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EASTERN KENTUCKY UNIVERSITY

Qualitative and Quantitative Analysis of Commercial DNA Swabs and Kits

Honors Thesis

Submitted

in Partial Fulfillment

of the

Requirements of HON 420

Spring 2016

By

Shelby Leigh Banks

Mentor

Dr. Jamie Daniel Fredericks

Department of Chemistry

Quantitative and Qualitative Analyses of DNA Swabs and Genomic DNA Kits

Shelby Leigh Banks

Dr. Jamie Daniel Fredericks, Department of Chemistry

With the use of DNA becoming increasingly more important in the field of forensics, the analysis of DNA extraction kits and collection swabs is significant. This researches main objective was to find a protocol that extracts the highest quantity and quality amount of DNA from human buccal swabs. Three extraction kits (Zymo Research Quick – gDNA MiniPrep Capped Column DNA Kit, a Bioline Isolate II Genomic DNA Kit, and Invitrogen PureLink Genomic DNA Kit) were tested with three different swabs (standard cotton swabs, Puritan foam swabs, and Isohelix DNA buccal swabs). Following procedures outline by the DNA kits distributors, DNA from buccal cells was extracted. The quality and quantity of the extracted DNA samples was measured by using a NanoDrop 2000 UV – Vis Spectrophotometer. Lastly, the samples were then processed with Rotor – Gene Q Real – Time Polymerase Chain Reaction Cycler to confirm the accuracy of the NanoDrop 2000 UV-Vis spectrophotometer measurements. Modifications were made to the protocol to ensure the aims of the research were satisfied. The experimental results showed that the Invitrogen PureLink Genomic DNA Kit protocol with only half of the PBS (250 μl of PBS) added to the samples extracted the highest quantity and quality amount of DNA with the Puritan foam swabs.

Keywords: forensic science, DNA extraction, DNA swab, buccal swab comparison, extraction kit comparison, Invitrogen PureLink, Bioline Isolate II, Zymo Research Quick, NanoDrop, Rotor – Gene

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1. Introduction

1.1 Background of DNA

What is DNA? DNA is the abbreviation for deoxyribonucleic acid. It is the hereditary material that is within all humans and most all other organisms. Nearly every cell in the human body has the same DNA and most DNA can be found in the nucleus while small amounts of DNA is located in the mitochondria. DNA that is found in the nucleus is called genomic DNA or gDNA. DNA that is found in the mitochondria is called mitochondrial DNA or mtDNA.

Human DNA consists of around three billion bases. More than 99 percent of those three billion basses are the exact same in all humans. It is the arrangement, or order, of those three billion bases that determines the information that is available for constructing or sustaining an organism.

The information that is in DNA is storied as a code. This code is made up of four chemical bases: adenine (A), thymine (T), guanine (G), and cytosine (C). The DNA bases pair up with each other, adenine pairs with thymine and cytosine

pairs with guanine. These pairs form units that are referred to as base pairs. Each chemical base, or DNA base, is attached to a sugar molecule as well as a phosphate molecule. Together, the base, the sugar molecule, and the phosphate molecule are called a nucleotide. Nucleotides are organized in two long parallel strands that twist around each other to form a spiral called a double helix (Figure 1) (Fouse, et al., 2015). The double helix structure is much like a ladder, where the base pairs form the ladder's rungs while the sugar and phosphate molecules form the ladder's rails

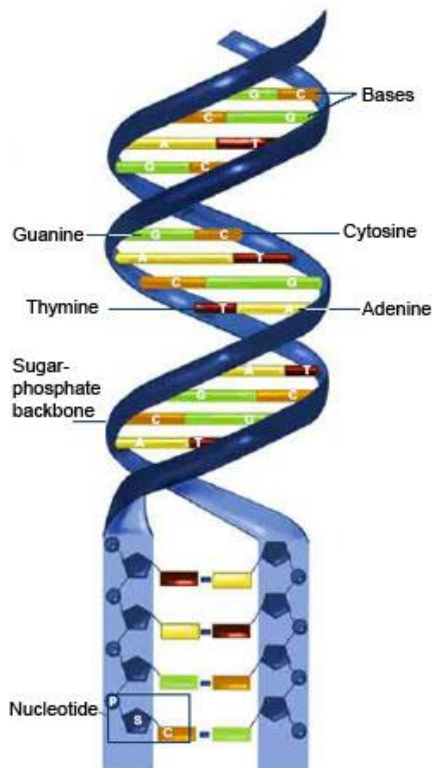

Figure 1: Structure of Genomic DNA
https://publications.nigms.nih.gov/thenewgenetics/chapter1.html

DNA has an important property in that it can make copies of itself. Each strand of DNA that is in the double helix has the ability to serve as a pattern for

replicating the arrangement of the bases. The ability to produce exact copies of itself is critical for producing new cells. All new cells that are created need to have an identical copy of the DNA that is present within the old cell.

DNA makes up genes which are the basic functional and physical component of heredity. Genes act as the instructions to make specific molecules called proteins. The genes vary in size from a couple hundred bases to more than a million bases, in humans. According to the Human Genome Project, humans have been estimated to have between 20,000 and 25,000 genes (Sawicki, et al., 2002). Each individual has two copies of each gene; one is inherited from each parent. The majority of genes are the same in all individuals but a small percentage of the genes differ slightly between people. A gene that contains small differences in the sequence of the DNA bases is called an allele. These alleles or differences are what contribute to the unique physical features that each person has (Adamowics, et al., 2014).

Within the nucleus of each cell there are DNA molecules that are tightly coiled around proteins (histones) and constitute structures called chromosomes. Each chromosome has a centromere, constriction point, which divides the chromosomes into two separate arms. The shorter of the arms is labeled the “p arm” while the longer arm of the chromosome is labeled the “q arm”. It is the location of the centromere on each chromosome that helps to describe the location of each specific gene.

Each cell, in humans, normally contains 23 pairs of chromosomes, a total of 46 chromosomes. Twenty-two of these 23 pairs are called autosomes and appear the same in both females and males. The last pair, or 23rd pair, are the sex chromosomes and are different between females and males. Males have only one copy of the X chromosome and one Y chromosome while females have two copies of the X chromosome (Housman, 1995).

