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The Effects of Commercial Cleaning Agents on Automated DNA Extraction Efficiency and

Genetic Profile Quality

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By

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The Effects of Commercial Cleaning Agents on Automated DNA Extraction Efficiency and Genetic Profile Quality

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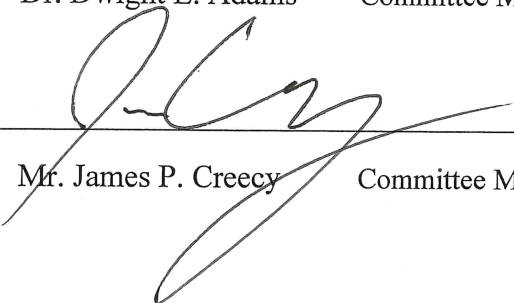APPROVED FOR THE W. ROGER WEBB FORENSIC SCIENCE INSTITUTE

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By _____

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Table of Contents

Abstract

As forensic DNA analysis has experienced countless advances in the past several decades, it has gained considerable notoriety among the general public, including those that are involved in the commission of crimes, leading to biological evidence that has been contaminated with various cleaning products in an attempt to conceal or destroy DNA evidence. This research examined the effects that three types of cleaning agents have on the ability of the Applied Biosystems® Automate *Express*™ Forensic DNA Extraction System to efficiently extract high quality DNA free from inhibiting compounds using the Prepfiler *Express*™ Forensic DNA Extraction Kit. This study further assessed the impact that these chemicals have on the entire forensic DNA analysis process through evaluation of the quality of genetic profiles using a quantitative scale. A dilution series (neat to 1:1000) was prepared from whole human blood, as well as from a bleach product containing sodium hydroxide, a quaternary ammonium-based multi-surface cleaner, and a carpet cleaner with hydrogen peroxide as the active ingredient. Each blood dilution was combined with each dilution of the three cleaning products and each of those samples was analyzed in triplicate. The amount of DNA extracted from bleach-treated samples was reduced compared to corresponding control samples due to destruction of the DNA prior to extraction. The quantification results from samples treated with both the ammonium-based cleaner and the hydrogen peroxide carpet cleaner were similar to controls. The automated system successfully removed inhibitory compounds from samples containing sodium hydroxide and quaternary ammonium compounds, but the blood samples containing the concentrated hydrogen peroxide cleaner showed increased inhibition. The genetic profile quality scores indicated that the ammonium-based cleaner had no effect on profiles regardless of the dilution ratio of the sample, while samples containing at least equal amounts of bleach and blood can be expected to

display extensive dropout of alleles. The inhibition previously mentioned due to the hydrogen peroxide carpet cleaner completely inhibited amplification in samples containing 1:100 or 1:1000 diluted blood treated with neat carpet cleaner. These results indicate that crime scene personnel should document any cleaning agents that may have contaminated biological evidence as it could significantly impact the results of DNA analysis depending on the type of product and its concentration in relation to the evidence.

List of Tables

List of Figures

1. INTRODUCTION

One of the most crucial and commonly encountered types of evidence during the forensic investigation of crimes is blood. The presence of blood can be an indicator of violence inflicted on one person by another, while the genetic information contained within the cells of blood can provide evidence to investigators that a suspect or victim may have been in a specific location or come in contact with a specific object. Since blood evidence can provide such probative information, criminals often go to great lengths to prevent its detection. Historical records have described the use of chemical tests to detect latent blood evidence in forensic investigations dating back to 1440 A.D. (Nam, Won, & Lee, 2014). More recently, with knowledge about the capabilities of forensic DNA testing becoming more widespread, criminals not only wish to conceal bloodstains, but also seek to destroy the identifying biological information found in blood evidence. Typically, the concealment of bloodstains is attempted through the use of commercially available cleaning solutions in an effort to remove any visible traces of blood. Utilizing these cleaning solutions leads to potentially compromised forensic samples that contain foreign chemical agents, often in significantly higher volumes than the blood itself. As forensic DNA technologies and methods continue to develop, research is necessary to investigate the effects these chemicals have at various stages in the DNA analysis process.

1.1 Automated DNA Extraction Technology

When analyzing DNA present in evidentiary blood samples, the first step in the process is the extraction of genetic material from white blood cells within the blood and the removal of contaminants and other cellular components (Li, 2008). Currently, more and more forensic biology labs are employing automated extraction systems to accomplish this. There are various

systems available that employ different extraction kits and methods of binding and purifying

DNA. All of the systems have reduced the time and labor required for this portion of DNA

analysis while providing more consistent processing of many types of samples (Rechsteiner,

2006). One such system is the Automate *Express*™ Forensic DNA Extraction System which uses

the PrepFiler *Express*™ Forensic DNA Extraction Kit to extract and purify DNA from a variety

of forensic samples using paramagnetic particles. After the cells in a sample have been lysed to

release the cellular DNA into solution, the system uses a multi-component surface chemistry to

first bind the DNA to the paramagnetic resin, then to hold the bound DNA stationary throughout

a series of washes using magnets, before finally eluting the purified DNA free from extraneous

substances. (Brevnov, Pawar, Mundt, Calandro, Furtado, & Shewale, 2009) Because the system

operates using a series of specific chemical interactions between the sample lysate, resin, and

buffers, the presence of a contaminating chemical substance within a sample that alters those

reactions could be detrimental to the system's performance.

1.2 Characteristics of Three Common Types of Cleaning Agents

Some of the most common chemical substances that may be present in samples of blood

collected from crime scenes are those found in commercial cleaning products due to the

attempted concealment of this evidence by those involved in the crime. Three common classes of

products that might be used to destroy evidence of a crime are bleach solutions containing

sodium hypochlorite, liquid disinfectant products that contain quaternary ammonium

compounds, and "stain-fighting" cleaners that have an active ingredient of hydrogen peroxide.

Bleach solutions containing sodium hypochlorite are alkaline solutions with a highly

basic pH, usually between 11and 12 for Clorox® brand solutions (The Clorox Company), that

have been studied for many years for their cytotoxic effects on bacteria. Studies have shown that sodium hypochlorite destroys these organisms by penetrating the cell's nucleus and damaging the DNA directly (Rosenkranz, 1973). This property has led to the use of sodium hypochlorite solutions for the decontamination of surfaces and equipment in forensic laboratories (Vandewoestyne, et al., 2011) and for the removal of extraneous DNA from the surfaces of forensic bone and tooth samples (Kemp & Smith, 2005). Due to its ability to induce significant damage to the DNA molecule, the presence of chemical solutions containing sodium hypochlorite in a forensic blood sample could have serious, detrimental effects on the ability to generate a viable genetic profile. These effects need to be quantified in a way that allows for a more accurate understanding of the limitations of current DNA technology.

Quaternary ammonium compounds, or QACs, are the active ingredient in various cleaning products including liquid surface disinfectants. The antimicrobial properties of QACs are due to their interaction with, and eventual destruction of, the lipid membranes of bacterial cells (McDonnell & Russell, 1999). Destruction of these membranes leads to leakage of cellular contents and degradation of nuclear DNA (Salton, 1968). QACs have also been shown to directly damage DNA in the form of lesions and strand breaks in both bacterial (Deutschle, Porkert, Reiter, Keck, & Riechelmann, 2006) and human corneal cells (Ye, et al., 2011) at concentrations far below those present in surface disinfecting solutions.

Hydrogen peroxide based cleaners have become increasingly popular as a less toxic alternative to chlorine-based bleaches for stain removal (Wentz, Lloyd, & Watt, 1975). Studies on the biological effects of hydrogen peroxide have shown that it can alter the concentrations of different types of ions within cells (Halliwell & Aruoma, 1991) and cause significant DNA damage through the generation of free radicals (Imlay & Linn, 1988). Hydrogen peroxide in

larger quantities has also been shown to inhibit the PCR amplification of DNA extracted from blood samples (Akane, 1996). These properties illustrate the potential of hydrogen peroxide to have a measurable, negative impact on DNA present in forensic blood samples, as well as the ability of a forensic examiner to successfully develop a genetic profile from treated blood samples. A study that examines the effects of hydrogen peroxide solutions on current forensic DNA analysis methods across a broad range of concentrations would provide DNA analysts with examples of the types of genetic profiles that could be obtained from samples that are treated with hydrogen peroxide cleaners.

1.3 Research Problem

The field of forensic DNA analysis has been continuously progressing since it first began in the 1980's through the development of new technologies and methodologies. This constant advancement has led to current practices which are extremely sensitive and efficient when compared with those used originally. Unfortunately, criminals have simultaneously become more aware of the importance of biological evidence to investigations. Their attempts to conceal and destroy this evidence result in forensic samples that contain various chemical compounds that have the potential for negative interaction with the DNA itself, as well as the various components of the DNA analysis process. Research is needed that examines the effects that these chemical cleaning agents have on more recently developed DNA technologies, such as the PrepFiler *Express*™ Forensic DNA Extraction Kit and the Automate *Express*™ Forensic DNA Extraction System. Further study of the effects that these chemicals have on genetic profiles obtained from treated samples is also necessary to provide quantifiable data over a range of concentrations to determine the point at which a significant decline in profile quality is observed.

