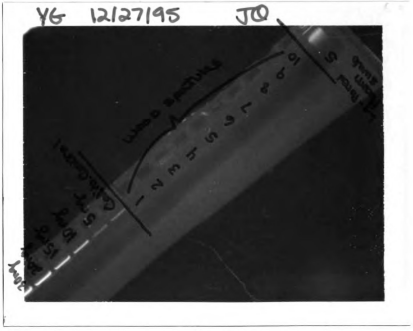

Figure 1.4-Yield Gel Photo (Large-Pored Foam Swabs)

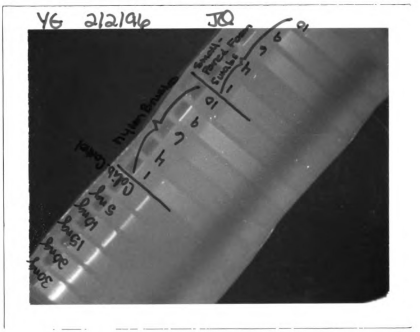

Figure 1.5-Yield Gel Photo (Nylon Brushes & Small-Pored Foam Swabs)

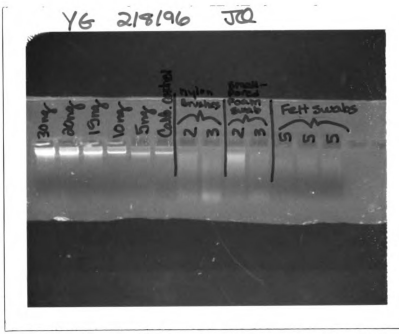

Figure 1.6-Yield Gel Photo (Nylon Brushes & Small-Pored Foam Swabs)

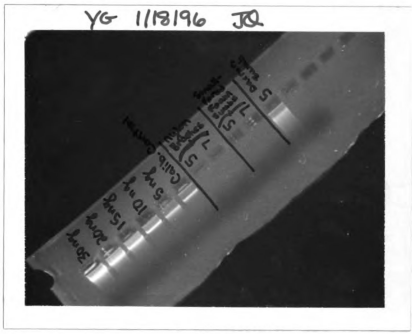

Figure 1.7-Yield Gel Photo (Nylon Brushes & Small-Pored Foam Swabs)

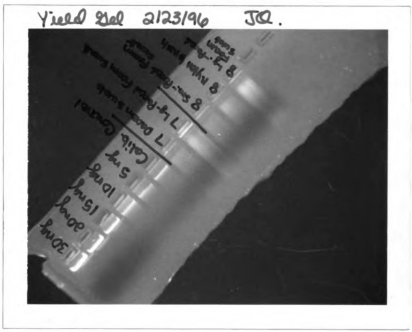

Figure 1.8-Yield Gel Photo (Large-Pored Foam Swabs, Small-Pored Foam Swabs & Nylon Brushes)

swabs, and nylon brushes were the only swabs which were restricted and run on test electrophoresis gel. All three types of swabs produced completely restricted DNA for the ten study participants, with the exception of one individual. One of the study participants scrubbed the inner cheek lining very hard with each of the swabs, causing them all to contain traces of blood. Because the swabs obtained by the other nine participants did not contain blood, the step which involved the Cell Lysis Buffer in the isolation procedure was skipped. This step, however, should have been included for the swabs which contained visible traces of blood. Because hemoglobin was left in the sample and can act as an interfering substance and because the buccal cells were treated harshly, poor test electrophoresis gel and analytical results were obtained. The test electrophoresis gel clearly showed that only low molecular weight DNA was restricted for swabs collected by this one study participant. The majority of the other swabs which were run on the test electrophoresis gel had to be diluted to appear equivalent to the 500ng Pst I test gel standard. Figure 2 contains photographs of the diluted samples reran on test electrophoresis gels.

The next set of results produced were those obtained by analyzing the lumigraphs. The lumigraphs were inspected and there was no evidence of extra bands or any other irregularity produced. The intensity of the sizing ladder was inspected and judged adequate, ensuring proper exposure

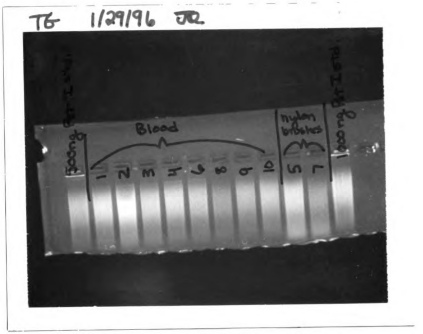

Figure 2.1-Test Gel Photo (Blood & Nylon Brushes)

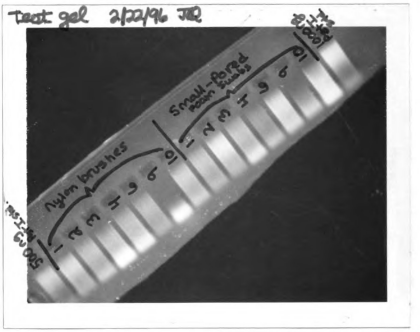

Figure 2.2-Test Gel Photo (Nylon Brushes & Small-Pored Foam Swabs)

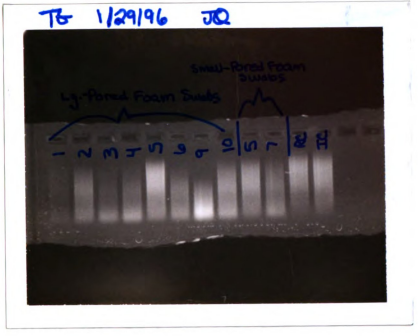

Figure 2.3-Test Gel Photo (Large-Pored Foam Swabs & Small-Pored Foam Swabs)

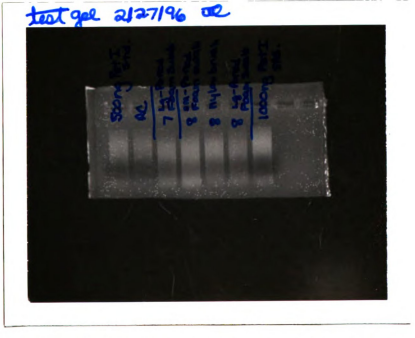

Figure 2.4-Test Gel Photo (Large-Pored Foam Swabs, Small-Pored Foam Swabs & Nylon Brushes)

length of the x-ray films. Then the lumigraphs were successfully analyzed with the LifePrint Sizing Program. The alleles measured for all three internal controls were within accepted limits. Table 2 contains the band sizes measured for these controls and their acceptable ranges. All samples on the lumigraphs that had visible bands present were analyzed. Of the three swabs used beyond the isolation step (large-pored foam swabs, small-pored foam swabs, and nylon brushes), there were nine study participants that produced adequate test electrophoresis gel results, upon which two DNA probes were used, thereby establishing a total of fifty-four sets of bands possible to analyze. Forty-two sets of visible bands were measured. For the eighteen possible sets of bands for each of the three types of swabs, eleven sets were obtained with the large-pored foam swabs, fourteen sets with the small-pored foam swabs, and seventeen sets with the nylon brushes. The twelve samples that produced no results on the lumigraphs were later reprecipitated and run undiluted on analytical gels to see if the samples were too diluted to produce visible bands. The second set of lumigraphs for these samples also produced no visible bands. Table 3 lists these analytical gel results.