The use of genomic DNA when applied to the forensic field has had a beneficial impact including the exoneration of innocent people, the identification of offenders, and the establishment of criminal databases. In 1974, James Bain of Lake Wales, Florida was convicted of rape, kidnapping, and burglary and sentenced to life in prison. The rapist left semen on the victim's underwear however this was before DNA testing was available. In 2001 a Florida statue made it possible for cases to be reopened for DNA testing. Bain was granted post-conviction DNA testing and the DNA that was found on the victim's underwear was sent to the DNA Diagnostics Center. The tests excluded Bain as a possible source of the DNA. In 2009, a judge signed the order that released Bain from prison after serving 35 years for a crime he did not commit (Smith, 2014). Because of genomic DNA testing, in 1987, a Florida rapist named Tommie Lee Andrews became the first person in the United States to be convicted as a result of DNA evidence and was sentenced to 22 years in jail (James, 2009). Lastly, genomic DNA has been used to generate the Combined DNA Index System (CODIS). This system is a database for the exchange and comparison of forensic DNA evidence from crime investigations. It contains

convicted offenders and arrestee DNA profiles from federal, state and local contributing forensic laboratories (Roewer, 2013). However, regardless of the anonymous nature of DNA profile data and security measure that are in place, simply just the misuse of the data or the mishandling of samples are possible threats to individuals rights.

Because of the current processes for generating a profile data, it is unlikely that databases are completely error free but the majorities of the errors are due to human error and are transcriptional in nature. New extraction and amplification methods could be the answer in preventing human error in DNA analysis. A research group at the California Department of Justice DNA Laboratory has developed a polymerase chain reaction (PCR) assay method that amplifies a specific target sequence that can vary in length. This allows for the even degraded DNA samples to be assessed (Swango, et al., 2005). So if samples become degraded in the laboratory from human error, they are still able to be analyzed with this new PCR assay. Simple and rapid extraction of human genomic DNA is still a holdup for analysis.

1.2 Objective of Research and Aims

The overall purpose of this research was to find a protocol that extracts the highest quantity and quality amount of DNA from human buccal swabs. This research could help minimize the amount of case work that is backlogged in both federal and privately owned labs. A case is backlogged when the samples have to wait to be analyzed. As samples are waiting to be analyzed they are held in

refrigeration. The longer the samples are kept in refrigeration there is an ever increasing chance that the samples could become degraded which leads to poor test results and inaccuracy. Some cases depend greatly on those DNA samples and if they become degraded then that case may go unsolved. Also, without the use of buccal swabs and extracted DNA, the databases that depend on DNA would not exist. If a buccal swab and extraction kit combination is able to be found that provides better use to the collection of DNA then the majority of errors that are due to human error may cease.

In order to find this protocol the researched focused on three main questions. The first question was which of the three swabs produced the highest yields of DNA. The second question was which of the three swabs tested with which DNA kit was the most cost effective. The last question was which kit had the timeliest protocol.

The three DNA kits that were tested were an inexpensive Zymo Research Quick – gDNA MiniPrep Capped Column DNA Kit, a Bioline Isolate II Genomic DNA Kit, and an Invitrogen PureLink Genomic DNA Kit. The three DNA swabs that were tested were a standard cotton swab that is comparatively inexpensive, a Puritan foam swab, and an Isohelix DNA buccal swab that is made of polystyrene. Isohelix DNA buccal swabs are designed to give increased yields of high quality genomic DNA but using a matrix design (Marshall, 2014).

The three different swab types that were tested were used to collect buccal cells. Buccal cells are the cells that are on the inside of one's cheek.

Those cells contain DNA and that DNA is extracted using the three DNA extraction kits that were tested.

This research is similar to research conducted by the Metropolitan Police Department Lab. The Metropolitan Police had traditionally used standard cotton swabs to retrieve DNA for the use of forensic profiling until a new nylon flocked swab had been generated. The new nylon flocked swab claimed that it increased sample recovery as well as release yields. The study that they conducted examined the standard cotton swab and the new swabs capability to retrieve DNA. Their results indicated that both of the swab types were capable of retrieving high percentages of DNA but the standard cotton swab with the spin – column extraction method had proven to be the most effective over the nylon swab(Brownlow, et al. 2012).

A lab in the United Kingdom also conducted research that compared the DNA retrieval capability of different extraction methods. Saliva samples were collected with a swab from the glue on envelopes. Their research concluded that BioRobot EZI extraction method yielded the highest concentration of extracted DNA (Roman, et al., 2009).

Lastly, a group of researchers at Comenius University in Bratislava Slovakia conducted research that focused on finding the most suitable method of collection for oral biological material. The used different swab types to collect the samples and then used both a phenol – chloroform extraction and a silica membrane based commercial kit for the extraction of the biological material. The

quantity of the DNA was measured with a real – time PCR cycler. Their research concluded that the neutral viscose transport swab with the silica membrane based commercial kit had the most suitable quantity of extracted DNA (Ipper, 2014).

The objective of this research is to find a protocol that extracts the highest quantity and quality amount of DNA from human buccal swabs. An aim of this research was to determine which of the three swabs produced the highest yields of DNA. Another aim of this research was determine which of the three swabs tested with which DNA kit was the most cost effective. The last aim of the research was to find which kit had the timeliest protocol.

2. Experimental

2.1 Part One

The first part of the researched involved analyzing all three extraction kits with each of the three swab types.

2.1.1 Extraction Kit Protocols

The first extraction kit that was analyzed was the Bioline Isolate II Genomic DNA Kit. The protocol that is published by the manufacturer for this kit is as follows: The swabs were placed in clean microfuge tube and 200 μl of Lysis Buffer G1 and 10 μl of Proteinase K was added. The tubes were wrapped in parafilm then incubated at 56° C for up to 24 hours. Following incubation, the sample tubes were spun down. 200 μl of Lysis Buffer G3 was added to the

samples and followed by an incubation period. The samples were vortexed then 200 µl of 96% - 100% Ethanol was added to the samples tubes and vortexed again. The samples were transferred and loaded into DNA spin column and collection tubes and then centrifuged. The flow – through in the collection tubes was discarded. 400 µl of Wash Buffer GW1 was added to the samples, then the samples were centrifuged and the flow – through was discarded. 400 µl of Wash Buffer GW2 was added to the samples then the samples were centrifuged once again. The DNA spin column was added to a new microfuge tube and100 µl of Elution Buffer G was added to the columns. The samples were incubated at room temperature for one minute and then centrifuged. The DNA that was extracted from the process above was collected in the microfuge tube (Bioline, 2012).