1.4 Purpose of Research

The aim of this research was to determine whether three common classes of commercial cleaning products have negative effects on current forensic DNA analysis processes when they are present in varying amounts in liquid blood samples. More specifically, this research focused on whether the Automate *Express*™ Forensic DNA Extraction System using the PrepFiler *Express*™ Forensic DNA Extraction Kit is able to effectively extract a comparable amount of purified DNA free from PCR inhibitors from samples that have been treated with these chemicals when compared with untreated control samples. This research also analyzed the impact that these three types of cleaning products have on overall genetic profile quality. This was accomplished by analyzing the profiles generated from a broad range of cleaner to blood ratios within each of the three treatments. This portion of the research was performed in order to determine the minimum ratio at which each cleaner begins to have a significant negative effect on the genetic profile. The results of this study will allow forensic DNA examiners employing current methods to have a better understanding of the capabilities and limitations of their technology when processing these types of compromised samples. That knowledge will allow analysts to provide more realistic expectations to criminal investigators of the quality of information that may be obtained from this evidence. This information can also be used by crime scene personnel to assist them when deciding which evidence to collect from a scene that will prove to be the most probative.

2. LITERATURE REVIEW

2.1 History of Forensic DNA Analysis

The potential applications for the analysis of DNA in the field of forensic science were recognized over thirty years ago. Since that time, the process of isolating and analyzing DNA for the purpose of human identification has been a topic of continuous research. Beginning with the observation and interpretation of the molecular structure of DNA in the 1950's and continuing through the current methods of microsatellite typing and automation of forensic genetic profiling, the field of forensic DNA analysis has experienced dramatic advancements during the course of the past three decades.[(Watson & Crick, 1953); (Butler J. , 2010)]

2.1.1 The Structure and Molecular Properties of DNA

The chemical structure of the deoxyribonucleic acid molecule was first elucidated in 1953 by James D. Watson and Francis Crick through X-ray diffraction as seen in Figure 1 (Watson & Crick, 1953). The individual unit of DNA is referred to as a "nucleotide," and consists of one of four nitrogenous bases attached to a deoxyribose sugar molecule and a phosphate group. As individual nucleotides are joined together, these sugar and phosphate groups alternate to form the "backbone" of each strand of DNA, illustrated in Figure 2. The four bases on the interior of the DNA molecule (adenine, thymine, guanine, and cytosine) bond to one another through either two hydrogen bonds, in the case of

Figure 1. Photo of the crystalline structure of DNA. X-ray fiber diagram of DNA molecule that shows the double helical form. Taken by R.E. Franklin and R. Gosling in 1953. **(Watson & Crick, 1953)**

adenine and thymine, or three hydrogen bonds, in the case of guanine and cytosine (Chargaff, 1951).This base pair specific bonding can be seen in Figure 3. These bonds between base pairs

allow two chains of DNA to hybridize to one another to form the double-stranded, double-helix

molecular conformation described by Watson and Crick that is the natural state of DNA within

the cell (Watson & Crick, 1953).
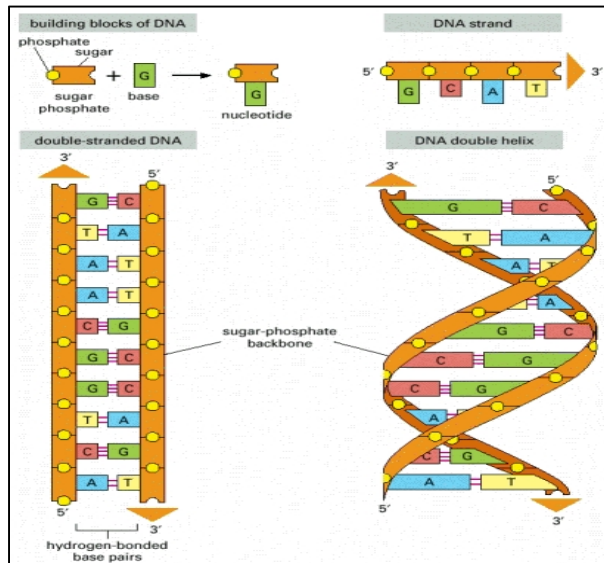
Figure 2. DNA and its individual components. DNA is comprised of nucleotides which each contain a phosphate, a sugar, and one of four different bases. The sugar-phosphate backbone is formed as multiple nucleotides join together to form one DNA strand. Two complementary strands of DNA then bond through base pairing to form a double helix. (Alberts, Johnson, Lewis, Raff, Roberts, & Walter, 2002)
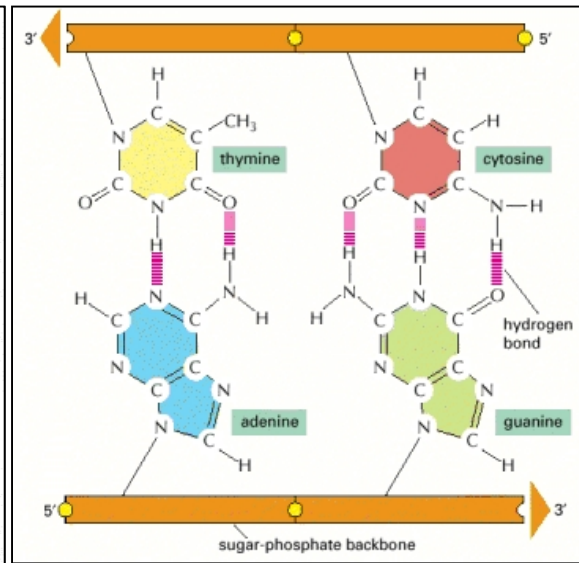
Figure 3. Complementary base pairing between two DNA strands. This diagram depicts the specific structures and bonding patterns of each of the bases found in DNA. Thymine binds to adenine through two hydrogen bonds, while cytosine binds guanine more strongly with three hydrogen bonds. (Alberts, Johnson, Lewis, Raff, Roberts, & Walter, 2002)

The directionality of DNA comes from the orientation of the carbon atoms within the

deoxyribose molecules found in the sugar-phosphate backbone. Within a single nucleotide, the

phosphate group is attached to the 5' carbon atom of the sugar molecule, while the base is bound

at the 1' carbon. The next nucleotide in the DNA sequence attaches to the hydroxyl group at the

3' position, so DNA sequences are traditionally written in the 5' to 3' direction. This is also the

direction that DNA polymerases synthesize new strands of DNA during the replication process.

(Littauer & Kornberg, 1957)

All of the DNA contained within each cell of an individual is referred to as a person's

genome. This genetic information is organized in humans into 22 pairs of autosomal

chromosomes and 2 sex-determining chromosomes,

seen in Figure 4, which are replicated identically and

passed on each time a cell divides through the process

of mitosis. As a result of this process, the genetic

information contained within all somatic, or body, cells

in humans is identical regardless of the tissue or body

fluid of origin. This is a very important quality of DNA

for the field of human identity testing. In humans and

other organisms that reproduced sexually, one
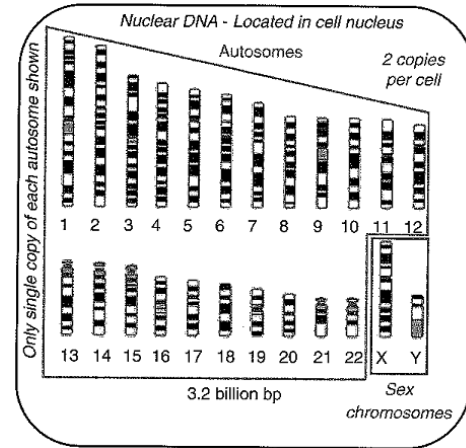
Figure 4. The chromosomes comprising the human genome. Each nucleated cell in the human body has 23 pairs of chromosomes, 22 autosomal pairs and one pair of sex chromosomes. The exception is sex cells which contain only one copy of each chromosome. **(Butler J. , 2010)**

chromosome from each of the 23 pairs is passed on to the offspring from the male, and the other

23 chromosomes are inherited from the female, leading to genetic variation between individuals.

In order for this to be accomplished, gamete cells, either sperm or egg cells, in humans contain

only half of an individual's genome organized into a single set of 23 chromosomes (Weismann,

1893) (Tjio & Levan, 1956). Within a person's genome, the DNA is organized into 30,000-

40,000 genes, and within these genes are two distinct types of regions. The first type are coding

regions called exons, which contain the genetic sequences that are actually translated to

synthesize all of the proteins found in the human body. The second type of DNA makes up the

noncoding regions, called introns that are not involved in the translation process. These regions

contain large amounts of repeating DNA sequences, and their function within the genome is still

a topic of research and debate (Berget, Moore, & Sharp, 1977; Chow, Gelinas, Broker, &

Roberts, 1977). It is these repetitive, noncoding DNA sequences that have been, and continue to

be, the focus of forensic biologists for the purposes of human identification.