The final results are related to the calculations made regarding swab comparisons. For each of the three swabs, allele measurements were compared to the corresponding blood samples. The difference was calculated between these alleles in order to reach conclusions that relate to the

Table 2-Allele Measurements for Internal Controls

Lifecodes' Probes	Control Ranges			Measurements		
	Isolation Control	Restriction Control	Allelic Control	Isolation Control	Restriction Control	Allelic Control
D12S11	12.75-12.25 7.30-7.01	12.75-12.25 7.30-7.01	16.08-15.45 7.67-7.37	12.434 7.120	12.431 7.174	15.748 (1) 7.438 15.781 (2) 7.515 15.905 (3) 7.537 15.866 (4) 7.499
D17S79	3.92-3.77 3.50-3.37	3.92-3.77 3.50-3.37	4.12-3.96 3.63-3.49	3.807 3.411	3.822 3.411	4.048 (1) 3.565 4.046 (2) 3.581 4.053 (3) 3.580 4.026 (4) 3.574

Table 3-Analytical Gel Results for Buccal Swabs

Note: 9 Study Participants
3 Swabs Used (Nylon brushes, Large-Pored Foam Swabs & Small-Pored Foam Swabs)
2 Probes Used (Lifecodes' D12S11 and D17S79)

54 Sets of Bands Possible

Swab Device:	Number of bands Observed: (18 sets possible per swab
Nylon Brushes	17
Small-Pored Foam Swabs	14
Large-Pored Foam Swabs	11

Note: All 42 sets of bands observed ''matched'' within the 2% range requirement for separate gels.

efficiency of the swabs. The percentage difference was also calculated for each swab and blood sample per study participant. For the swab and blood sample to have been considered a ''match'', the percentage difference had to be less than 2%. All of the forty-two sets of bands produced differences less than 2%. Tables 4-6 contain all the allele measurements for each of the three swabs, as well as their comparisons to the alleles obtained with blood samples. Figures 3-6 are duplicates of the original lumigraphs produced.

Table 4-Nylon Brush Alleles vs Blood Alleles

				NYLON BRUSHES					
				Allele Measurements for Swab Comparison					
		D12S11					D17S79		
	Blood	Buccal	Difference	% Diff.		Blood	Buccal	Difference	% Diff.
1	12.486	12.476	0.01	0.08		4.076	4.029	0.047	1.15
	7.886	7.808	0.078	0.99		3.825	3.785	0.04	1.05
2	12.069	12.057	0.012	0.1		4.117	4.053	0.064	1.57
	11.117	11.056	0.061	0.55		3.847	3.803	0.044	1.15
3	11.376	*				3.818	3.773	0.045	1.19
	10.861	*				3.36	3.367	0.007	0.21
4	10.039	9.88	0.159	1.6		3.811	3.806	0.005	0.13
	7.531	7.465	0.066	0.88		3.811	3.806	0.005	0.13
5	11.169	11.156	0.013	0.12		3.587	3.593	0.006	0.17
	10.534	10.442	0.092	0.88		3.337	3.297	0.04	1.2
6	12.209	12.115	0.094	0.77		3.83	3.797	0.033	0.87
	12.209	12.115	0.094	0.77		3.582	3.513	0.069	1.95
7	12.535	12.405	0.013	0.1		3.834	3.829	0.005	0.13
	7.178	7.129	0.049	0.68		3.438	3.426	0.12	0.35
8	12.501	12.435	0.066	0.53		3.839	3.839	0	0
	6.61	6.541	0.069	1.05		3.635	3.644	0.009	0.25
9	11.642	11.515	0.127	1.1		3.815	3.789	0.026	0.68
	6.087	6.032	0.055	0.91		3.593	3.58	0.013	0.36
		*No Results Obtained.							

Table 5-Small-Pored Foam Swab Alleles vs Blood Alleles

			SMALL-PORED (BEIGE) FOAM SWABS					
			Allele Measurements for Swab Comparison					
		D12S11				D17S79		
	Blood	Buccal	Difference	% Diff.	Blood	Buccal	Difference	% Diff.
1	12.486	*			4.076	4.036	0.04	0.99
	7.886	*			3.825	3.787	0.038	1
2	12.069	12.116	0.047	0.39	4.117	4.057	0.06	1.48
	11.117	11.01	0.107	0.97	3.847	3.839	0.008	0.21
3	11.376	11.295	0.081	0.72	3.818	3.816	0.002	0.05
	10.861	10.688	0.173	1.62	3.36	3.366	0.006	0.18
4	10.039	9.891	0.148	1.5	3.811	3.817	0.006	0.16
	7.531	7.438	0.093	1.25	3.811	3.817	0.006	0.16
5	11.169	11.09	0.079	0.71	3.587	3.606	0.019	0.53
	10.534	10.371	0.163	1.57	3.337	3.318	0.019	0.57
6	12.209	*			3.83	3.822	0.008	0.21
	12.209	*			3.582	3.536	0.046	1.3
7	12.535	12.422	0.113	0.91	3.834	3.823	0.011	0.29
	7.178	7.112	0.066	0.93	3.438	3.405	0.033	0.97
8	12.501	12.501	0	0	3.839	3.811	0.028	0.73
	6.61	6.601	0.009	0.14	3.635	3.594	0.041	1.14
9	11.642	*			3.815	*		
	6.087	*			3.593	*		
		*No Results Obtained.						

Table 6-Large-Pored Foam Swab Alleles vs Blood Alleles

			LARGE-PORED (PINK) FOAM SWABS					
			Allele Measurements for Swab Comparison					
		D12S11				D17S79		
	Blood	Buccal	Difference	% Diff.	Blood	Buccal	Difference	% Diff.
1	12.486	12.491	0.005	0.04	4.076	4.035	0.041	1.02
	7.886	7.867	0.019	0.24	3.825	3.807	0.018	0.47
2	12.069	*			4.117	4.053	0.064	1.58
	11.117	*			3.847	3.836	0.011	0.29
3	11.376	*			3.818	3.829	0.011	0.29
	10.861	*			3.36	3.349	0.011	0.33
4	10.039	*			3.811	*		
	7.531	*			3.811	*		
5	11.169	11.178	0.009	0.08	3.587	3.589	0.002	0.06
	10.534	10.503	0.031	0.3	3.337	3.334	0.003	0.09
6	12.209	*			3.83	*		
	12.209	*			3.582	*		
7	12.535	12.473	0.062	0.5	3.834	3.854	0.02	0.52
	7.178	7.15	0.028	0.39	3.438	3.425	0.013	0.38
8	12.501	12.501	0	0	3.839	3.832	0.007	0.18
	6.61	6.546	0.064	0.98	3.635	3.599	0.036	1
9	11.642	*			3.815	3.83	0.015	0.39
	6.087	*			3.593	3.593	0	0
		*No Results Obtained.						