The second extraction kit that was analyzed was the Invitrogen PureLink Genomic DNA Kit. The protocol that is published by the manufacturer for this kit is as follows: The swabs were placed in a clean microfuge tube and 500 µl of 10X phosphate buffered saline, 20 µl of Proteinase K, and 500 µl of Lysis / Binding Buffer were added to the sample and incubated at 55° C for 24 °. The swab was removed from the tube then the sample tube was centrifuged for 1 minute at 10,000 g. 200 µl of 95% Ethanol was added to the sample then it was vortexed. A spin column was added to a collection tube and the prepared Lysate was added to the spin column. The spin column and collection tube combination was centrifuged for 1 minute at 10,000 g at room temperature then the flow – through was discarded. Next, 500 µl of Wash Buffer 1 was added, the spin column and collection tube combination was centrifuged for 1 minute at 10,000 g

at room temperature and the flow – through was discarded. Next, 500 µl of Wash Buffer 2 was added, the spin column and collection tube combination was centrifuged for 3 minutes at 10,000 g at room temperature and the flow – through was discarded. The spin column was added to a new microfuge tube and 200 µl of Elution Buffer was added to the spin column. The sample incubated at room temperature for one minute then centrifuged for one minutes at max speed at room temperature. The column was removed and discarded since the extracted DNA was collected in the microfuge tube.

The last kit that was analyzed was the Zymo Research Quick – gDNA MiniPrep Capped Column DNA Kit. The protocol that is published by the manufacturer for this kit is as follows: The swabs were placed in a clean microfuge tube and 500 µl of Genomic Lysis Buffer then the samples were vortexed for a few seconds. The samples were incubated at room temperature for between five and ten minutes. A spin column was added to a collection tube and the samples were added to the spin column. The samples contained within the spin column and the collection tube were centrifuged for one minute and then the flow – through was discarded. 200 µl of DNA Pre – Wash Buffer was added to the spin column then centrifuged for one minute. 500 µl of g – DNA Wash Buffer was added to the sample then centrifuged for one minute. The spin column was then transferred to a clean microfuge tube and 50 µl of DNA Elution Buffer was added to the spin column. The samples were incubated at room temperature for two to five minutes. Following the incubation period, the samples

were centrifuged to elute the DNA. The column was removed and discarded for the DNA was collected in the microfuge tube.

2.1.2 Quality Measurements

All of the samples qualities were analyzed with the NanoDrop Spectrophotometer 2000. The NanoDrop measures how pure a sample is. Nucleic acids and proteins have absorbance maxima at 260 nanometers (nm) and 280 nm respectively. A sample is considered pure if the ratio of absorbance ($\frac{A260}{A280}$) reads between 1.8 and 2.0. That means that the kit extracted the DNA from the swab but not the junk that the swab could have collected in the mouth. If the ratio of absorbance is lower than it may indicate that there is a high presence of proteins, phenol or other contaminants that absorb more strongly near 280 nm (Thermo Scientific, 2005). As displayed in the sample spectrum (Figure 2), the ratio of absorbance for the sample is 1.90. This example spectrum shows that the sample was relatively pure.

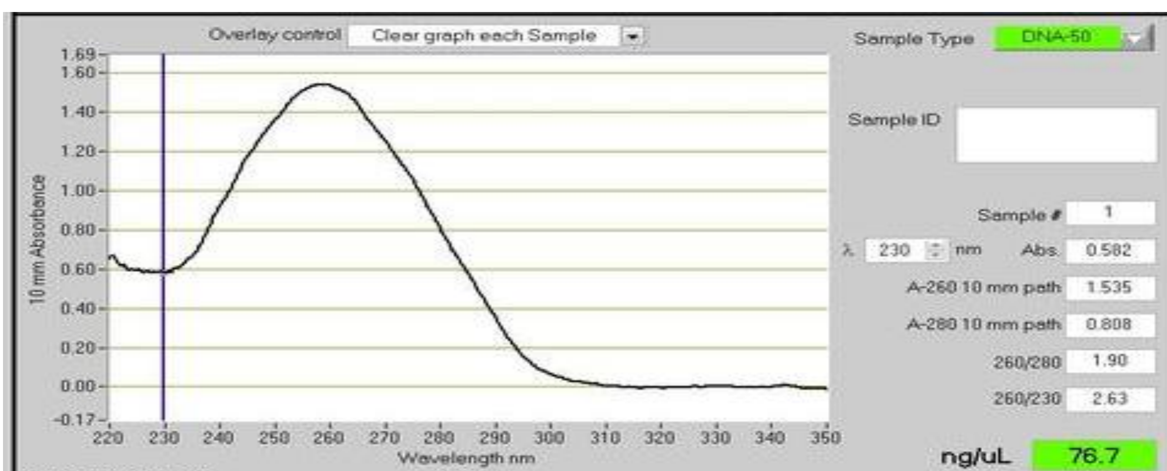

Figure 2: Example of Spectrum Displaying the Ratio of Absorbance for a Pure Sample
http://www.u.arizona.edu/~gwatts/azcc/InterpretingSpec.pdf

2.1.3 Quantity Measurements

Following the analysis of the quality, the quantities of the samples were analyzed with the NanoDrop Spectrophotometer 2000 and the Rotor – Gene Q Real – Time PCR Cycler. Both instruments measure how concentrated the extracted samples were. That means how much DNA was extracted from the buccal swab and is contained within the sample tube. However the NanoDrop is not the most reliable instrument to use to measure quantity so to verify the results the samples were tested with the Rotor – Gene which is more reliable.

2.2 Part Two

The second part of the research involved analyzing the Invitrogen PureLink Genomic DNA Kit with only the Puritan foam swab and the Isohelix swab. Analysis of the swabs that were collected followed the procedure outlined above. The quantities of the samples were analyzed with the NanoDrop Spectrophotometer 2000 and the Rotor – Gene Q Real – Time PCR Cycler.

2.3 Part Three

In order to answer all of the research questions, a modification to the protocol is necessary in order to find the most cost effect and timeliest protocol. The modification that was made to the protocol was with the amount of PBS that was added to the samples. In order to extract the DNA, the cells must be broken open to release the DNA. The image (Figure 3) shows a cell breaking open to release the DNA that is inside of the cell. This is commonly referred to as lyse the cell or cell lysis. PBS stands for phosphate buffered saline. PBS is added to the

samples to help lyse the cells. The protocol instructed that 500 µl of PBS to be added to each of the samples.