2.1.2 RFLP using VNTRs

The beginning of forensic DNA analysis came with the introduction of restriction fragment length polymorphism (RFLP) analysis to detect differences in variable number tandem repeats (VNTRs). In certain hypervariable regions within the genome, these short sequences of DNA, also called "minisatellites," are repeated in tandem and the number of repeats present at one location on a chromosome is referred to as an "allele." Since each somatic cell contains matched pairs of chromosomes, each person will have two alleles present at each locus. Allelic variation between individuals occurs in these hypervariable regions as different individuals have different numbers of repeats at each locus. (Jeffreys, Wilson, & Thein, March 1985)

In order to determine the number of repeats present at a given locus, Jeffreys used restriction enzymes, or endonucleases, that cut DNA strands at a specific location on either side of the VNTR in question. This resulted in a DNA fragment of a specific length, based upon how many repeats were present (Jeffreys et al., 1985). The size of the specific fragment was determined through gel electrophoresis, in which an electrical charge is applied to agarose gel, causing DNA to migrate from one end of the gel towards the other due to the overall negative charge of the molecule. Smaller DNA fragments are able to move through the gel more quickly than larger fragments, so the size of a specific fragment can be determined based upon its position on the gel relative to a sizing ladder comprised of fragments of known lengths,
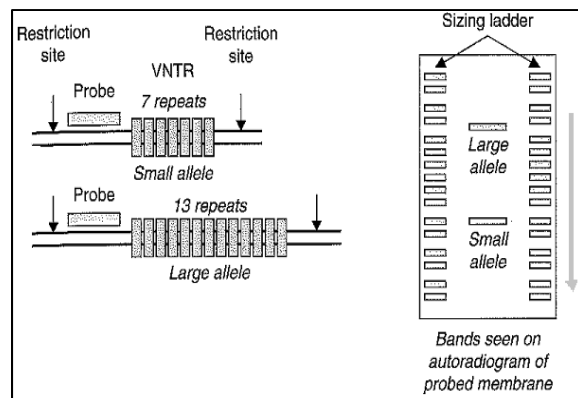
Figure 5. Restriction Fragment Length Polymorphism Analysis. DNA from an individual with two different sized alleles is digested with restriction enzymes that cut the DNA into fragments of different sizes. These are then separated based on size on a gel before being transferred to a membrane and washed with radioactive probes to allow visualization. **(Butler J. , 2010)**

illustrated in Figure 5 (Aaij & Borst, 1972). Using various radioactive probes that hybridize to different DNA fragments, multiple VNTRs could be visualized on a single autoradiogram using X-rays. The combination of alleles from multiple VNTR loci was termed an individual's "DNA fingerprint" by Jeffreys. (Jeffreys, Wilson, & Thein, July 1985)

The forensic application of the RFLP process was recognized almost immediately after it was developed, and it was first applied to forensic type samples by its developers within the same year (Gill, Jeffreys, & Werrett, 1985). The process of RFLP typing was further validated in 1991 in a study that exposed biological samples to a variety of environmental, chemical and biological insults, including chlorine bleach, to examine what effect these treatments had on the quality and reliability of the resulting RFLP pattern. While the study concluded that RFLP analysis was a valid method that produced no false results under any of these conditions, multiple samples produced no pattern whatsoever after being exposed to these types of conditions and contaminants (Adams, et al., 1991). Despite the fact that RFLP analysis was highly discriminating, it has become largely obsolete with regards to forensic DNA analysis due to its lengthy analysis process and the fact that it requires greater than 50 ng of intact, high molecular weight DNA to develop a reliable profile. Since DNA recovered from evidentiary samples has often been degraded by a variety of environmental insults prior to analysis, this makes RFLP less effective in a forensic capacity. (Butler J. , 2010)

2.1.3 The Polymerase Chain Reaction

A crucial advancement in the history of forensic DNA analysis was the development of the polymerase chain reaction, or PCR. This process, developed by Kary Mullis at the Cetus Corporation, was first described in an article by Saiki et al. in late 1985 (Saiki, et al., 1985) and
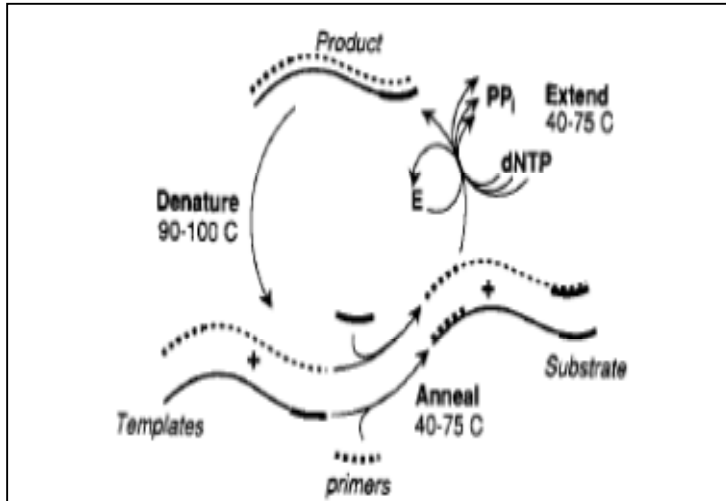
Figure 6. A model of one cycle of the polymerase chain reaction. Each cycle first requires the denaturation of double-stranded template DNA, followed by annealing of forward and reverse primers, then the extension of the new DNA segment by the DNA polymerase. Then the product and template strands are denatured from one another and the process begins again. **(Bloch, 1991)**

was later patented by Mullis in 1987 (Mullis K. B., 1987). PCR allows for the copying of a single sequence of DNA through multiple cycles in order to generate millions of identical copies. This is accomplished through a series of amplification cycles that involve the manipulation of the reaction temperature. First, the temperature is raised to denature double stranded DNA, then lowered to allow fluorescently labeled primers to anneal to the DNA region of interest. DNA polymerase then extends the new, complementary strand of DNA through the addition of deoxynucleotide triphosphates (dNTPs), and the process begins anew with the denaturing of the template and newly synthesized strands from one another so that they can each be used as template strands in the next amplification cycle. The process is represented in Figure 6, and these steps are repeated continuously until millions of copies of the original DNA sequence exist in the sample (Mullis, Faloona, Scharf, Saiki, Horn, & Erlich, 1986). The process was later improved through the use of a thermally stable DNA polymerase known as *Taq* polymerase, allowing the entire process to be carried out in one sealed tube since the polymerase was no longer inactivated by the extremely high denaturing temperatures (Moretti, Koons, & Budowle, 1998). *Taq* polymerase is isolated from the thermophilic *Thermus aquaticus* bacterium that was first discovered inhabiting thermal springs in Yellowstone National Park and elsewhere in California (Brock & Freeze, 1969).

The PCR process was critical to the advancement of forensic DNA typing, as analysts could now generate millions of copies of the DNA present in extremely low quantity or degraded biological evidence samples. This represented a significant improvement over RFLP methods that required intact DNA samples that were several thousand bases long. PCR amplification capabilities necessitated the development of a profiling system that utilized much smaller, but still highly variable DNA sequences, which led to the advancements discussed in the following section. (Li, 2008)

2.1.4 Short Tandem Repeat Profiling

Short tandem repeats (STRs), or microsatellites, are small segments of noncoding DNA that contain a 2-7 base pair sequence that is repeated a variable number of times. These repetitive DNA markers were first studied in the late 1980's and the advantages of applying STRs to forensic DNA typing were established as early as 1988 (Craig, Fowler, Burgoyne, Scott, & Harding, 1988). Although a single STR locus does not provide the same level of discrimination as a typical VNTR locus, the relatively small size of each STR allows several separate loci to be amplified simultaneously through multiplex PCR which exponentially increases the discriminating power (Edwards & Gibbs, 1994). In an effort to standardize the STR loci being typed in forensic labs, the Federal Bureau of Investigation

Figure 7. The 13 CODIS core STR loci with chromosomal positions. These 13 loci were chosen by the FBI as the standardized set used to generate a genetic profile that can be entered into the U.S. federal DNA database, the Combined DNA Indexing System. **(Butler & Reeder, Short Tandem Repeat DNA Internet DataBase,**

designated 13 specific loci, pictured in Figure 7, as the "core" loci to be used to generate a complete genetic profile for comparison and for entry into the U.S. national DNA database, the

Combined DNA Indexing System (CODIS). Multiplex systems were validated in the late 1990's

that incorporated these 13 loci, allowing forensic analysts to now generate standardized genetic

profiles from samples containing only a fraction of the amount of DNA required for previous

DNA typing methods. Furthermore, the development of fluorescent tags and capillary

electrophoresis made STR typing significantly safer and more efficient than RFLP analysis

(Moretti, Baumstark, Defenbaugh, Keys, Smerick, & Budowle, 2001). All of these advantages

have led to worldwide acceptance of STR typing as the optimum method for forensic DNA

analysis for more than 20 years.

2.2 Current Forensic DNA Analysis Methods

The history of forensic DNA analysis over the past three decades that was described in

the previous sections has culminated in the current processes employed by public and private

labs worldwide. The general steps involved in the forensic DNA analysis process include

extraction of DNA from biological samples, quantification of the amount of DNA present in a

sample, amplification of the DNA, and finally, separation and detection of the individual

amplified DNA fragments to generate a genetic profile. There are various specific methods and

technologies available for each of these four steps, however, the processes described in the

following sections are only those currently employed by the Forensic Biology Unit at the

Oklahoma State Bureau of Investigation's Forensic Science Center, where this research study

was conducted.

2.2.1 Extraction of DNA with the Prepfiler *Express*™ Forensic DNA Extraction Kit

The initial step in any type of forensic DNA analysis is extraction of the DNA itself from

all other biological material and contaminants present in an evidentiary sample. In the current

study this is accomplished through the use of the Prepfiler *Express*™ Forensic DNA Extraction Kit used in conjunction with the Automate *Express*™ Forensic DNA Extraction System. The original Prepfiler™ extraction kit was developed by Applied Biosystems® in 2008 and binds the DNA from lysed cells to the surface of magnetic particles in order to keep it stable during multiple wash steps that remove the rest of the cellular material that can interfere with downstream PCR processes. The purified DNA is then removed from the particles using an elution buffer with a different chemical composition (Brevnov, Pawar, Mundt, Furtado, & Shewale, 2008). The manual system was validated using a variety of samples including liquid blood samples and samples with various known PCR inhibitors. The authors used the results from quantification data to conclude that the PrepFiler™ kit efficiently removed all PCR inhibitors that had been introduced to the samples. The study also compared the PrepFiler™ kit to several other extraction methods, and showed that it produced higher concentrations of extracted DNA from a variety of different types of samples. (Brevnov, Pawar, Mundt, Calandro, Furtado, & Shewale, 2009)

An additional benefit to the PrepFiler™ chemistry is that it was able to be modified for an automated DNA extraction system. For this purpose, Applied Biosystems developed the PrepFiler Express™ Forensic DNA Extraction kit for use in the Automate Express™ DNA Extraction System. A preliminary validation of this system concluded that it was able to improve the quality of DNA obtained by removing potential inhibitors, while also significantly reducing the amount of time required for DNA extraction (Balsa, et al., 2011) These results were similar to those obtained in a more extensive study of the system conducted in 2012 which determined the Automate Express™ System was able to extract DNA of sufficient quantity and quality, with PCR inhibitors effectively removed, for all case-type samples that were tested (Liu, et al., 2012).