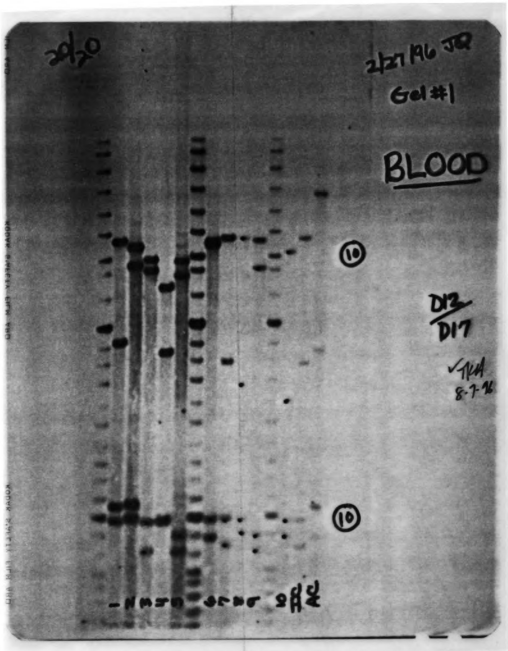

Figure 3-Analytical Gel #1 (Blood)

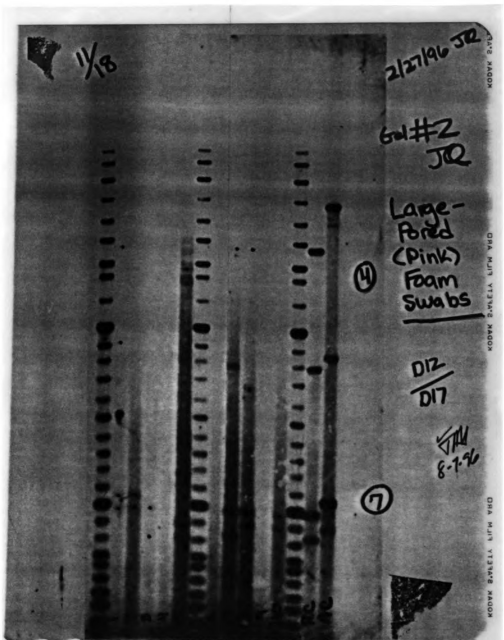

Figure 4-Analytical Gel #2 (Large-Pored Foam Swabs)

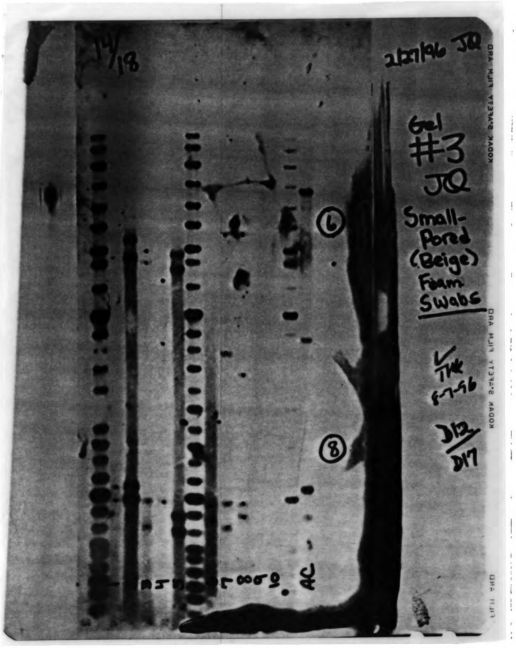

Figure 5-Analytical Gel #3 (Small-Pored Foam Swabs)

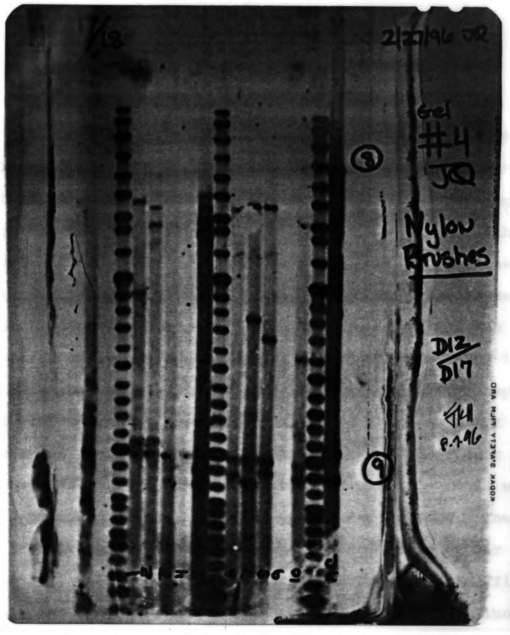

Figure 6-Analytical Gel #4 (Nylon Brushes)

Chapter 5

CONCLUSIONS AND RECOMMENDATIONS

There were five initial methods used to extract the collected buccal cells from the swabs. The most successful method involved rinsing the swabs in saline and rubbing them together to remove the cells. The less successful methods involved using solutions that were intended to lyse the cells while still attached to the swabs, in order to collect their contents. The saline rinse method, however, was used to first collect the whole buccal cells then to later isolate the DNA inside. After the isolation step was completed and yield electrophoresis gels were analyzed, three of the six types of swabs were eliminated based on their poor results. There were faint or no results seen, or there was only degraded or low molecular weight DNA present. The lack of isolated high molecular weight DNA is due to either a failure in collecting enough buccal cells or with difficulty isolating the DNA present in the collected cells. Also, with the isolation step it's very important to note that Cell Lysis Buffer must be used with any swab that contains traces of blood.

All of the forty-two sets of bands found on the lumigraphs were measured and the percent difference was

calculated for the particular swab and the corresponding blood sample. Each of these percent difference calculations were less than 2%, which established them as ''matches''. Therefore, this research confirms the findings of previous studies which state that buccal cell are as capable of producing accurate and reliable DNA results as blood samples. The twelve samples that produced no results were reprecipitated and run again undiluted on analytical gels. This confirmed that the lack of results was due to insufficient amount of DNA present in the samples, not to improper amounts of sample loaded into the gels. This reduces the chance of error associated with performing the RFLP procedure and confirms that the problem is with the original collection steps involving the buccal swabs. This would include a buccal swab device that produced poor DNA results due to low buccal cell collection and\or extreme abrasiveness by the swab which caused damage to the DNA present.

When reviewing the final results of the lumigraphs, it is clear that nylon brushes produced the greatest number of visible bands. The nylon brushes were also the easiest to work with when extracting buccal cells. Study participants commented that the nylon brushes were less bulky to use than other swabs, and that they were painless during buccal cell collection. The nylon brushes are also one of the most cost effective buccal swab devices. Therefore, based on all of the findings of this research, nylon brushes are the

recommended device to collect buccal cells for the purpose of RFLP DNA analysis.

There are a few recommendations to be made for the future use of buccal swabs for RFLP DNA testing. There are some practical comments which address the actual collection. First, it is highly recommended that more than four buccal swabs be collected, six to eight swabs prove to be more efficient with actual cases. Since this research project, Sparrow Hospital paternity laboratory has implemented this buccal swab procedure. It has been found that four swabs do not always produce consistent results. Perhaps the study participants in this research project were more thorough when collecting the buccal cells than actual patients and clients involved in the testing. Secondly, it is recommend that buccal swabs collected off-site be sent back to the laboratory by express delivery service. This is because not all swabs were being properly dried as the collection procedure instructed before being sent back to the laboratory. If swabs are received shortly after collection, then possibilities of bacterial contamination due to improper drying can be minimized.

Recommendations for future research projects using buccal swabs would include studies involving cadavers. Success with this type of sample collection would prove invaluable for DNA testing on bodies found at crime scenes. Also valuable, would be a similar comparative study of different types of vaginal swabs used in collecting samples

with rape kits. Perhaps certain types of swabs would be more successful in collecting sample material. Finally, future studies involving buccal swabs should include any differences in DNA results between people who possess dental caries and those who do not. It may be suggested that people with more dental decay may contain greater bacterial levels in their mouth which could possibly affect the quality and quantity of DNA obtained with buccal swabs.

APPENDIX A

APPENDIX A

BUCCAL CELL EXTRACTION METHODS

A. CELL LYSIS BUFFER RINSE METHOD

1. In a sterile plastic petri dish thoroughly rinse all collected buccal swabs with Lifecodes' Cell Lysis Buffer. This is done by submerging the swabs in the Cell Lysis Buffer and rubbing them together to cause the buccal cells to come off.