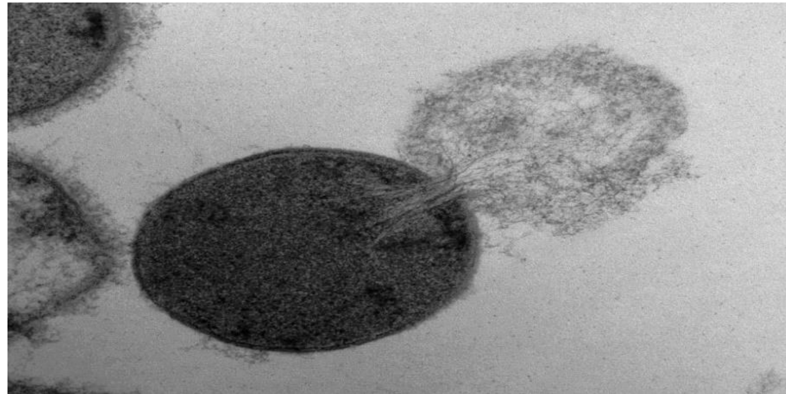

Figure 3: Image of Cell Lysis
http://www.news.gatech.edu/2013/01/09/study-quantifies-size-holes-antibacterials-create-cell-walls-kill-bacteria

2.3.1 Extraction Kit Protocol

The third part of the research involved adding a modification to the protocol outlined above for the Invitrogen PureLink Genomic DNA Kit. The modification that was made to the protocol was only 250 µl of PBS was added to half of the samples and no PBS was added to the other half of the samples. The modified protocol is as follows: The swab was placed in a clean microfuge tube and 20 µl of Proteinase K and 500 µl of Lysis / Binding Buffer were added to the sample. 250 µl of 10X phosphate buffered saline was added to half of the samples and 0 µl of 10X phosphate buffered saline was added to the other half of the samples. The samples were incubated at 55° C for 24 °. The swab was removed from the tube then the sample tube was centrifuged for 1 minute at 10,000 g. 200 µl of 95% Ethanol was added to the sample then it was vortexed. A spin column was added to a collection tube and the prepared Lysate was

added to the spin column. The spin column and collection tube combination was centrifuged for 1 minute at 10,000 g at room temperature then the flow – through was discarded. Next, 500 µl of Wash Buffer 1 was added, the spin column and collection tube combination was centrifuged for 1 minute at 10,000 g at room temperature and the flow – through was discarded. Next, 500 µl of Wash Buffer 2 was added, the spin column and collection tube combination was centrifuged for 3 minutes at 10,000 g at room temperature and the flow – through was discarded. The spin column was added to a new microfuge tube and 200 µl of Elution Buffer was added to the spin column. The sample incubated at room temperature for one minute then centrifuged for one minutes at max speed at room temperature. The column was removed and discarded since the extracted DNA was then in the microfuge tube.

2.3.2 Quantity Measurements

The effect that the addition of phosphate buffered saline had on the samples when it was added was analyzed. The quantities of the samples were analyzed with the NanoDrop Spectrophotometer 2000 and the Rotor – Gene Q Real – Time PCR Cycler.

2.4 Part Four

The final part of the research involved using the part of the modification from the third part of the research.

2.4.1 Extraction Kit Protocol

The modification to the protocol involved only adding 250 µl of PBS to each sample. The modified protocol is as follows: The swab was placed in a clean microfuge tube and 250 µl of 10X phosphate buffered saline, 20 µl of Proteinase K, and 500 µl of Lysis / Binding Buffer were added to the sample and incubated at 55° C for 24 °. The swab was removed from the tube then the sample tube was centrifuged for 1 minute at 10,000 g. 200 µl of 95% Ethanol was added to the sample then it was vortexed. A spin column was added to a collection tube and the prepared Lysate was added to the spin column. The spin column and collection tube combination was centrifuged for 1 minute at 10,000 g at room temperature then the flow – through was discarded. Next, 500 µl of Wash Buffer 1 was added, the spin column and collection tube combination was centrifuged for 1 minute at 10,000 g at room temperature and the flow – through was discarded. Next, 500 µl of Wash Buffer 2 was added, the spin column and collection tube combination was centrifuged for 3 minutes at 10,000 g at room temperature and the flow – through was discarded. The spin column was added to a new microfuge tube and 200 µl of Elution Buffer was added to the spin column. The sample incubated at room temperature for one minute then centrifuged for one minutes at max speed at room temperature. The column was removed and discarded since the extracted DNA was then in the microfuge tube.

2.4.2 Quantity Measurements

The quantities of the samples were analyzed with the NanoDrop Spectrophotometer 2000 and the Rotor – Gene Q Real – Time PCR Cycler.

3. Results and Discussion

The first part of the research involved using DNA from one individual for samples. The swab was rubbed on the inside of the check to collect buccal cells. A total of 18 swabs were collected over the course of two weeks. Each swab was used for two trials with each extraction kit. So 2 cotton swabs, 2 puritan swabs, and 2 isohelix swabs were used with each of the three kits, the Bioline Isolate II Genomic DNA Kit, Invitrogen PureLink Genomic DNA Kit, and the Zymo Research Quick – gDNA MiniPrep Capped Column DNA Kit. The procedures that were provided with the DNA kits were followed to extract the DNA out of the buccal cells. The quality of the extracted DNA was measured (Table 1).

Table 1: Quality and Quantity Measurements for Extracted DNA from Specified Extraction Kit and Swab Type

Extraction Kit	Swab Type	Quality (NanoDrop)	Quantity (NanoDrop) (ng)	Quantity (Rotor – Gene) (ng)
Bioline	Puritan	1.92	4.80	8.98
	Cotton	1.33	3.30	5.76
	Isohelix	1.62	4.00	8.02
Invitrogen	Puritan	2.04	7.35	72.89
	Cotton	1.96	1.25	25.22
	Isohelix	2.03	1.45	35.45
Zymo	Puritan	2.11	51.5	13.35
	Cotton	1.47	17.7	6.45
	Isohelix	1.51	19.6	7.12

According to Table 1, the overall quality of the extracted DNA is higher with the Invitrogen PureLink Genomic DNA Kit and with the puritan foam swab.

According to the NanoDrop Spectrophotometer 2000 the Invitrogen PureLink Genomic DNA Kit had the overall highest quantity and the puritan swab had the overall highest quantity. Also, the Invitrogen PureLink Genomic DNA Kit and the puritan swab had the highest quantity according to the Rotor – Gene Q Real – Time PCR Cycler

The quality of the extracted DNA was measured with the NanoDrop Spectrophotometer 2000 (Figure 4).