In an additional study, this automated system was also compared to two other extraction methods

and the Automate Express™ System using the PrepFiler Express™ kit recovered higher

quantities of DNA as well as more efficiently removing PCR inhibitors. (Bogas, Balsa, Carvalho,

Anjos, Pinheiro, & Corte-Real, 2011)

Since this extraction system is relies upon an optimized, multi-component surface

chemistry in order to successfully bind, wash and elute purified DNA (Brevnov, Pawar, Mundt,

Calandro, Furtado, & Shewale, 2009), it is reasonable to suspect that a sample containing

additional chemical solutions, i.e. bleach, disinfectants, etc., might affect the system's ability to

effectively extract high quantities of DNA while simultaneously removing PCR inhibitors. The

current research investigated this question using samples containing various concentrations of

contaminating chemical solutions.

2.2.2 Quantification of DNA through Real-Time PCR Using the Quantifiler® Human DNA Quantification Kit

Once the purified DNA has been extracted from a sample, the amount of DNA present

must be determined in order to generate the optimal initial DNA concentration in the

downstream PCR reaction. One of the most effective methods available to accomplish this is

Real-Time PCR, which was first introduced in 1992 by scientists at Roche Molecular Systems

Inc. Originally, the method was developed as a way of monitoring the successful amplification of

DNA through PCR while the reaction was occurring, eliminating the need for labor intensive

confirmatory tests for PCR products after the amplification process was completed. This

monitoring method was accomplished by the addition of ethidium bromide to the reaction, a

compound whose fluorescence increases in the presence of double-stranded DNA. Recording the

amount of fluorescence present throughout the reaction indicated whether the amount of DNA present was increasing after each PCR cycle. (Higuchi, Dollinger, Walsh, & Griffith, 1992)

This method has since been improved and optimized for the purposes of forensic human identification resulting in the current process which utilizes the Quantifiler® Human DNA Quantification Kit (Applied Biosytems®) and analyzes samples on a 7500 Real-Time PCR System (Applied Biosystems®). This kit capitalizes upon the 5' exonuclease activity of the Taq polymerase described in Section 2.1.3 of this chapter. This polymerase activity cleaves double-stranded DNA in the 5' → 3' direction releasing individual nucleotides and small DNA fragments (Holland, Abramson, Watson, & Gelfand, 1991). To take advantage of this activity, the Quantifiler® kit contains a probe, called the Taqman® probe, which hybridizes to a small section of the target sequence which is a portion of the human telomerase reverse transcriptase gene that was selected due to its singularity and similarity in size to the STR loci amplified downstream in forensic analysis. This

Figure 8. Detection of PCR amplification through fluorescent probes. In the initial phases of the reaction, two dyes, a reporter dye and a quencher dye, are both attached to the probe and the emission of the reporter is quenched. As the DNA polymerase extends the new DNA product, it uses its 5' nuclease activity to cleave the reporter dye from the probe, allowing it to fluoresce and be detected. **(Applied Biosystems, 2001)**

probe contains a fluorescent "reporter" dye on the 5' end and a second "quencher" dye on the 3' end. As long as the probe is intact, the quencher dye suppresses the fluorescence of the reporter dye using Förster-type energy transfer, however, when the Taq polymerase encounters the probe during synthesis of the new PCR product, the exonuclease activity releases the reporter

dye on the 5' end first, and the resulting fluorescent signal can be recorded (pictured in Figure 8).

The intensity of the fluorescent signal increases with each PCR cycle as more and more

Taqman[®] probes are released from the increasing number of DNA template strands. Eventually,

the signal will reach a specific threshold where it can be reliably distinguished from all

background fluorescence. The cycle at which the fluorescence crosses this line is called the

"Threshold Cycle", or C_T, and the C_T for a sample is inversely proportional to the amount of

DNA template present at the beginning of the PCR process. In other words, the more DNA is

present in a sample prior to PCR, the lower the C_T for the given sample will be. The C_T value for

a sample can be compared to a standard curve generated from samples with known DNA

concentrations in order to determine the exact concentration of DNA present, reported in ng of

DNA/µL of extract. (Green, Roinestad, Boland, & Hennessy, 2005)

An additional feature of the Quantifiler[®] kit is the inclusion of an Internal PCR Control

(IPC) that allows for the detection of substances inhibiting amplification during the

quantification process. The IPC system consists of a synthetic DNA sequence, primers to

specifically amplify this synthetic sequence, and a probe similar to the one described previously

that is labeled with a different reporter dye. If a sample truly contains little to no DNA, then this

synthetic DNA sequence will display normal amplification with a C_T value between 20 and 30

cycles. If the sample displays an artificially low quantification value due to PCR inhibition

however, the amplification of the IPC sequence will also be inhibited leading to a higher C_T

value (Applied Biosystems, 2006). In forensic casework, these results would be used to

determine if a sample needed to be re-purified or re-extracted before moving on to STR

amplification, but in a research context the IPC can be used to determine the effectiveness of an

extraction method at removing inhibitors that have been added to a sample as seen in the

validation of the Prepfiler *Express*® system. (Balsa, et al., 2011)

2.2.3 Multiplex PCR Amplification using the AmpFLSTR® Identifiler® Plus PCR Amplification Kit

Once the amount of DNA present in a given sample has been determined, it can be

effectively amplified using PCR. Currently, many forensic biology laboratories employ the

AmpF*l*STR® Identifiler® Plus PCR Amplification Kit to accomplish this. The Identifiler® Plus

kit is a multiplex STR assay, which means multiple loci are all amplified simultaneously. The

loci amplified are 15 tetranucleotide repeat loci, including the 13 core CODIS loci, and the

Amelogenin marker used for gender-determination (Applied Biosystems, 2009). The kit contains

fluorescently labeled forward and reverse primers, an allelic ladder containing amplified alleles

commonly reported at each locus, control DNA 9947A with a known profile, and an

amplification master mix made up of Taq DNA polymerase, deoxynucleotide triphosphates

(dNTPs), salts, and sodium azide. (Applied Biosystems, 2009)

Once the primers and reaction mix have been combined with the correct amount of a

sample extract to target approximately 1 ng of DNA, the mixture is placed onto a thermal cycler

where it undergoes an initial incubation at 95°C in order to activate the AmpliTaq Gold® DNA

Polymerase, followed by 28 PCR cycles. Each cycle features a 94°C step to denature the double-

stranded DNA, before moving to 59° to allow annealing of the primers to the DNA template and

elongation of the newly synthesized DNA by the polymerase. Following the 28 amplification

cycles, the reaction is held at 60°C for 10 minutes to allow for complete extension of each DNA

fragment. These standard thermal cycling parameters were determined during the course of the

validation of this PCR kit by Wang et al. This validation also established that this kit displayed

greater sensitivity and a better ability to overcome PCR inhibitors when compared to previous

amplification kits. (Wang, Chang, Lagace, Calandro, & Hennessy, 2012)

2.2.4 Detection of PCR Products Using Capillary Electrophoresis

After the original DNA template in a sample has been amplified using the Identifiler®

Plus kit to produce millions of copies, the individual STR loci must be separated from one

another in order to generate an individual's genetic profile. In the field of forensic science, this is

currently accomplished through the use of capillary electrophoresis. This separation method was

first applied to forensic STR fragments in the early 1990's and operates in general by forcing the fluorescently-labeled DNA fragments through a narrow glass capillary filled with a sieving polymer (McCord, Jung, & Holleran, 1993). The negatively charged DNA migrates from the cathode end of the capillary to the anode end once an electrical current has been applied, and as it

Figure 9. Components of a capillary electrophoresis system. Within a capillary electrophoresis system, DNA migrates from one end (cathode) of the capillary to the other (anode) when an electrical current is applied. As it migrates, the DNA fragments are separated based upon their sizes before passing through a detection window where a laser excites the fluorescent labels and a charge-coupled camera records the emissions. **(Butler & McCord, 2004)**

moves, the smaller fragments are able to pass through the sieving polymer more quickly while

the larger fragments get caught and are slowed down. This leads to separation of the individual

PCR products by the time they reach the detection window in the capillary where the fluorescent tags are excited with a laser light source causing them to emit light which is then translated into an electrical signal that can be recorded by a computer to be compared to a sizing standard that is run simultaneously, giving the exact size of the DNA fragments in question. These fragments are then separated first by which color of fluorescent dye was used to label them, and then by their size within each dye channel in order to generate an electropherogram. A general depiction of this type of system is shown in Figure 9. (Butler & McCord, 2004)

2.3 DNA Damage and Amplification Inhibition

In forensic DNA analysis, the successful recovery of a useful STR profile is not always possible, even in evidentiary samples that contain a sufficient amount of biological material. Two of the major causes for this lack of success are often DNA degradation and inhibition of PCR amplification. DNA degradation can occur due to various agents that are endogenous, occurring naturally within an organism, or exogenous, originating from outside sources (De Bont & van Larebeke, 2004). This damage to the DNA molecule comes in various forms including single- or double-strand cleavage, chemical modification of the individual bases, and depurination, in which adenine and guanine bases are released from the molecule (Lindahl, 1993). In a living organism, these types of damage can often be repaired by specific enzymes to prevent incorrect or incomplete replication (Sancar, Lindsey-Boltz, Unsal-Kacmaz, & Linn, 2004), however in non-replicating cells that have been removed from the body, like the ones comprising forensic biological samples, these enzymes are typically no longer active and the damage is permanent.