2. After all swabs have been rinsed, use a plastic disposable pipet to transfer the Cell Lysis Buffer\ buccal cell mixture to a sterile 14ml plastic centrifuge tube.

3. Centrifuge the Cell Lysis Buffer\buccal cell mixture at 1000rpm for ten minutes at room temperature.

4. With a plastic disposable pipet remove the supernatant and discard. Transfer the buccal cell pellet to a properly labeled 1.7 ml plastic microcentrifuge tube.

5. Centrifuge the tube in a microcentrifuge for two minutes on high speed (14,000 x g) at room temperature.

6. With a plastic disposable pipet decant the supernatant and discard.

7. Add 1.0ml Lifecodes' Protein Lysis Buffer to the microcentrifuge tube. Vortex the contents of the tube for two minutes. Use a pipetor and plastic tips to manually resuspend the pellet and to break up any remaining clumps of the pellet. Allow to sit on ice for ten minutes.

8. Microcentrifuge for two minutes on high speed at room temperature. Decant the supernatant and blot the tube dry. Keep the tubes on ice.

9. Immediately before use, prepare a master mix of 225ul Protein Lysis Buffer and 25ul PRO-K per sample, plus two extra aliquots to compensate for pipetting tolerances. Master mix MUST ALWAYS BE KEPT ON ICE.

10. Processing one sample at a time: Add 250ul of master mix to the pellets. Use a pipetor and small plastic tips to thoroughly resuspend the pellet. Vortex the tube contents for two minutes.

11. Place the microcentrifuge tubes in a 65°C heat block. Begin a two hour incubation after the last tube is added to the heat block. Vortex the tubes every 15-20 minutes to resuspend the pellet.

12. Vortex the tubes vigorously for 30 seconds following the complete incubation. Microcentrifuge for two minutes on high speed at room temperature.

NOTE: The procedure may be stopped at this point and samples may be stored at 4°C.

13. To continue, determine the DNA quantity and quality from a yield electrophoresis gel analysis by following Lifecodes' RFLP DNA testing protocol.

B. MASTER MIX (PROTEIN LYSIS BUFFER AND PRO-K) METHOD

1. In a sterile plastic petri dish use a sterile scalpel knife to cut off the swab heads. Place the pieces of the swabs into a 5ml plastic tube.

2. Immediately before use, prepare a master mix of Lifecodes' Protein Lysis Buffer and Pro-K (made with a proportion of 1:9 Pro-K to Protein Lysis Buffer) allowing for 2ml per sample tube. Master mix MUST ALWAYS BE KEPT ON ICE.

3. Add 2ml of master mix per tube and submerge all pieces of swab in the solution.

4. Place tubes in a 65°C heat block. Begin the two hour incubation after the last tube is added to the heat block. Vortex the tubes contents every 15-20 minutes. Use a sterile plastic pipet to push swab pieces down into the bottom of the tube and in the solution.

5. After the completed incubation, use a sterile plastic pipet to express the supernatant from the swab pieces. Transfer the supernatant to a properly labeled micro-centrifuge tube.

6. Vortex the contents of the tube vigorously for 30 seconds. Microcentrifuge for two minutes on high speed at room temperature.

NOTE: The procedure may be stopped at this point and samples may be stored at 4°C.

7. To continue, determine the DNA quantity and quality from a yield electrophoresis gel analysis by following Lifecodes' RFLP DNA testing protocol.

C. GIBCO SPINEASE BASKET\TUBE METHOD

1. With a sterile scalpel knife, cut the swab head off and into small pieces and place into a Gibco Spinease basket\tube.

2. Fill the basket\tube containing the swab pieces with sterile saline and allow to sit at room temperature for ten minutes (for the swab pieces to absorb the saline).

3. Vortex the basket\tubes vigorously for five minutes. Microcentrifuge the basket\tubes for two minutes on high speed at room temperature.

4. Use a plastic disposable pipet to transfer the saline\ buccal cell mixture collected in the bottom of the basket\tubes to a properly labeled sterile 1.7ml microcentrifuge tube.

5. Repeat this saline rinse three times with the same buccal swab pieces to assure a thorough collection of buccal cells.

6. Microcentrifuge the tubes that contain the saline\ buccal cell mixtures for two minutes on high speed at room temperature.

7. Use a plastic disposable pipet to discard the saline supernatant.

8. Add 1.0ml Protein Lysis Buffer to the buccal cell pellets that remain in the microcentrifuge tubes. Vortex for two minutes. Use a pipetor with plastic tips to manually break up any remaining clumps of the pellet. Allow to sit on ice for ten minutes.

9. This procedure continues exactly as the protocol for using the Cell Lysis Buffer extraction method, beginning with step #8.

D. SALINE RINSE METHOD

1. In a sterile plastic petri dish thoroughly rinse all collected buccal cells off the swabs with sterile saline. This is done by scrubbing two swabs together while immersed in 10ml of sterile saline. Using a sterile plastic disposable pipet transfer the saline\ buccal cell mixture into a sterile 14ml plastic centrifuge tube.

2. Centrifuge the saline\buccal cell mixture at 1000rpm for ten minutes at room temperature.

3. Use a plastic disposable pipet to remove the saline supernatant and discard. Transfer the buccal cell pellet to a properly labeled 1.7ml microcentrifuge tube.

4. This procedure continues exactly as the protocol for using the Cell Lysis Buffer exaction method, beginning with step #5.

E. MANUAL BUCCAL CELL EXTRACTION METHOD

1. Using sterile metal tweezers pull the swabs heads off and place into a sterile plastic petri dish.

2. Add 5ml of sterile saline and use the tweezers to pull apart the swab material to make the buccal cells come off the swabs.

3. Use a plastic disposable pipet to transfer the saline\ buccal cell mixture to 14ml sterile plastic centrifuge tube.

4. Re-rinse the swab heads a second time with sterile saline and again use tweezers to manipulate off the buccal cells. Add the additional saline\buccal cell mixture to the centrifuge tube.

5. Centrifuge the saline\buccal cell mixture for ten minutes at 1000rpm at room temperature.

6. Use a plastic disposable pipet to remove the saline supernatant and discard. Transfer the buccal cell pellet to a properly labeled 1.7ml microcentrifuge tube.

7. This procedure continues exactly as the protocol for using the Cell Lysis Buffer exaction method, beginning with step #5.

APPENDIX B

APPENDIX B

RFLP DNA PROCEDURE

The first step of the RFLP DNA tying procedure was to isolate DNA from cells. The nucleated cells in obtained samples were subjected to strong detergents which are hypotonic buffered solutions that break open the cellular membranes and expose the nucleus. These same solutions cause non-nucleated red blood cells to lyse, and therefore eliminate hemoglobin which can interfere with the RFLP method. Lifecodes' Cell Lysis Buffer was used for this purpose. It is important to note that the nuclear membranes which encase the nucleus and contain DNA were still intact at this point. This first step using Cell Lysis Buffer was only used with the blood samples because of the amount of red cells present. From the beginning of the isolation step to the end of the RFLP procedure, all samples were kept at 4° C. This was to avoid the introduction of bacterial nucleases that could destroy the integrity of the DNA contained in the sample. Processing of the buccal cells began here, with an incubation in Lifecodes' Protein Lysis Buffer. The next step was to incubate all the samples in a ''master mix'' of Lifecodes' Protein Lysis Buffer (a buffered salt solution) and Proteinase K (a digestive enzyme) for two hours at 65° C. This caused the nuclear membrane to lyse and removed endogenous nucleases as well

extraneous cellular proteins found in the sample. The purpose of the isolation step was to obtain free DNA that can later be digested with restriction enzymes. An isolation control was assayed. It is one of the three major quality control points throughout the RFLP procedure. Processing of this control began at the isolation step and it was run along with the samples. In the end, the DNA band patterns results should match the established known data for the particular lot of isolation control used. This assures that the isolation steps were performed properly.