According to the NanoDrop the puritan swab had an overall average quality of 2.0 meaning the extracted sample was on average pure. The cotton swab had an overall average quality of 1.6 and the isohelix swab had an overall average quality of 1.7. The average of both the cotton swab and the isohelix swab were not pure. As displayed in Figure 4, the puritan swab produced the most pure samples and the Invitrogen kit produced the most purse samples.

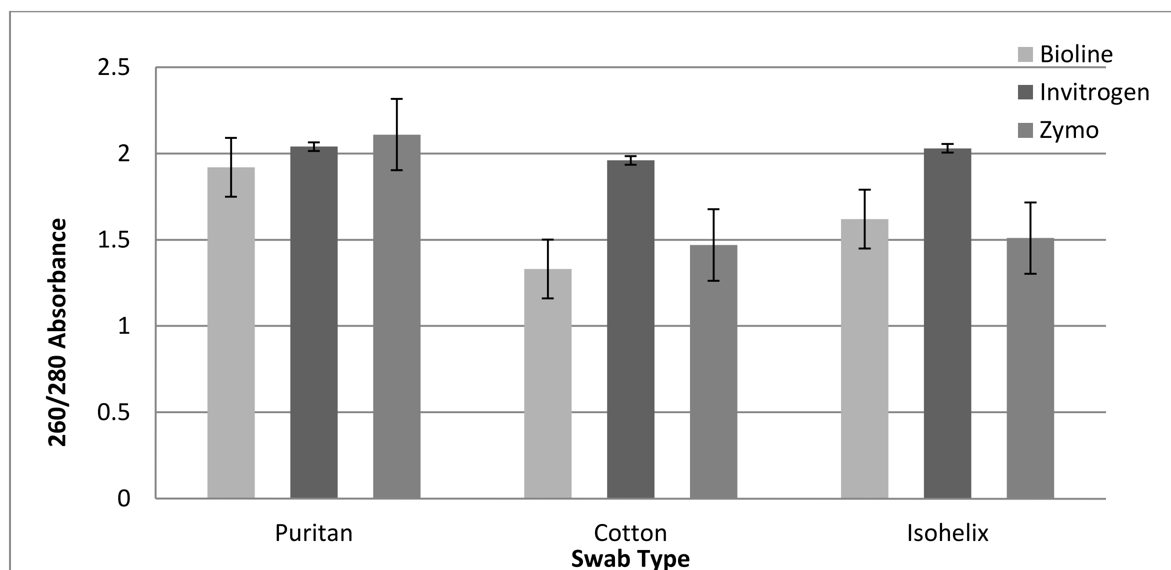

Figure 4: NanoDrop Spectrophotometer 2000 Quality Measurements for Extracted DNA Samples

The quantity of the extracted DNA was also measured for the extracted DNA samples (Table 1). The quantity of the extracted DNA was tested with both the NanoDrop Spectrophotometer 2000 (Figure 5) and Rotor – Gene Q Real – Time PCR Cycler (Figure 6). The thermal cycles at which the Rotor – Gene Q Real – Time PCR Cycler was programmed to run are displayed in Table 2.

According to the NanoDrop the puritan swab had an overall average quantity of 21.4 ng of DNA. The cotton swab had an overall average quantity of 7.6 ng of DNA and the isohelix swab had an overall average quantity of 8.4 ng of DNA. As displayed in Figure 5, the puritan swab collected the highest concentration of DNA while the cotton swab collected the lowest. And the Invitrogen kit extracted more DNA than the other two kits tested.

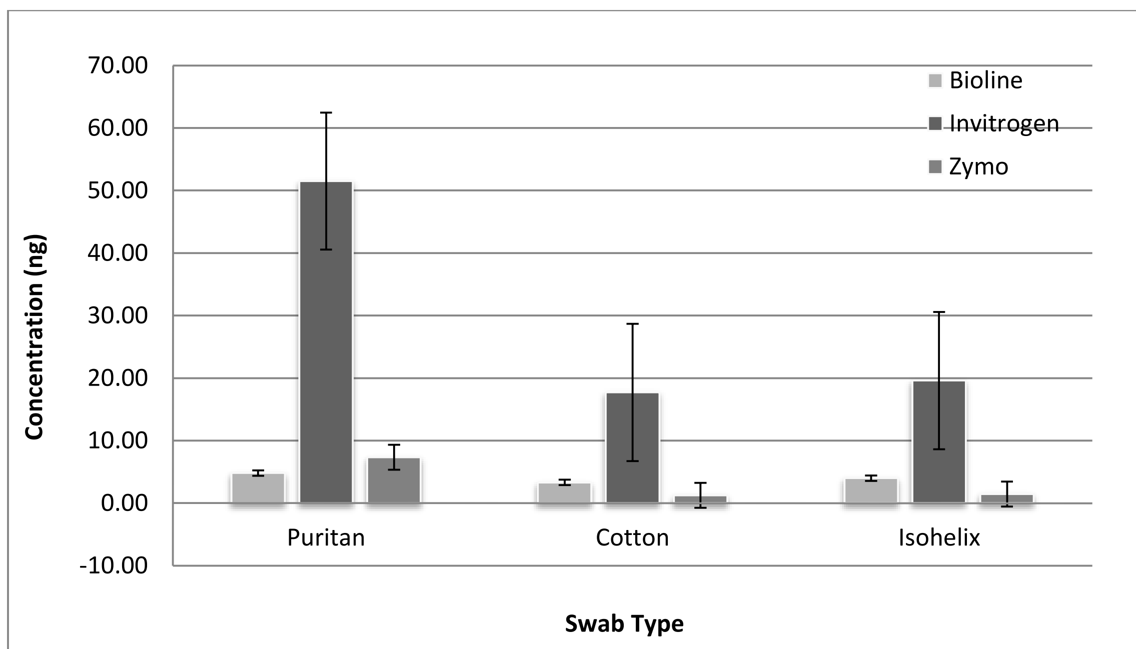

Figure 5: NanoDrop Spectrophotometer 2000 Quantity Measurements of Extracted DNA Samples

According to the Rotor – Gene Q Real – Time PCR Cycler the puritan swab had an overall average quantity of 10.35 ng of DNA. The cotton swab had an overall average quantity of 4.86 ng and the isohelix swab had an overall average quantity of 8.51 ng. Displayed in Figure 6, the puritan swab collected the highest concentrations of DNA while the cotton swab collected the lowest and the Invitrogen kit extracted the most DNA.