The second major challenge in attempting to generate a complete and useful forensic DNA profile is the inhibition of the PCR amplification process by substances that are present within forensic samples following DNA extraction. Some common examples of these types of substances include humic acid in soil, heme in blood, heavy metals, urea, collagen, certain types of carbohydrates, and even excessive amounts of DNA itself. Inhibitors can affect all components of the PCR process including interfering with primer binding, altering Mg^{2+} concentration, inhibiting the action of the DNA polymerase, or modifying the free nucleotides or DNA template, all of which can lead to heterozygous peak height imbalance, allelic dropout, or complete failure of the amplification reaction (Alaeddini, 2012). Three commonly encountered chemical compounds that can cause both of these particular issues in forensic samples are reviewed in the following sections. Each of the chemicals discussed are the major active ingredient in various types of commercially available cleaning products that might be used to conceal or destroy biological evidence.

2.3.1 Sodium Hypochlorite

One of the most common agents used for cleaning and disinfection purposes is chlorine bleach. The most common type of chlorine bleach available today has an active ingredient of sodium hypochlorite. Chlorine was first discovered in 1774 by Swedish chemist C.W. Scheele, and the first liquid bleaching agent was produced in 1785 in France, but liquid bleach did not become commercially marketed in the United States until the early 1900's (Baldwin, 1927). In the 1970's, Hayatsu et al. studied the effects that sodium hypochlorite had specifically on nucleic acids. They studied the changes produced in the UV spectra of isolated nucleosides after treatment with both 10 ppm and 100 ppm sodium hypochlorite and determined that the chemical structure of each individual base was altered to generate products that extensively reduced UV

absorption. The study went on to attempt to identify which specific products were made, but for the purposes of DNA amplification and analysis any alteration to the bases present in the DNA molecule would render it useless for human identification, regardless of what the specific products were. (Hayatsu, Pan, & Ukita, 1971)

In 1973, a study was conducted to examine the effects of sodium hypochlorite on DNA in its natural state within the cell. The authors exposed bacterial cells that were deficient in DNA polymerase I, the enzyme responsible for repairing damaged DNA, to sodium hypochlorite and found that the growth of this bacterial strain was inhibited to a greater extent than normal bacteria exposed to the same treatment. This data showed that the inhibition was due to damage inflicted directly to the DNA of the organism by sodium hypochlorite, rather than just damage to other cellular structures (Rosenkranz, 1973). This attribute of sodium hypochlorite led to the use of dilute chlorine bleach as the preferred method for the decontamination of forensic lab spaces and equipment as it was able to alter or destroy exogenous DNA to the point that it was no longer able to amplified using PCR. Vandewoestyne et al. found that the number of alleles detected was significantly lower in samples collected from various lab areas and equipment after such decontamination procedures as opposed to before (Vandewoestyne, et al., 2011). An additional forensic application of the DNA-damaging properties of sodium hypochlorite was explored in two similar studies in which the solution was used to remove contaminating DNA from the surface of both forensic (Rennick, Fenton, & Foran, 2005) and ancient (Kemp & Smith, 2005) skeletal remains and teeth. The first study examined the effects of sodium hypochlorite decontamination on the endogenous DNA that an analyst would want to successfully extract and determined that the bleach treatments degraded this DNA significantly. The second study was more focused on the successful removal of contaminating DNA from the surface of ancient

remains, however, the authors did come to a somewhat contradictory conclusion that sodium

hypochlorite did not damage the endogenous DNA of the bones, but did destroy the exogenous

DNA.

All of the research discussed thus far regarding the effect of sodium hypochlorite on

DNA has used isolated nucleotides, epithelial cells, or DNA found in bones and teeth; a final

study of relevance to this project examines various types of DNA damage, including that

inflicted by sodium hypochlorite solutions, in liquid blood samples. The authors determined that

when liquid blood was immersed in a commonly used 10% bleach solution (0.6% sodium

hypochlorite), full STR profiles were still obtained, even after a 2 hour incubation period. When

the concentration of sodium hypochlorite was increased to 3%, however, allelic dropout occurred

after a 1 hour incubation, and complete profile loss was seen after 2 hours. (Ambers, Turnbough,

Benjamin, King, & Budowle, 2014)

A final study that examined the negative effects of chlorine bleach on forensic DNA

analysis was conducted in 2011. This study examined two types of commercial disinfecting

solutions and compared them to Clorox® bleach in order to determine if they could be used as an

alternative method for the sterilization of forensic laboratory workspaces and equipment. During

the course of this study, different concentrations of each solution were added to DNA that had

previously been extracted from a blood sample, and the extracts were then quantified and

amplified. The results from both the Internal PCR Control recorded during quantification as well

as the final STR profiles support the conclusion that chlorine bleach is highly inhibitive to the

PCR process when present in a forensic sample following the extraction process. (O'Brien &

Figarelli, 2011)

2.3.2 Quaternary Ammonium Compounds

The second type of cleaning agent of concern to this research consists of solutions that contain a class of chemicals called quaternary ammonium compounds, or QACs. The specific type of QAC found in the disinfectant cleaner selected for this research is alkyl dimethyl benzyl ammonium chloride (Reckitt Benckiser, 2013), generally known as benzalkonium chloride (BAC), pictured in Figure 10. QACs are classified as "cationic surfactants", and consist of a hydrophobic,

Figure 10. The chemical structure of benzalkonium chloride (BAC). BAC is a type of quaternary ammonium compound in which the nitrogen is bound to a benzyl group, two methyl groups and an even-numbered alkyl chain. (ChEBI: The Database and Ontology of Chemical Entities of Biological Interest, 2014)

hydrocarbon group and a positively-charged, hydrophilic group. The value of QACs as antiseptics and disinfectant cleaners comes from their interactions with the cell membranes of infectious agents eventually leading to cell lysis and death (McDonnell & Russell, 1999). The specific mechanism of this interaction has been described by Salton in the following five steps: "(1) Adsorption and penetration of porous wall (2) Interaction with lipid-protein membrane; reaction with hydrophobic proteins, oriented lipids; membrane disorganization (3) Leakage of low molecular weight metabolites, e.g. amino acids, purines, pyrimidines, nucleotides, ions (4) Degradation of proteins, nucleic acids (5) Lysis due to wall-degrading autolytic enzymes" (Salton, 1968).

Since the major commercial value of QACs lies in their antimicrobial properties, most of the research conducted using these compounds has examined the toxic effects on bacterial cell viability following exposure to QACs. A study of this type by Deutschle et al., however, also included an investigation of the genotoxic effects of QACs, specifically benzalkonium chloride.

The authors describe genotoxicity as the ability of an agent, chemical or otherwise, to cause damage specifically to DNA, including strand breaks. They found that exposure to increasing levels of BAC led to DNA single-strand breaks as well as other DNA lesions that are precursors to strand breaks (Deutschle, Porkert, Reiter, Keck, & Riechelmann, 2006). An additional study published in 2007 studied the effects of QACs on both mammalian and plant cells. This study documented increased amounts of DNA damage following exposure to two different types of QACs (Ferk, et al., 2007).

One final study of BAC examined the effects of the compound on DNA found in human cells, specifically human corneal epithelial cells. The cells were exposed to doses of BAC ranging from only 0.00005% to 0.001% before being tested for the presence of both single- and double-strand DNA breaks. The authors found that as the concentration of BAC was increased, the amount of both types of DNA damage increased as well. The study went on to determine that even after 24 hours of cell recovery from these treatments, the damage was only partially repaired through natural cellular mechanisms (Ye, et al., 2011). It is notable that the maximum concentration of BAC used in the previously described experiment was approximately 100 times more diluted than the concentration of BAC present in the disinfectant cleaning product selected for this research.

2.3.3 Hydrogen Peroxide

Another type of disinfecting agent that has become common more recently consists of solutions containing hydrogen peroxide as the active ingredient. Hydrogen peroxide was first reported in the early 19th century and was first marketed as a disinfectant approximately 50 years later (Block, 2001). Because the compound has very low toxicity to humans, animals, and the

environment in general, it is useful not only to destroy bacteria and viruses on materials that may

come in contact with food or drink, but also for the sterilization of the consumables themselves

such as drinking water (Yoshpe-Purer & Eylan, 1968) or milk (Naguib & Hussein, 1972).