To determine the quantity and quality of the free DNA present after the isolation steps, a yield electrophoresis gel was prepared. A portion of the sample containing the DNA was mixed with a yield gel loading buffer which contained bromophenol blue. This blue dye binds to the DNA and allows it to be visualized during electrophoresis. Samples were then loaded into yield electrophoresis gels made of agarose. Appendix C outlines the procedure used to prepare the agarose gels. The yield electrophoresis gels contain ethidium bromide which also binds to the DNA and will fluoresce under ultraviolet light. The purpose of the bromophenol blue was to allow visual monitoring of the electrophoresis process. The ethidium bromide was used to visualize the electrophoretically separated DNA fragments. The band size and intensity of the fragments were compared to a set of yield calibrators which were run along with the samples. These calibrators contained known amounts of DNA,

ranging from 50ng-300ng. They were validated by properly running a calibrator control which contained 50ng of DNA. By visual comparison, the amount of DNA present in the samples was estimated. If the samples contained much less than 50ng of DNA, additional sample would need to be processed and combined with the first aliquot. If the sample contained more than 300ng of DNA, it would have to be diluted with Protein Lysis Buffer. It is important to note that a yield electrophoresis gel cannot distinguish between human and non-human DNA. It is possible that bacterial DNA could have contaminated a sample, and it wouldn't be known until the hybridization steps which use human specific DNA probes. The quality of DNA was also assessed by visual inspection of the yield electrophoresis gel. High molecular weight DNA is represented by a tightly formed band near the top well on the gel. Degraded lower molecular weight DNA will travel farther away from the loading well on the gel, and appear as a smear because of the series of different fragment lengths. The yield gel was electrophoresed for one hour at 50 volts. After electrophoresis, under an ultraviolet light source, a permanent record of the yield gel was made by a photograph. Figure 1 contains photographs of the yield electrophoresis gels.

The restriction step took place after it was assessed that enough high molecular weight DNA was present. Because the restriction digestion of 5ug of DNA per sample is necessary for the probe system being used and the yielded

sample concentrations were much higher, the DNA had to be appropriately diluted with distilled water. Lifecodes uses the restriction enzyme Pst I which recognizes the six nucleotide sequence CTGCAG in the targeted DNA. Next a ''master mix'' of nuclease-free water, digestion buffer, spermidine, and Pst I was added to each sample and incubated at 37°C for two hours. After the DNA was digested, any protein impurities were precipitated out with conditioning salts that contained lithium chloride. It's important to precipitate out non-DNA proteins to prevent bandshifting during the analytical gel electrophoresis step. Then the DNA was precipitated out with a series of 95% and 70% ethanol washes. Finally, the DNA was resolubilized with sterile water. A restriction control must be assayed with every batch of samples to ensure accurate restriction. Processing of this control begins with the restriction steps.

To determine the completeness of the restriction digestion with Pst I, a test electrophoresis gel was run. Incomplete or partial digestion may occur due to a poor restriction enzyme to DNA ratio, incorrect incubation temperature, or insufficient incubation time. A portion of each restricted DNA sample was mixed with loading buffer to visually inspect the electrophoresis process. These mixtures were loaded into the same agarose gels used for the yield electrophoresis gels. Appendix C contains details on

making agarose gels. Once samples were loaded into the test gel, they electrophoresed at 50 volts for one hour. Because test electrophoresis gels also contain ethidium bromide, the digestion patterns were visualized under an ultra-violet light source. A completely digested sample appears as a uniform smear. An incompletely digested sample appears as a tight band that remained near the loading well. DNA samples that are not completely digested can be reprecipitated in ethanol and redigested with Pst I and run again on a test electrophoresis gel. With the samples, two Pst I test gel standards were run. One is equivalent to 500ng of Pst I digested DNA and the other is equivalent to 1000ng. By comparing the intensity of the sample bands to the two standards, an estimation of the amount of digested DNA in each sample was made. The reliability of the Pst I restriction enzyme was confirmed by obtaining expected results for the restriction control which was run with the samples. The restriction control should appear equivalent to the 500ng Pst I test gel standard. Samples that did not have approximately 500ng of restricted DNA had to either be diluted with loading buffer or have additional aliquots of restricted DNA added. All diluted samples were rerun on test electrophoresis gels to verify their concentrations. Once again, a permanent record was made by photographing the test electrophoresis gels. Figure 2 contains photographs of test electrophoresis gels.

The next step of the RFLP procedure involves the analytical gel electrophoresis. This is when restricted DNA fragments get sorted by size. An allelic control which produces known band patterns, was also run on every analytical gel to assure that electrophoresis conditions were acceptable. The isolation and restriction controls which have been processed along with the samples, were only loaded onto one gel to continue their RFLP testing. All samples and controls were mixed with specific amounts of loading buffer and loaded onto 12cm x 27cm analytical gels. Refer to Appendix C for instructions on preparing analytical gels. Sizing standards were loaded between samples. These sizing standards contain bacterial viral DNA fragments of known lengths in loading buffer. When run on analytical gels they create a sizing ladder from large to small on the final lumigraphs, which are used to determine DNA fragment size of the samples. The samples and controls were loaded at the negative electrode end of the agarose gel. The agarose gels were placed in electrophoresis gel boxes with Lifecodes' Gel Buffer. For sixty-five hours the gels were allowed to electrophorese at 14.5 volts. The electrical current flowing through the gels cause the negatively charged DNA fragments to migrate towards the positive electrode. The smallest DNA fragments migrate faster and are found farther down the gel. The larger fragments travel slower and are located closer to the wells they were loaded into. After the gels had electrophoresed for an hour

circulatory pumps were turned on. They were used to circulate the gel buffer to maintain an even current throughout all gels contained in the box.

After electrophoresis, the separated DNA fragments were transferred from the agarose gels to the surface of a thin nylon membrane. This step is called Southern Blotting. It's necessary to transfer the DNA because the agarose gel is too fragile for the next step that involves hybridization. To prepare for the next step, the separated DNA fragments must be made single-stranded. This was done by soaking the gels in a denaturing solution for approximately half an hour. The denaturing solution is very alkaline and contains a caustic soda (sodium hydroxide) which causes double-stranded DNA to separate. The gels were then soaked in a neutralization buffer which is a strong buffered solution containing a high salt concentration. This is necessary because the DNA will not bind to the nylon membrane in an alkaline environment. The transfer set-up for each gel involved three sponges and two sheets of blotting paper saturated in neutralization solution. The gels were gently laid on top and any air bubbles between the layers were carefully removed, to ensure complete contact. The labeled nylon membrane was placed on top of the gel and air bubbles removed. Two additional blotting papers saturated with neutralization solution were placed on the nylon membrane. Finally, two inch stacks of paper towels were placed on top next with a Plexiglas weight. The

transfer occurred for three hours. The DNA fragments were transferred by capillary action due to the neutralization solution which "pulled" the DNA fragments from the gel onto the nylon membrane. The membrane is a permanent record of the separated DNA fragments. After the transfer, the membranes were baked at 80° C for one hour and exposed to ultra-violet light for ninety seconds. This baking and UV light cross-linking caused the DNA on the membrane to become fixed.