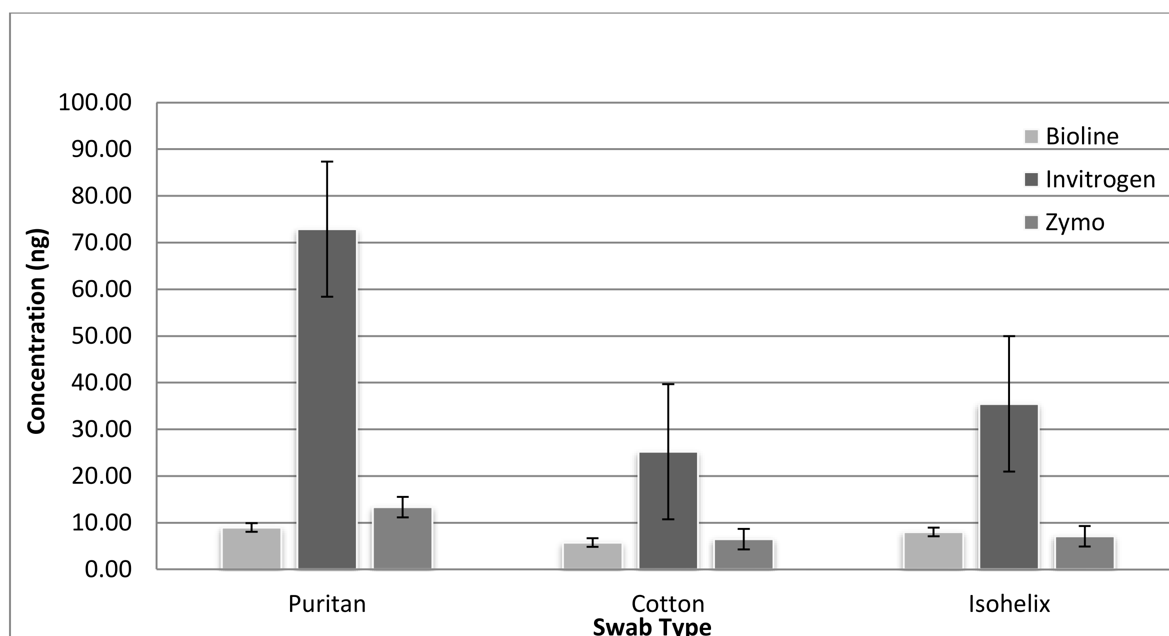

Figure 6: Rotor - Gene Q Real - Time PCR Cycler Quantity Measurements of Extracted DNA Samples

Table 2: Programmed Thermal Cycles for Rotor - Gene Q Real - Time PCR Cycler

Cycles	Temperature	Time	Notes
1	95 °C	2 minutes	Polymerase Activation
40	95 °C 58 °C 72 °C	5 seconds 10 seconds 20 seconds	Denaturation Annealing Extension

The cotton swab had the poorest quality and quantity results from Part 1 tests. Therefore cotton swab was not used in Part 2 of the research. The Invitrogen PureLink Genomic DNA Kit had the highest quality and highest quantity from Part 1 testing and therefore that is the only extraction kit that was tested in Part 2 of the research. Part 2 of the research involved using samples that were provided by volunteers. Twelve volunteers rubbed the inside of their cheek with the puritan swab and the isohelix swab to collect their buccal cells. One puritan swab and one isohelix swab was collected from each individual over the course of 2 days, totaling 24 swabs.

The quantity of the samples was measured with the NanoDrop and Rotor – Gene (Figure 7).

According to the NanoDrop the puritan swab had an overall average quantity of 2.63 ng and the isohelix swab had an overall average quantity of 2.39 ng. According to the Rotor – Rotor – Gene Q Real – Time PCR Cycler the puritan swab had an overall average quantity of 7.11 ng and the isohelix swab had an overall average quantity of 3.87 ng. As displayed in Figure 7, the puritan swab still had higher concentrations of DNA than the isohelix swab when used with the Invitrogen PureLink Genomic DNA Kit.

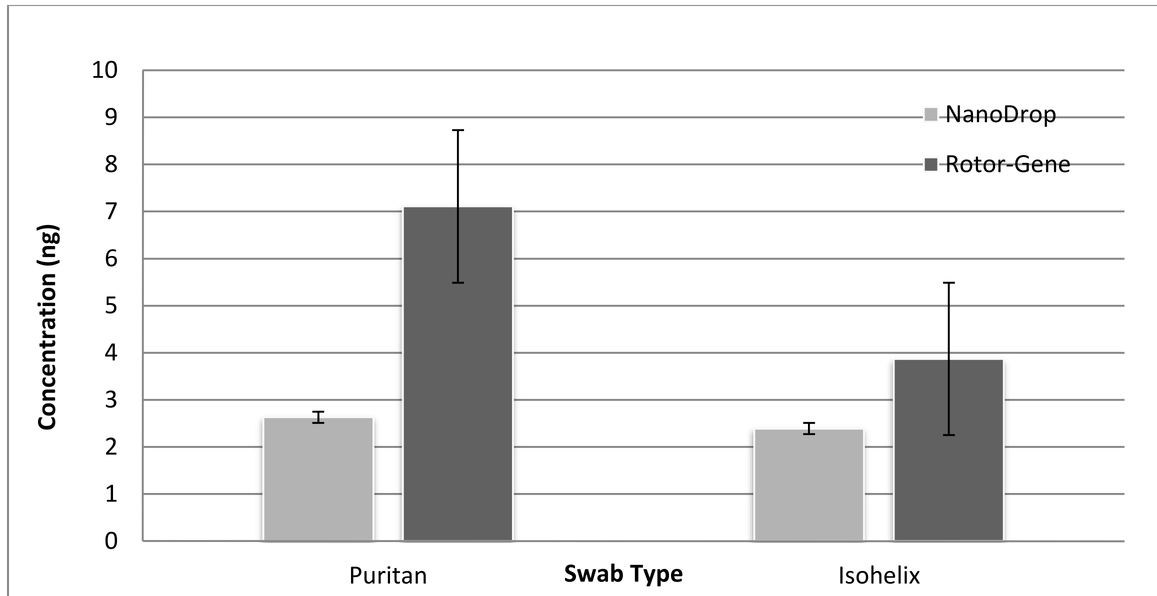

Figure 7: NanoDrop Spectrophotometer 2000 and Rotor - Gene Q Real - Time PCR Cycler Quantity Measurements of Extracted DNA Samples

The Invitrogen PureLink Genomic DNA Kit produced high concentrations of DNA with both the puritan swab and the isohelix swab.