Hydrogen peroxide occurs naturally within human cells, and its biocidal properties are evident as

it is one of the compounds that destroy infectious agents in both the mucous membranes and in

the bloodstream. (Block, 2001)

Although hydrogen peroxide is a compound that occurs naturally within cells, exposure

to increased levels of the chemical can damage cells in various ways including disruption of

membrane lipids leading to cell lysis, as well as damage directly to the cellular DNA. The latter

of these types of damage is of particular interest to researchers in both medical and forensic

science. The negative effects that are exerted upon cells by hydrogen peroxide and other related

chemical species have been termed "oxidative stress," and, in the case of DNA, this stress

typically comes in the form of free radicals that attack both the sugar-phosphate backbone of the

molecule and the individual nitrogenous bases (Imlay & Linn, 1988). Two mechanisms by which

DNA damage is caused within the cell from hydrogen peroxide exposure were put forth by

Halliwell and Aruoma. In the first theory, increased hydrogen peroxide exposure causes

increased release of copper or iron ions within the cell. These ions then bind directly to cellular

DNA before being oxidized by hydrogen peroxide to generate the hydroxyl radical. This

powerful free radical then attacks the DNA that is bound to the metal ions and induces strand

breakage and base modification. The second mechanism of DNA damage proposed by the

authors describes the inhibition of the cell's ability to extrude excess calcium ions (Ca^{2+}). This

leads to an increase in intracellular free Ca^{2+} ions, which in turn leads to the activation of

endonucleases present within the cell. These endonucleases then proceed to sever DNA strands

causing fragmentation without any base modification (Halliwell & Aruoma, 1991). Despite

several studies describing the damaging effects of hydrogen peroxide on DNA, when these

principles were applied to forensic samples in a study conducted in 2014, the investigators were

unable to induce significant damage to the DNA in either whole human blood or bloodstains on

fabric samples. No significant interference with STR typing results was seen in either set of

samples with the exception of a slight reduction in allele peak heights. (Ambers, Turnbough,

Benjamin, King, & Budowle, 2014)

A final property of hydrogen peroxide that is of particular interest with regards to

forensic blood evidence relates to inhibition of the PCR process. In a series of studies conducted

in Japan in the 1990's, hydrogen peroxide was examined with respect to its effect on PCR

amplification of DNA extracted from blood and bloodstains. The studies were able to produce

evidence that when large quantities of hydrogen peroxide are present in the reaction, it inhibits

amplification, however, when DNA extracts were treated with smaller volumes of hydrogen

peroxide and the chemical was then precipitated out of the sample prior to amplification, the

PCR efficiency was actually increased. This was likely due to the fact that hydrogen peroxide is

able to decompose the heme compound found in blood that can inhibit PCR (Akane, 1996). The

current literature illustrates that the possible effects of hydrogen peroxide with regards to DNA

damage and interference with forensic analysis methods are varied and dependent upon many

factors, including concentration, other compounds present in a sample, and time of exposure.

Research is needed that controls and isolates these variables while providing a more accurate

model of the possible interaction between blood and hydrogen peroxide that might occur in a

forensic setting.

3. MATERIALS AND METHODS

3.1 Preparation of Dilution Series, Control Samples, and Experimental Samples

A series of five dilutions of whole blood was prepared by combining whole human blood with phosphate buffered saline (PBS). The blood sample was a single-source, male sample obtained from Innovative Research™ (Novi, MI), lot number 23 51253, and contained sodium ethylenediamminetetraacetate (NaEDTA) as an anticoagulant. The dilution series consisted of a neat blood sample, a 1:2 blood:PBS sample, a 1:10 blood:PBS sample, a 1:100 blood:PBS sample, and a 1:1000 blood:PBS sample. The volumes used to prepare these dilutions are listed in Table 1. Once prepared, the five blood dilutions were stored at ~2°C.

Table 2: Volumes of Blood and PBS Used to Prepare Dilution Series

Dilution	Volume of Blood Used (mL)	Volume of PBS Used (mL)	Total Volume (mL)
Neat	10.0	0	10.0
1:2	3.5	7.0	10.5
1:10	1.0	10.0	11.0
1:100	0.1	10.0	10.1
1:1000	0.01	10.0	10.01

A dilution set was prepared in the same manner as above for each one of three commercial cleaning products. The first cleaning solution selected was Clorox® Regular-Bleach (The Clorox Company, Oakland, CA), which has an active ingredient of 8.25% sodium hypochlorite. The second product was Lysol® Multi-Surface All-Purpose Cleaner (Reckitt Benckiser LLC, Slough, Berkshire, UK), containing an active ingredient of 0.1076% dimethyl

benzyl ammonium chlorides, which belong to the group of chemical compounds referred to as quaternary ammonium compounds, or QAC's. The final cleaning product chosen was Resolve® Carpet Cleaner (Reckitt Benckiser LLC, Slough, Berkshire, UK), which has an active ingredient of <2.5% hydrogen peroxide. The cleaners were diluted with ultrapure, deionized water, rather than PBS. Once prepared, these three dilution sets were stored at room temperature.

A set of untreated blood samples from each of the five dilutions was prepared to be used as control samples. Approximately 200 µL of each blood dilution was combined with 200 µL of ultrapure deionized water in a 1.5 mL microcentrifuge tube. Water was added to simulate effects that might occur in the treated samples due to dilution of the sample by the additional liquid of each cleaning product. A reagent blank for the control sample set was prepared by combining 200 µL of PBS and 200 µL of deionized water. Each sample was vortexed thoroughly and stored at room temperature for 1 hour before beginning the DNA extraction protocol described in the subsequent section.

Three sets of experimental samples were prepared by combining 200 µL of each blood dilution with 200 µL of each cleaning product dilution in a 1.5 mL microcentrifuge tube. For example, experimental set 2 consisted of samples of blood combined with bleach, therefore Sample 2A contained 200 µL of neat blood combined with 200 µL of neat bleach. Sample 2B consisted of 200 µL of neat blood combined with 200 µL of 1:2 bleach dilution, etc. A complete list of all samples prepared in the course of this study is depicted in Appendix A. A reagent blank was prepared for each of the three treated sample sets by combining 200 µL of each of the neat cleaning solutions with 200 µL of deionized water. All samples were vortexed and stored at room temperature for 1 hour before being processed using the DNA extraction protocol described in the subsequent section.

3.2 DNA Extraction

Three replicates from each of the samples from Set 1 (control samples), Set 2 (treated with bleach), Set 3 (treated with Lysol®), and Set 4 (treated with Resolve®) were extracted using the PrepFiler *Express*™ Forensic DNA Extraction Kit chemistry with the Automate *Express*™ Forensic DNA Extraction System instrument. A reagent blank was also extracted in triplicate for each sample set, with the exception of Set 1 which contained only one reagent blank sample. One of these three blanks was extracted during each run on the Automate *Express*™ instruments. Samples were extracted according to the protocol found in the PrepFiler *Express*™ Forensic DNA Extraction Kit User Guide (Life Technologies Corporation®, 2010). Prepfiler Lysep™ columns were inserted into each of the hinge less Prepfiler™ sample tubes. Next, 40 μL of each sample was pipetted into a PrepFiler® Lysep Column/hinge less tube assembly, as depicted in Figure 11. Approximately 500 μL of PrepFiler® lysis buffer and 5 μL of 1M Dithiothreitol

Figure 11. Prepfiler® Lysep Column/hinge less tube assembly. Sample lysate is transferred from the Lysep column to the hinge less sample tube when the thin filter at the base of the column breaks upon centrifugation. The sample tube can then be separated and placed directly into the instrument.

(DTT) were added individually to each sample. The samples were then placed onto an Eppendorf Thermomixer® C (Hamburg, Germany) and incubated at 70°C while shaking at 750 rpm for 40 minutes. After incubating, each column/tube assembly was centrifuged for two minutes at ~10400 rpm to transfer the sample lysate to the hinge less sample tube. The Lysep™ columns were then removed from the sample tubes and discarded.

Preparation of the Automate *Express*™ instrument is depicted below in Figure 12. The cartridge rack was loaded first with 13 Prepfiler *Express*™ reagent cartridges before being placed on the

instrument. Each of the hinge less sample tubes containing the sample lysates was placed into

row S of the tip and tube rack. Row T2 was loaded with Automate *Express*™ Tips inserted into

tip holders, and Row E was loaded with labeled Prepfiler™ Elution Tubes before the tip and tube