The next step of the RFLP procedure involved nucleic acid hybridization. The targeted DNA fragments which were fixed to the membrane, were detected by hybridizing them with DNA probes of identical sequence. DNA probes are single-stranded DNA which are complimentary to the core sequence of the VNTR target. The two probes used in this study were supplied by Lifecodes. The probes used were chemiluminescent probes. They are alkaline phosphatase (AP) labeled and will create a chemiluminescent reaction when sprayed with a chemical called LumiPhos 480. This reaction will cause a development of x-ray film upon exposure and appears as darken bands. The D12S11 probe was used because it has an allelic size range from 3.0-26.0 kb, and it includes larger sized VNTRs. The D17S79 probe has a smaller allelic size range from 2.0-7.0 kb, and was used because it includes smaller sized VNTRs. Because these two probes cover opposite allelic ranges, they were contained in a

single hybridizing solution. The first step involved placing the fixed membranes into the probing solution and incubating them at 55°C for twenty minutes. Then a series of washes were performed using solutions with varying stringency conditions to remove any probe non-specifically bound to DNA or the membrane. Stringency conditions involve the temperature and ionic strength of the wash solutions. Stringency is important because it affects the sensitivity and specificity of hybridization. High stringency is accomplished with high temperatures and low ionic strength solutions. It promotes specific and complete binding of the probes to the targeted DNA sequences, and removes any probe weakly bound in mismatches. The first two washes were with a slightly lower stringency solution which removed any residual loosely bound probe. The second two washes were with a higher stringency solution that removed probe which was bound in mismatches. After these washes the membranes were placed into Lifecodes' Quick-Light Buffer, which is an alkaline reaction buffer that supports the chemiluminescent reaction. The membranes were then sprayed with LumiPhos 480 which contains a chemiluminescent substance reacts with the alkaline phosphatase on the probes in the reaction buffer, to begin the chemiluminescent reaction. The membranes were sealed in plastic folders and placed into x-ray film cassettes. In a darkroom, x-ray film was added to the cassettes and exposed to the chemiluminescent reaction

occurring on the membranes. The x-ray film was developed in an automated film processor after twenty-one hours of exposure. The DNA profiles were seen on the lumigraphs as patterns of blackened bands. Figures 3-6 contain duplicate copies of the four lumigraphs produced.

The final step in the RFLP procedure was the visual inspection and interpretation of the lumigraphs. The visual inspection was performed first to ensure the quality of results obtained. All samples were checked for the number of bands produced by each DNA probe, which represented the number of hybridized alleles. Each sample should only have one or two alleles per probe. This is based on classical Mendelain genetics, which explains that an individual inherits one allele from its' mother and one from its' father. Therefore, only one or two bands can represent an allele of a single locus. There would be one band on the lumigraph if the individual inherited the exact same allele from both of its' parents. If more than two bands appear per sample, a possibility of contamination or mixing of samples could have occurred. Multiple bands could have also been produced if problems with restriction digestion occurred. If partial digestion occurred with samples it may appear as though extra alleles were present. Extra bands on the lumigraph can also happen when star activity takes place. Star activity is the result of excessive restriction by the restriction enzyme used. To be assured of the complete and specific digestion of samples, the internal

controls (isolation, restriction, and allelic controls) were all checked for the presence of extra bands. The lumighaphs were also evaluated for the proper length of exposure to the chemiluminescent reaction. If the lumigraphs appear faint, then additional developments of x-ray film need to be done with longer exposure times.

After the lumigraphs had been inspected and the quality of results assured, the process of interpretation began. The lumigraphs were analyzed by using the LifePrint Sizing Program which is computer-aided. The program assists in measuring the sizes of the bands on the lumigraphs. The band sizes are measured based on the inverse relationship that exists between the fragment's size and the migrational distance traveled by the fragment during electrophoresis. The larger sized fragments which contain more copies of the targeted VNTR repeats, move slower and less distance. The smaller fragments with fewer repeat sequences will travel faster and farther down the gel during electrophoresis. To establish the exact relationship between migrational distance and size, bands must be compared to molecular weight standards. The bands on the lumigraphs were read by a digitizing light box attached to a computer with the Lifeprint Sizing Program. The positions of the bands on the lumigraphs were digitized then analyzed by the sizing program. By using the LifePrint Sizing Program the bands produced on the lumigraphs were compared to the Lifecodes sizing ladder. This ladder is made up of a series of

molecular weight standards which produce fragments of known size. Accurate measurements of sizing ladder alleles reflect a properly performed RFLP procedure. The measured alleles for the sizing standards must be within 2% of their known values to ensure accurate sample results. After the allele measurements were verified for the sizing standards, then allele measurements were made for the sample bands. The measurements for the sample bands were compared to the known positions of the sizing standards. The band positions were then interpreted by their corresponding fragment size. All the lumigraphs were read and all the band sizes were recorded. To validate sample results, the band sizes for each of the three internal controls (isolation, restriction, and allelic controls) were compared to their known values. Table 2 contains the measured and expected alleles for all three internal controls. Appendix D contains instructions for using the Lifeprint Sizing Program for lumigraph analysis. For exact sample and reagent amounts, dilutions, and detailed procedural steps of the RFLP method refer to Lifecodes' Quick-Light Paternity Identity Manual.

APPENDIX C

APPENDIX C

PROCEDURE FOR THE PREPARATION OF AGAROSE GELS

NOTE: Yield gels, test gels, and analytical gels all utilize 0.6% agarose. These instructions are for preparing one 12 x 15cm (100ml) or one 12 x 27cm (200ml) gel. For multiple gels, the amounts of agarose and buffer need to be adjusted proportionally. Yield gels and test gels can be made in either 12 x 15cm or 12 x 27cm gel trays. Analytical gels must always be made in 12 x 27cm gel trays.

NOTE: Lifecodes Gel Buffer concentrate must be diluted 1:40 with distilled water before use.

1. Determine the number and sizes of gels needed.

2. Prepare gel trays by tapping the two open ends.

3. Measure diluted Gel Buffer (see table below for amounts) in a graduated cylinder and pour it into an Erlenmeyer flask large enough to hold at least twice the volume of the buffer to be added.

	YIELD/TEST GELS		ANALYTICAL GELS
	Sm100ml)	Lg(200ml)	Lg(200ml)
Amount agarose	0.6g	1.2g	1.2g
Amount Gel Buffer	100ml	200ml	200ml
Ethidium Bromide	5ul	10ul	----

4. Weigh the correct amount of agarose and add it to the flask, then swirl gently. Heat on top of a heated stir plate until all agarose crystals are completely dissolved. The solution should be colorless.

5. After heated, pour the solution into a graduated cylinder to check the volume. Add distilled water to replace any volume lost during the heating.

6. For yield and test gels, return the solution to the flask and add 5ul of ethidium bromide per 100ml of gel. Then swirl the flask to mix thoroughly.

7. Pour the liquid agarose into the center of the gel tray. The trays must be on level surfaces. Remove any bubbles in the agarose with a small metal spatula. Place plastic combs into position (one to four combs may be used for yield and test gels).

8. To identify analytical gels, a small piece of paper with the gel number (written in pencil) is dropped into the bottom right hand corner immediately after pouring gels. This label will not interfere with the electrophoresis or blotting processes.

9. Allow gels to solidify for sixty minutes before use. The combs are removed just before the gels are to be loaded. Gels can be stored in a plastic bag in a refrigerator for a maximum of three days.