The third part of the research involved using samples provided by a volunteer. The swabs collected from the volunteer were used with the Invitrogen PureLink Genomic DNA Kit protocol described above but with a slight modification.

Twelve samples were used that were provided by a volunteer over the course of a week. Six puritan swab samples were collected and six isohelix swab samples were collected. Some of the samples were analyzed with the original protocol but majority of the samples were analyzed with a modified protocol. Two puritan swab samples and two isohelix swab samples were analyzed with the

500 µl of phosphate buffered saline added to the samples. Two more puritan swab samples and two more isohelix swab samples were analyzed with the 250 µl of phosphate buffered saline added to the samples. Two different puritan swabs and two different isohelix swabs were analyzed with no phosphate buffered saline added to the samples. Table 2 displays the final quantity of the extracted DNA with the modified protocols measured by the NanoDrop Spectrophotometer 2000 and the Rotor – Gene Q Real – Time PCR Cycler.

As displayed in Table 3, the quantity of the extracted DNA according to the NanoDrop and the Rotor – Gene Q Real – Time PCR Cycler are higher when 250 µl of PBS is added to the sample versus when 500 µl of PBS is added and when no PBS has been added to the samples.

Table 3: NanoDrop Spectrophotometer 2000 and Rotor – Gene Q Real – Time PCR Cycler Measurements of Quantity of Extracted DNA with the Original and Modified Protocol

Swab Type	Amount of PBS Added (µl)	Quantity (NanoDrop)(ng)	Quantity (Rotor – Gene) (ng)
Puritan	500	1.88	1.66
	500	2.04	1.76
Isohelix	500	1.62	1.07
	500	1.43	1.11
Puritan	250	2.06	1.89
	250	1.72	1.67
Isohelix	250	1.09	0.73
	250	1.12	1.05
Puritan	0	1.41	1.68
	0	1.23	1.21
Isohelix	0	0.55	1.07
	0	0.30	0.71

The fourth and final part of the research involved using samples provided by volunteers. The swabs collected from volunteers were used with the Invitrogen PureLink Genomic DNA Kit protocol described above but with only 250 µl of PBS added all to the samples. Again twelve volunteers rubbed the inside of their cheek with the puritan swab and the isohelix swab to collect their buccal cells. Two puritan and two isohelix swabs were collected from each individual over the course of two weeks, totaling 48 swabs. Then those swabs were analyzed with the Invitrogen PureLink Genomic DNA Kit but with the modified protocol of only 250 µl of PBS added. The quantity of the samples was measured with the NanoDrop Spectrophotometer 2000 and the Rotor – Gene Q Real – Time PCR Cycler (Figure 8).

According to the NanoDrop the puritan swab had an overall average quantity of 7.94 ng and the isohelix swab had an overall average quantity of 7.54 ng. According to the Rotor – Rotor – Gene Q Real – Time PCR Cycler the puritan swab had an overall average quantity of 11.99 ng and the isohelix swab had an overall average quantity of 9.44 ng. As displayed in Figure 8, the puritan swab still continued to have higher concentrations of DNA than the isohelix swab when used with the Invitrogen PureLink Genomic DNA Kit. Also, even when half of the amount of PBS was added, the Invitrogen PureLink Genomic DNA Kit still extracted high concentrations of DNA. In fact, these concentrations (Figure 8) are higher than the concentrations when the recommended amount of 500 µl of PBS was added to the samples (Figure 7).

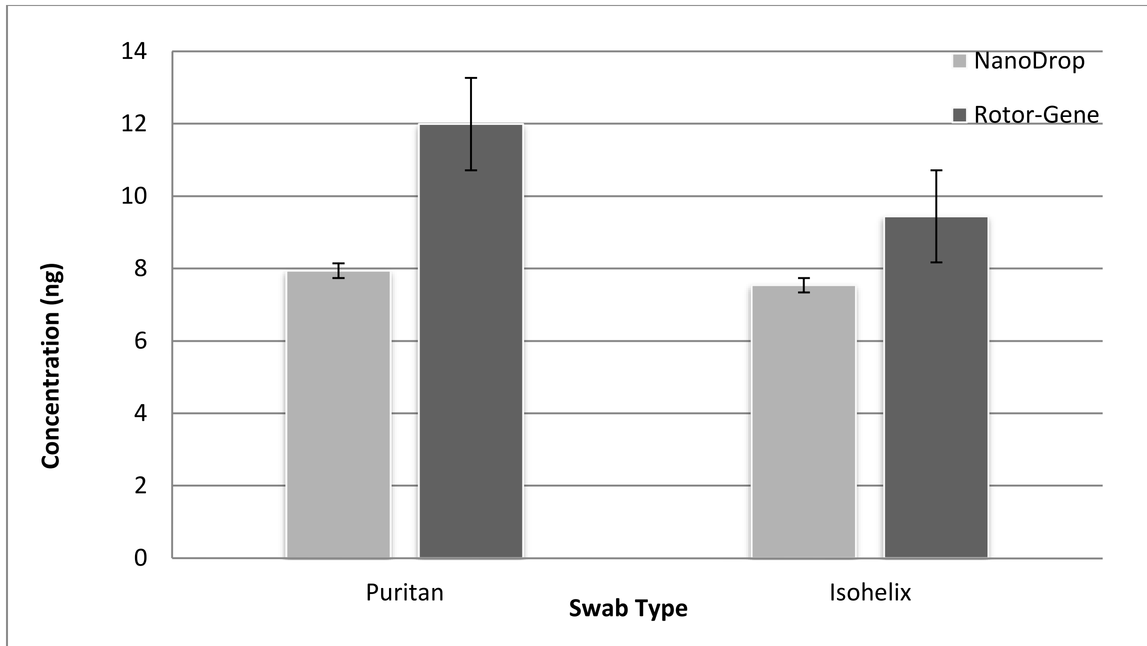

Figure 8: NanoDrop Spectrophotometer 2000 and Rotor - Gene Q Real - Time PCR Cycler Quantity Measurements of Extracted DNA Samples

The published standards were looked up for the three kits that were analyzed to compare the research results (Table 4). According to the manufacturers the Zymo Research Quick – gDNA MiniPrep Capped Column DNA Kit had the shortest completion time. The Zymo Research Quick – gDNA MiniPrep Capped Column DNA Kit and the Invitrogen PureLink Genomic DNA Kit had the most pure reported quality. The Bioline Isolate II Genomic DNA Kit had the highest reported quantity of DNA. Unfortunately, the Invitrogen PureLink Genomic DNA Kit did not report their quantity. It would appear as if the Zymo Research Quick – gDNA MiniPrep Capped Column DNA Kit should have been the most ideal protocol to follow to get the most pure samples in the shortest

amount of time and still have high concentrations of DNA. However, this research yielded different results.