rack was loaded on to the instrument.
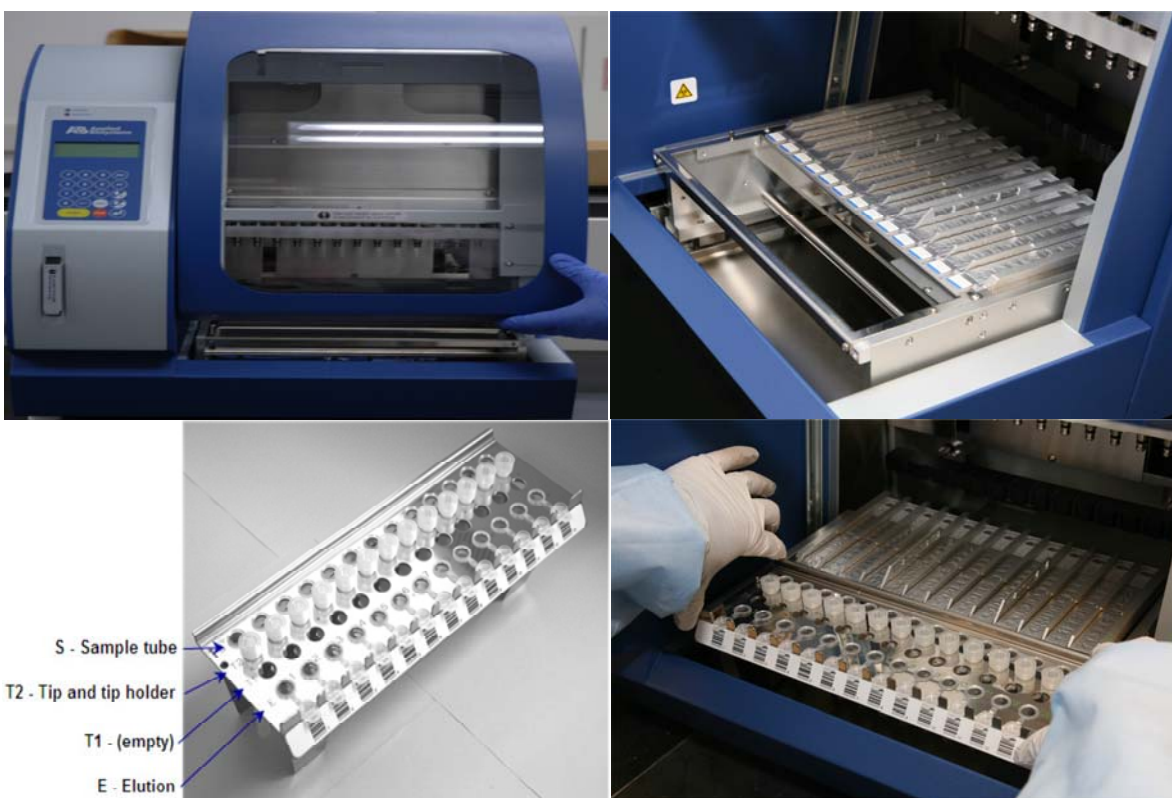
Figure 12. Preparation of the Automate *Express*™ system prior to DNA extraction. Figures depicting the Automate *Express*™ system (upper left), the cartridge rack with 13 Prepfiler *Express*™ reagent cartridges that is loaded first (upper right), the tip and tube rack with sample tubes containing lysate in row S and elution tubes that will contain DNA extract in row E (lower left), and the instrument with both loaded racks placed inside (lower right).

The "PF Express" protocol on the Automate *Express*™ system was selected and, once completed,

the elution tubes containing approximately 50 µL of purified DNA extract were closed and

removed from the instrument. These DNA extracts were stored at approximately 2°C, while all

other plastics were removed from the instrument and discarded.

3.3 DNA Quantification

Each one of the purified DNA extracts was quantified using the Quantifiler® Human

DNA Quantification Kit (Applied Biosystems®) on an Applied Biosystems® 7500 Real-Time

PCR System according to the "DNA Quantification" protocol found in the Oklahoma State

Bureau of Investigation's Forensic Biology Unit Policy Manual, Revision 4 (2014). Prior to

quantification, 1:20 dilutions of each sample containing neat blood and 1:10 dilutions of each

sample containing 1:2 blood were prepared. This was done to simplify the targeting of 1 ng of

DNA downstream during amplification in the likely event that these samples contained high

amounts of DNA. A serial dilution set of standards with known DNA concentrations was

prepared by diluting the 200 ng/µL stock DNA solution contained in the Quantifiler® kit with

TE^{-4} buffer (Tris-HCl + EDTA). These standards were used to generate a standard curve for

comparison, and the concentrations of each of the eight standard solutions were as follows:

Standard A- 50.000 ng/µL, Standard B- 16.700 ng/µl, Standard C- 5.560 ng/µL, Standard D-

1.850 ng/µL, Standard E- 0.620 ng/µL, Standard F- 0.210 ng/µL, Standard G- 0.068 ng/µL, and

Standard H- 0.023 ng/µL. A master mix was prepared by combining 10.5 µL of the Quantifiler™

Human Primer Mix per sample with 12.5 µL of the Quantifiler™ PCR Reaction Mix per sample.

This master mix was dispensed into each necessary well of a MicroAmp® Optical 96-Well

Reaction Plate (Applied Biosystems), followed by 2 µL of each standard, sample, or reagent

blank. Plates were then sealed using a MicroAmp® Optical Adhesive Film and centrifuged

briefly at approximately 3700 rpm to remove all air bubbles from the bottom of the wells.

The reaction plates were loaded onto an Applied Biosystems® 7500 Real-Time PCR

System and the appropriate standard and sample names were entered into the plate set-up in the

7500 Software program. The samples were first subjected to a 10 minute hold at 95°C to activate

the Taq polymerase before undergoing 40 PCR cycles each consisting of 15 seconds at 95°C

followed by 1 minute at 60°C. After ensuring that none of the negative control samples showed

any detectable DNA and that the standard curve was acceptable (R^2 value $\geq$ 0.98), the

concentration value for each sample was then used to target the appropriate amount of DNA in

the following amplification protocol. These quantification values are depicted in Appendix C.

3.4 DNA Amplification

Following quantification, each of the sample sets were amplified using the AmpF*l*STR®

Identifiler® Plus PCR Amplification Kit on an Applied Biosystems® GeneAmp® PCR System

9700 according to the "DNA Amplification (Identifiler Plus)" protocol found in the Oklahoma

State Bureau of Investigation's Forensic Biology Unit Policy Manual, Revision 4 (2014). A

master mix was prepared by combining 10 µL of the AmpFLSTR® Identifiler® Plus Kit Master

Mix per sample and 5 µL of the AmpFLSTR® Identifiler® Plus Kit Primer Set per sample.

Approximately 15 µL of the prepared master mix was added to each 0.2 µL PCR amplification

tube. The appropriate volume of each sample needed to target approximately 1 ng of DNA was

then added, before finally adding the necessary volume of TE^{-4} buffer to bring each total reaction

volume to approximately 25 µL. For samples that had a quantification value that was either

undetectable or too low to allow for the addition of 1 ng of DNA to the reaction, the maximum

volume of 10 µL of the sample was added. The tubes were placed onto an Applied Biosystems®

GeneAmp® PCR System 9700 thermal cycler and amplified using the "ID Plus (28 cycle)"

thermal cycling protocol validated internally by the OSBI's Forensic Biology Unit. This protocol

consists of a 10 minute initial activation hold at 95°C to activate the *Taq* polymerase, followed

by 28 cycles at 94°C for 20 seconds and 59°C for 3 minutes. The final step of the PCR protocol

was an extension step at 60°C for 10 minutes before the samples were returned to 4°C where

they remained until they were removed from the instrument and stored in a -20°C freezer. The

thermal cycling parameters are depicted in Table 2.

Table 2: Thermal Cycling Protocol for Identifiler® Plus Amplification

Initial Incubation Step	Cycle (28 cycles)		Final Extension	Final Hold
	Denature	Anneal/Extend		
HOLD	CYCLE		HOLD	HOLD
95°C	94°C	59°C	60°C	4°C
10 min	20 sec	3 min	10 min	∞

3.5 Genetic Analysis

The amplified DNA products for each set of experimental samples were analyzed using

capillary electrophoresis on an Applied Biosystems™ 3500 Series Genetic Analyzer. A master

mix was prepared by combining 8.5 µL of Hi-Di™ Formamide per sample with 0.5 µL of

GeneScan™ 600 LIZ® dye Size Standard v2.0 per sample. Approximately 9 µL of this master

mix was dispensed into each necessary well of a MicroAmp® Optical 96-Well Reaction Plate,

followed by 1 µL of each of the amplified DNA products, including reagent blanks and

amplification controls. In addition to these samples, 1 µL of the AmpF*l*STR® Identifiler® Plus

Allelic Ladder was also added to one well on the plate. After the plate was covered with a 96-

well septa, it was centrifuged briefly at approximately 3700 rpm to remove any air bubbles from

the bottom of the wells. The plate was heat denatured by being placed onto a heat block at 95°C

for 3 minutes, before being snap cooled at 0°C for an additional 3 minutes. The plate was then

loaded onto the genetic analyzer, the sample names and types were entered into the 3500

detection software, and the appropriate run parameters were selected (15 second injection at a

voltage of 15 kV).

3.6 Data Analysis

The resulting electropherograms were analyzed using GeneMapper® *ID-X* Software v1.4 from Life Technologies™. The negative amplification controls and reagent blanks were examined to ensure that no peaks were present above the analytical threshold designated for each dye channel. These thresholds were selected during the course of the OSBI's internal validation of the 3500 instrument and were as follows: blue dye (PET®)- 100 RFU (relative fluorescence units), green dye (NED™)- 190 RFU, yellow dye (VIC®)- 200 RFU, and red dye (FAM®)- 250 RFU. All other samples were analyzed to determine if there was allelic dropout or imbalance between the heights of the two peaks present at each of the heterozygous loci. The peak height (measured in relative fluorescence units) of the smaller of the two peaks present at each heterozygous locus had to be at least 50% of the peak height for the larger of the two peaks in order to be considered "balanced," according to the casework procedures of the OSBI Forensic Biology Unit. Each sample was given a score from 0 to 28 for the number of alleles present on the electropherogram, and a score from 0 to 12 for the number of heterozygous loci that were balanced (above 50%). Any artifacts visible on the electropherograms were also noted along with the likely cause of each artifact.