APPENDIX D

APPENDIX D

LIFEPRINT SIZING PROGRAM FOR LUMIGRAPH ANALYSIS

1. Call the DNA directory to the PC. After the C:\> type: cd\DNAPHI91.

2. Begin the DNA sizing program. After the C:\DNAPHI91> type: D.

3. ''LIFEPRINT (tm) SIZING PROGRAM FOR PATERNITY'' will appear on the screen. Press the digitizer button once to ensure proper hook-up. If a beeping sound is not heard, check connections and start the program again.

4. The computer will prompt you for optional information. Press ''enter'' to skip or type the day's date, press ''enter''. Type the date the gel was run, press ''enter''; type the initials of the person who ran the gel, press ''enter''. If the information was entered correctly, press ''enter''; if any information is incorrect, move the cursor to ''no'' and type new information in the appropriate areas.

5. The program requires the input of a gel number, which will become part of the file name under which the data is stored. Type the gel number without using a hyphen and the last two numbers of the current year, press ''enter''.

6. The program also requires the initials of the person who is sizing the lumigraph, and this becomes the other part of the file name. Type the initials and than press ''enter''.

7. The next screen will present the name of the file currently being created. If any lumigraphs have previously been sized under the same file name, you have the choice to:
   a. overwrite the previous named file,
   b. add what you are currently sizing to the existing file, or
   c. rename the file that you are currently creating by reentering the gel number and you initials.

   Choose the appropriate option, and press ''enter''. If there are no existing files with the same name, simply press ''enter''.

8. The next screen requests that the number of cases on the lumigraph be entered. The program will automatically flank each case with standard size marker lanes. Type the number of cases and type ''enter''.

9. For the first case on the lumigraph (starting on the left side), choose the option that accurately describes the type of case. Move the cursor to the appropriate number and type ''enter''. Next, type the case number and press ''enter''.

   Note: If you choose option four ''none of the above'' you will be expected to first enter the number of lanes on the lumigraph that the case occupies then enter the case number. After the case number is entered each lane is displayed on the monitor. Then it is necessary to type in a suffix for each lane in the order as it appears on the lumigraph. For paternity cases use the following suffixes: -10 for mothers, -20 for children, and -30 for alleged fathers. After each suffix, press ''enter''.

10. After each case is described, there is an option to enter the race of the mother and alleged father for paternity cases. Move the cursor to the appropriate choice and press ''enter''.

11. The next screen gives the option to edit information regarding case numbers and race. If all information is correct press ''yes''.

12. The next screen allows for the probe to be chosen, whose alleles (represented by bands on the lumigraph) for each sample lane will be sized. Move the cursor to the appropriate choice and press ''enter''.

13. On the next screen are numbers corresponding to the sizing standards used. To determine what standards need to be included, look for the highest allele and the lowest allele of the probe currently being sized (in all of the sample lanes on the lumigraph). Then choose a standard band which is higher than the highest allele as the standard with the greatest size, and do the same for the standard with the least size. Choose a range that includes at least seven size standard bands, but no greater than twenty-five bands to get an accurate determination for the goodness of fit.

SIZING THE STANDARDS

14. The proper order for sizing the bands on the lumigraph, is to start with the band of greatest size and to proceed sizing each band in numeric order until you reach the one with the smallest size. When "ENTER STANDARD #____" appears on the screen, center the cross-hairs of the mouse on the appropriate band in the standard lane and press the button on the mouse.

15. Complete the sizing of all the bands in the lane. The screen will then provide information in columns, under the heading "LANE NO 1". The goodness of fit is displayed below the numbers on the columns. The goodness of fit must be below 2.00 in order to proceed. If the goodness of fit is greater than 2.00, answer "no" to the question that asks if you want to go on. The standard lane can be resized. If the goodness of fit is less than 2.00, answer "yes" and "enter".

16. Repeat the sizing procedure for the remaining standard lanes.

SIZING THE ALLELES IN THE SAMPLE LANES

17. For each sample lane, the screen displays a table with the following headline: "Lane No__DIGTIZE sample #__" The lane number corresponds to one of the seventeen possible lanes from the gels, in which samples were loaded.

18. The next step involves sizing the sample lanes. If the sample lane contains only one band (homozygous) for the particular probe being used, center the mouse on the band and hit the button one time. If the sample lane contains two bands (heterozygous) size each band once, starting with the greatest size and proceeding to the lowest size.

19. Once the lowest sized band is finished, the cross-hairs on the mouse must be placed within one inch of the left hand side of the digitizer and press the button. This will indicate that the sizing of the lane is complete, and allows the next sample lane to be sized. Results are to be recorded on corresponding worksheets before proceeding the next allele.

20. Repeat the same procedure for all sample lanes until the last sample on the lumigraph has been completed, which should always be the control lane.

21. The sizes of the alleles that are determined for the control lane must be within 2% variation compared to the expected sizes, otherwise repeat the sizing.

    Note: If the control can not be sized within the expected range, the entire gel must be re-run. No sizing are to be used from a gel where the standards may be incorrect.

22. After completing the sizing of all samples, if the lumigraph contains more than one probe system, the opportunity arises to size the alleles of additional probe systems. By answering "no" to the question that asks if you are done, you are brought back to the screen that asks for information about the probe system being used. If there are no additional probes, answer "yes" to the question of being done.

23. Print the sizing information if necessary .

APPENDIX E

APPENDIX E

REAGENTS AND SUPPLIES

A. REAGENTS FROM LIFECODES, INC.

1. Cell Lysis Buffer: contains sucrose, magnesium chloride, and Triton X-100 in Tris buffer. Store at 2-8° C.

2. Protein Lysis Buffer: contains sodium EDTA and sodium chloride in Tris buffer. Store at 2-8° C.

3. Pro-K: contains Proteinase K in Tris buffer. Store at -20° C.

4. Gel Buffer: contains sodium EDTA in Tris-acetate buffer as a 40X concentrate. Dilute 1:40 with distilled water to prepare a working gel buffer. Store at room temperature.

5. Yield Calibrator set: includes calibrators containing lambda DNA in loading buffer at concentrations of 30ng\ul, 20ng\ul, 15ng\ul, 10ng\ul, and 5ng\ul. Store at 2-8° C.

6. Calibrator Control: contains 10ng\ul of high molecular weight human DNA in loading buffer. Store at 2-8° C.

7. Ethidium Bromide: in a 10mg\ml concentration. Store at room temperature. (Caution: Mutagen)

8. Conditioning Salt: contains lithium chloride. Store at room temperature.

9. Pst I Enzyme: contains 50 U\ul Pst I, Tris-hydrochloric acid, sodium chloride, EDTA, bovine serum albumin, and B-mercaptoethanol in Tris buffer. Store at -20° C.

10. Digestion Buffer: contains sodium chloride, magnesium chloride, bovine serum albumin and B-mercaptoethanol in Tris buffer. Store at -20° C.

11. Spermidine: contains a diluted aqueous solution of spermidine. Store at -20° C.

12. Hybridization Solution: contains polyethylene glycol, sodium chloride, sodium phosphate, EDTA, sodium dodecyl sulfate, sodium heparin and herring testes DNA. Store at 2-8° C.

13. Wash Component A (SSPE): contains sodium chloride, sodium phosphate and EDTA as a 25X concentrate. Contains sodium azide. Store at room temperature.

14. Wash Component B (SDS): contains sodium lauryl sulfate (SDS) as a 20% solution. Contains sodium azide. Store at room temperature.