Table 4: Published Standards for Extraction Kits

Extraction Kit	Completion Time	Reported Quality	Reported Quantity
Zymo Research Quick – gDNA MiniPrep Capped Column	15 minutes	$> 1.8 \frac{A_{260}}{A_{280}}$	≤ 25 µg
Bioline Isolate II Genomic DNA Kit	80 minutes	$1.7 – 1.9 \frac{A_{260}}{A_{280}}$	20 – 35 µg
Invitrogen PureLink Genomic DNA Kit	45 minutes	$> 1.8 \frac{A_{260}}{A_{280}}$	Unreported

According to this research the puritan foam swab when used with the Invitrogen PureLink Genomic DNA Kit produced the highest yields of DNA, was the timeliest protocol and was the most cost effective when half the amount of PBS was added to the sample.

The reason that the modified protocol is the most cost effective is that is uses less PBS. A bottle of PBS costs $40.00 / bottle and when it is used as the protocol suggest then it will last for at most 1,000 samples. However, if the bottle was used with the modified protocol, half the amount of PBS added or 250 µl of PBS, then it would last for twice as many samples, or for at most 2,000 samples.

4. Conclusions

4.1 Learned and Discovered

The overall purpose of this research was to find a protocol that extracts the highest quantity and quality amount of DNA from human buccal swabs. This research showed that the Invitrogen PureLink Genomic DNA Kit protocol with only half of the PBS (250 μl of PBS) added to the samples extracted the highest quantity and quality amount of DNA from the Puritan foam swabs. In the process of finding this protocol, all three of the guiding research questions.

4.2 Future Research and Goals

The Invitrogen PureLink Genomic DNA Kit protocol states that the samples should be incubated for between 3 and 24 hours. Further research could be completed to see that if samples that are incubated for 3 hours produce approximately the same results than if they were incubated for 24 hours. This could make the protocol even timelier than the modified protocol. Testing the Invitrogen PureLink Genomic DNA Kit with touch DNA could also be completed. The touch DNA method analyzes skin cells that are left behind when you touch an item (vanOorschot, et al. 2010). To do this, a volunteer would touch a sanitized surface with a hand then use a swab to collect the cells. The procedure outlined by the distributor would be followed in order to analyze the kits ability to extract the DNA from the collected cells. Another direction that this research could be taken in the future is with possibly testing a different type of extraction kit such as chelex.

Chelex is an ion exchange resin that is added to nuclease free water to achieve a certain percent solution (weight per volume) (Adamowicz, et al., 2014).

It is composed of styrene divinylbenzene copolymers. Chelex works by remove Mg^{2+} from the reaction which then results in nucleases to be inactivated and the DNA is protected. That then allows for the DNA to be extracted (Myers and Adkins, 2008).

Chelex is described as being a fast and cost effective method for DNA extraction (Rogers, et al., 2007). Samples are added to a tube of chelex and vortexed for 10 – 15 seconds. The tubes are centrifuged briefly (10 – 15 seconds) at high speed at room temperature and then incubated for 20 minutes at 95° C. After the incubation period, the samples are vortexed again for 10 – 15 seconds then centrifuged again at high speed at room temperature. The supernatant is then pipetted off because that is the portion that contains the eluted DNA (Durdiakova, et al., 2012).

Since chelex is a more cost effective technique, it can be inconsistent at times. Determining if samples are best if used immediately or allowed to sit overnight before using them will help with consistency in the results. Also, repeating the procedure above for a second time could lead to more consistent results. Lastly, the concentration of chelex used can vary. Determining the suspension of chelex in nuclease free water will help to achieve consistent results.

There are current methods using micro – filters require multiple handling steps in part because the salt conditions must be controlled in order for the attraction and the elution of DNA in the porous silica (Hanselle, et al., 2003).

There is a new method of human genomic DNA extraction from buccal swab samples. In this new method, DNA is attracted onto a gold – coated microchip by an electric field as well as capillary action. The capture DNA is then eluted by thermal heating at a temperature of 70° C. A device was designed that could hold four of these gold – coated microchips. The DNA that was extracted using the microchips was quantified by real – time polymerase chain reaction (qPCR). In comparison to the traditional commercial kits, the new gold – coated microchip extraction has an equivalent yield of DNA extraction and was accomplished in fewer steps (Yang, et al., 2014). Although this new extraction method has proven to be timely, it is however extremely expensive in comparison to the traditional commercial extraction kits. Future research with this method could lead to even better DNA extraction than current protocols.

This research could not only be used for human genomic DNA but also for broiler chickens. The department of Food Microbiology and Hygiene in the Netherlands conducted research with carcass skin of broiler chickens. They were comparing the bacterial counts of the chicken's skin. They used a dry standard cotton swab and a moistened standard cotton swab to collect Enterobacteriaceae and Salmonella. Their research concluded that there was no difference between the two sampling methods (the dry and moistened swabs) in the total counts both bacteria. They also concluded that there were very low concentrations of both bacteria when collected with both the dry and the moistened cotton swab. However, if the researchers possibly used a different type of swab, such as the

puritan foam swab, they may see a more accurate representation of the bacteria count on the carcass skin of the boiler chickens (Notermans, et al.,1976).

This research could be the answer that researchers at the Arch Pathology Lab are looking for. Their research is focused on developing a noninvasive sampling method to collect cells for DNA testing in the clinical laboratory setting. Their goal is have an increase in the participation rate of population genetic studies. Their current sampling method to collect cells for DNA testing is from whole blood collection. The use of buccal cell collection is painless compared to the venipuncture and finger pricks that are currently being used (Heath, et al., 2001).

This research has shown that the most expensive swab, the isohelix swab, does not mean it will perform the best since the puritan foam swab has proven to be the better option for DNA collection. Also, the most expensive extraction kit, the Bioline Isolate II Genomic DNA Kit, does not mean it will extract the best quality and quantity amount of DNA since the Invitrogen PureLink Genomic DNA Kit has proven to result in the best quality and quantity extractions.

A future goal for this research would be to take the modified protocol and continue to make modifications so to find an even timelier method. This timelier method could even lead to the protocol being more cost effective. Being able to extract high concentrations of pure DNA in the shortest amount of time and not being too costly will be beneficial to privately own labs as well as federal labs.

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