15. Loading Buffer: contains Ficoll 400, bromophenol blue, and sodium EDTA in Tris-acetate buffer. Store at room temperature.

16. Pst Test Gel Standard: contains 50ng\ul of Pst I digested K562 human DNA in Loading Buffer. Store at -20° C.

17. Allelic Control: contains 50ng\ul of Pst I digested K562 human DNA in Loading Buffer. Store at -20° C.

18. Sizing Standards: contains DNA fragments of various lengths originating from PhiX, M13, Lambda and T7 phage in Loading Buffer. Store at -20° C.

19. Lumi-Phos 480: Store at 2-8° C. (Avoid aerosol)

20. I.D. Na Agarose: Store at room temperature in a dry location.

21. Quick-Light Buffer: contains sodium azide. Store at room temperature.

22. Probe Solution I (D12S11/D17S79): contains enzyme labeled DNA probes D12S11 and D17S79 plus enzyme labeled phage (PhiX, M13, Lambda, T7) for the molecular weight markers, in Hybridization Solution. Store at 2-8° C.

23. Probe Solution II (D4S163): contains enzyme labeled DNA probe D4S163 plus enzyme labeled phage probes (PhiX, M13, Lambda, T7) for the molecular weight markers, in Hybridization Solution. Store at 2-8° C.

B. REAGENTS OTHER THAN LIFECODES, INC.

1. Denaturing Solution: contains sodium chloride, sodium hydroxide, and distilled water. Store at room temp.

2. Neutralization Solution: contains sodium chloride, trizma base, trizma HCl, and distilled water. Store at room temperature.

3. Sodium chloride: SP Cat. #7581-12. Store at room temperature.

4. Sodium hydroxide: SP Cat. #7708-2.5. Store at room temperature in an air-tight container.

5. Trizma base (Tris): Sigma Cat. #T-1503. Store at room temperature.

6. Trizma-HCL (Tris-HCl): Sigma Cat. #3253. Store at room temperature.

7. Ethanol-95%: Store at room temperature in a flammable container.

8. Isolation Control: contains approximately 2.5 X 10^6 lyophilized human cells per vial. Each lot is prepared in-house. Store at 2-8° C.

9. Restriction Control: contains approximately 100ng\ul high molecular weight human DNA in EDTA and Tris buffer. Each lot is prepared in-house. Store at 2-8° C.

10. Sterile saline: Baxter, Inc. Store at room temperature.

11. Dacron swabs: Baxter, Inc.

12. Felt swabs: Life Technologies, Inc. (C.E.P. Swabs)

13. Flat wooden spatulas: Baxter, Inc.

14. Large-pored foam swabs (pink): Sage Products (Toothette Oral Swab)

15. Small-pored foam swabs (beige): Texwipe Products (Clean Tip High Sorbency Swabs)

16. Nylon brushes: Cytobrush Plus (product #1101)

APPENDIX F

APPENDIX F

GLOSSARY

AGAR-A polysaccharide extracted from certain seaweeds.

AGAROSE-Support medium made with agar for electrophoresis.

ALLELE-One of two or more alternative forms of a gene occupying the same locus on homologous chromosomes.

BAND-The visual immage representing a particular DNA fragment on a lumigraph.

BAND SHIFT-The phenomenon in which DNA fragments in one lane of a gel migrate at a rate different from that of identical fragments in other lanes of the same gel.

BASE-Four chemical units (adenine, thymine, guanine, and cytosine) whose order in DNA molecules controls the genetic code.

BASE PAIR-Partnership of adenine with thymine or cytosine with guanine in the DNA double helix.

BUCCAL CELLS-Cells derived from the inner cheek lining. These cells are present in the saliva or can be gently scraped from the inner cheek surface.

CONTROLS-Tests performed in parallel with experimental samples and designed to demonstrate that a procedure worked correctly.

DEGRADATION-The breaking down of DNA by chemical or physical means.

DENATURATION-Conversion of DNA from double-stranded to single-stranded state by use of heat or high pH.

DIGESTED DNA-DNA cleaved by the action of restriction enzymes.

DNA(deoxyribonucleic acid)-Double-stranded moulecule that carries the genetic information in living organisms.

DNA PROBE-A short segment of single-stranded DNA labeled with a radioactive or chemical tag used to detect the presence of a particular DNA sequence through hybridization to its complementary sequence.

ELECTROPHORESIS-Technique for the separation of molecules through their movement on a support medium such as agarose under the influence of an electrical charge.

ENZYME-Protein that speeds up the rate of chemical reactions in the body but is unaltered itself in the reaction.

ETHIDIUM BROMIDE-An organic molecule that binds to DNA and flouresces under ultraviolet light and is used to identify DNA.

ETHYLENEDIAMINE TETRAACETIC ACID (EDTA)-A chemical preservative added to blood collection tubes that chelates magnesium so nucleases are invactivated, which prevents the degradation of DNA present in a sample.

GEL-A semisoft matrix (usually agarose) used in electrophoresis to separate molecules.

GENOMIC DNA-DNA sequence as it appears in cells.

HETEROZYGOUS-Having different alleles at a particular locus; for most forensic probes, the lumigraph displays two bands.

HOMOZYGOUS-Having the same allele at a particular locus; for most forensic probes, the lumigraph displays a single band.

HYBRIDIZATION-Process of complementary base pairing between two single strands of DNA.

HYPERVARIABLE REGION-A segment of a chromosome characterized by considerable variation in the number of tandem repeats at one or more loci.

KILOBASE (kb)-Unit of 1000 base pairs of DNA.

LOCUS-Position a gene occupies on a chromosome.

LUMIGRAPH-A photographic recording of the positions on X-ray film where a chemiluminescent reaction has occured. Positions reflect where DNA probes have hybridized with complementary sequences.

MISMATCH-Bases that do not match in complementary DNA strands.

MULTILOCUS-Refers to a number of different loci or positions in the genome.

NUCLEOTIDE-Combination of a base with a sugar and phosphoric acid.

NUCLEUS-The genome-containing membrane-bound structure in cells.

POLYMERASE CHAIN REACTION (PCR)-Process by which a small amount of DNA can be amplified to yield a larger quantity of DNA.

POLYMORPHISM-Occurrence in a population of two or more genetically determined alternative phenotypes.

RESTRICTION ENZYME-Derived from bacteria and causes the cleavage of DNA at specific points.

RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP)-Variation in the size of DNA fragments produced by restriction enzyme digestion of a genomic DNA. Pattern is recognized using a probe after electrophoresis, Southern blotting, and hybridization.

SIZE MARKER-DNA fragment of known size used to calibrate an electrophoretic gel.

SOUTHERN BLOT-Procedure for transferring denatured DNA from an agarose gel to a membrane where it can be hybridized with a complementary DNA probe.

STAR ACTIVITY-Relaxation of the strict recognition sequence of a restriction enzyme specifically resulting in the production of additional cleavages within DNA.

STRINGENCY-Conditions of hybridization that increase the specificity of binding between two single-strand portions of DNA, usually the probe and an immobilized fragment. Increasing the temperature or decreasing the ionic strength results in increased stringency.

TANDEM REPEATS-Multiple copies of an identical DNA sequence arranged in direct succession in a particular region of a chromosome.

TARGET DNA-The DNA sequence to be hybridized to a specific probe.

VARIABLE NUMBER OF TANDEM REPEATS (VNTR)-Copies of identical sequence DNA fragments (30-50bp) arranged in direct succession within a chromosome. The number of copies varies in random fashion at any locus from one individual to another.

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