4. RESULTS

4.1 Control Data

The results of Set 1, the untreated blood samples of each dilution, were used as control data to be compared to each one of the three experimental sets of treated samples. The first category of data recorded for the control samples was the quantification results, which show the concentration of DNA present in each sample following the extraction process. The second type of data recorded for the control samples consisted of Cycle Threshold (C_t) values for the Internal PCR Control (IPC) included in the Quantifiler$^®$ Human DNA Quantification Kit. The C_t value for a sample indicates the number of PCR cycles required for the amount of DNA in that sample to exceed a specific threshold. The more DNA that is present within the sample prior to the PCR process, the lower the C_t value, as fewer PCR cycles are required to produce enough DNA copies to surpass the threshold. In the case of the IPC for the Quantifiler$^®$ kit, the C_t value is used as an indicator of whether or not there is a substance present in the sample that is inhibiting the PCR process, leading to less efficient amplification of the DNA that is present. This inhibition will lead to a high C_t value for the IPC as well as for the sample itself, whereas in a sample that simply had low amounts of DNA present without inhibition, the C_t for the sample would be high while the IPC C_t value would be similar to what was seen in the quantification standards. Both the quantification and the IPC results for each of the individual replicates of Set 1, samples 1A1-1E3, along with the results for the dilutions of each extract are reported in Appendix B. The values from each of the three replicates from each of the blood dilutions were averaged and these values, both the DNA concentrations and the IPC C_t's, for each control sample are recorded in Table 3.

Table 3: Average Quantification and IPC C_t Values for Control Samples

Sample Name	Blood Dilution	Average Quantification Result (ng/µL)	Average IPC Result (C_t)
1A	Neat	5.840 ± 0.676	27.29 ± 0.097
1B	1:2	1.703 ± 0.135	27.36 ± 0.115
1C	1:10	0.401 ± 0.024	27.17 ± 0.015
1D	1:100	0.062 ± 0.008	27.14 ± 0.032
1E	1:1000	0.003 ± 0.001	27.33 ± 0.116

The remainder of the data reported for Sample Set 1 consists of scores based upon the quality of the genetic profile that was obtained for each sample. These scores were determined by analyzing the electropherogram for each sample that was generated by the Genemapper® *ID-X* software. The scoring system was based upon the genotype of the donor from which the samples were obtained. This genotype is listed below in Table 4.

Table 4: Genetic Profile of Blood Donor # 59556

Genetic Locus	Alleles
D8S1179	16
D21S11	29
D7S820	11,13
CSF1PO	12
D3S1358	15,17
TH01	7,9.3
D13S317	11,12
D16S539	8,10
D2S1338	22,24
D19S433	12.2,13
vWA	16,18
TPOX	8
D18S51	13.2,16
Amelogenin	X,Y
D5S818	12,14
FGA	22.3*,23

*Microvariant allele

It should be noted that the profile contains an off ladder allele at the FGA locus, more specifically this allele is a microvariant, or an incomplete tetranucleotide repeat for a given

allele. The calculation for this particular microvariant is displayed below and was confirmed

through the Variant Allele Reports of the NIST standard reference database SRD 130. (Butler &

Reeder, Variant Allele Reports, 2006)

Microvariant Calculation:

Size of microvariant allele: 236.26 bp

Size of allele "22" on allelic ladder: 233.22 bp

236.26 bp – 233.22 bp = 3.04 bp → microvariant allele = 22.3

Microvariants are reported as the number of complete repeat units and are designated as an

integer, in this case, 22, and any partial repeat is designated as a decimal, followed by the

number of bases in the partial repeat, giving this allele the designation of 22.3.

Using the full profile listed in Table 4, two different scores were given to each sample.

Since there are 28 total alleles present in the known profile, a score of 0 to 28 was given to each

sample based upon the number of alleles present above the analytical threshold that were called

by the Genemapper® *ID-X* software. This score was used to illustrate the level of allelic dropout

present in each sample. The second score given to each sample was used to display the

consistency of peak heights throughout the profile. This was done using the methods listed in

Section 3.6 "Data Analysis," and resulted in a score from 0 to 12 for each sample. While the

individual scores for each sample in Set 1 are displayed in Appendix C, the average numbers of

allele peaks present for each blood dilution, as well as the average number of heterozygous loci

with over 50% peak height balance are depicted in Table 5.

Table 5: Average Genetic Profile Scores for Control Samples

Sample Name	Blood Dilution	Average Number of Alleles Present	Average Number of Heterozygous Loci Over 50%
1A	Neat	28 ± 0	12 ± 0
1B	1:2	28 ± 0	12 ± 0
1C	1:10	28 ± 0	12 ± 0
1D	1:100	28 ± 0	12 ± 0
1E	1:1000	25.33 ± 1.53	8.67 ± 0.58

The reagent blank for Sample Set 1, containing only Phosphate Buffered Saline and ultrapure, deionized water, was analyzed using all of the same procedures and reported no detectable quantification results and had no alleles present on the electropherogram. The positive amplification control displayed all of the expected peaks, indicating efficient and accurate amplification, while the negative amplification control displayed no peaks above the analytical threshold, indicating no contamination of the amplification reagents by extraneous DNA.

4.2 Samples Treated with Clorox® Regular-Bleach

For Sample Set 2, liquid blood treated with Clorox® Regular-Bleach, 78 total samples were analyzed. The combination of each dilution of blood with each dilution of bleach resulted in 25 different mixtures, and three replicates from each one of these mixtures were extracted and analyzed. A reagent blank was also prepared containing only neat bleach and ultrapure, deionized water, and three replicates of this negative control were also analyzed in the same manner. Based upon the color changes observed, it was apparent in several samples that the bleach was destroying the red blood cells in the samples by lysing the cell membranes. Samples 2A and 2B both turned a very dark brown color with slight foaming visible, while several of the

samples containing diluted blood turned from a bright, translucent red solution to an almost

colorless, transparent solution with small amounts of precipitate present. Examples of these

changes are illustrated in Figure 13.

Figure 13. Comparison of physical appearance of control vs. bleach-treated samples. Control sample 1B (upper left)- 1:2 blood dilution vs. Sample 2F (upper right)- 1:2 blood mixed with neat bleach; Control sample 1C (lower left)- 1:10 blood dilution vs. Samples 2K and 2L (lower right)- 1:10 blood mixed with neat bleach and 1:10 blood mixed with 1:2 bleach, respectively.

The dramatic changes that the samples in Set 2 underwent as a result of the treatment with bleach are evidenced quantitatively as well beginning with the quantification results showing the amount of DNA that was extracted from each sample. The individual quantification results for each of these 78 samples as well as any dilutions that were prepared from them can be found in Appendix B.

The results from the three replicates of each sample were averaged and these values were

compared to the quantification values for each corresponding control sample from Set 1,

illustrated below in Table 6.

Table 6: Average Quantification Values for Sample Set 2 vs. Control Samples

Sample	Description	Average Quantification Result (ng DNA/µL)	Average Quantification Value for Control Samples (ng DNA/µL)
2A	Neat Blood + Neat Bleach	0.518 ± 0.041	5.840 ± 0.676 (Neat Blood)
2B	Neat Blood + 1:2 Bleach	2.010 ± 0.017	
2C	Neat Blood + 1:10 Bleach	4.840 ± 0.234	
2D	Neat Blood + 1:100 Bleach	5.123 ± 0.532	
2E	Neat Blood + 1:1000 Bleach	5.293 ± 0.177	

Sample	Description	Average Quantification Result (ng DNA/µL)	Average Quantification Value for Control Samples (ng DNA/µL)
2F	1:2 Blood + Neat Bleach	Undet.	1.703 ± 0.135 (1:2 Blood)
2G	1:2 Blood + 1:2 Bleach	0.242 ± 0.012	
2H	1:2 Blood + 1:10 Bleach	1.297 ± 0.080	
2I	1:2 Blood + 1:100 Bleach	1.873 ± 0.083	
2J	1:2 Blood + 1:1000 Bleach	1.840 ± 0.056	
2K	1:10 Blood + Neat Bleach	Undet.	0.401 ± 0.024 (1:10 Blood)
2L	1:10 Blood + 1:2 Bleach	Undet.	
2M	1:10 Blood + 1:10 Bleach	0.001 ± 0.001	
2N	1:10 Blood + 1:100 Bleach	0.571 ± 0.035	
2O	1:10 Blood + 1:1000 Bleach	0.459 ± 0.019	
2P	1:100 Blood + Neat Bleach	Undet.	0.062 ± 0.008 (1:100 Blood)
2Q	1:100 Blood + 1:2 Bleach	Undet.	
2R	1:100 Blood + 1:10 Bleach	Undet.	
2S	1:100 Blood + 1:100 Bleach	Undet.	
2T	1:100 Blood + 1:1000 Bleach	0.055 ± 0.011	
2U	1:1000 Blood + Neat Bleach	Undet.	0.003 ± 0.001 (1:1000 Blood)
2V	1:1000 Blood + 1:2 Bleach	Undet.	
2W	1:1000 Blood + 1:10 Bleach	Undet.	
2X	1:1000 Blood + 1:100 Bleach	Undet.	
2Y	1:1000 Blood + 1:1000 Bleach	Undet.	

During the quantification process, the C_t values for the IPC analyzed alongside each of the samples in Set 2 were recorded and can be viewed with the quantification results in Appendix B. These values indicate whether there are increased, decreased, or similar levels of inhibition present in these bleach-treated samples when compared with the untreated, control samples of the same dilution of blood. The IPC C_t values for each of the three replicates of each blood/bleach mixture were averaged together and compared to the average IPC results from the control samples. The following graphs in Figure 14 illustrate that the average IPC result for every single bleach-treated sample was consistently lower than that of the corresponding control sample, with only two exceptions. The average IPC result for samples containing 1:10 blood treated with 1:10 bleach was 0.07 cycles higher than the control, while the average IPC value for 1:100 blood/1:1000 bleach samples was 0.12 cycles higher than that of the control.
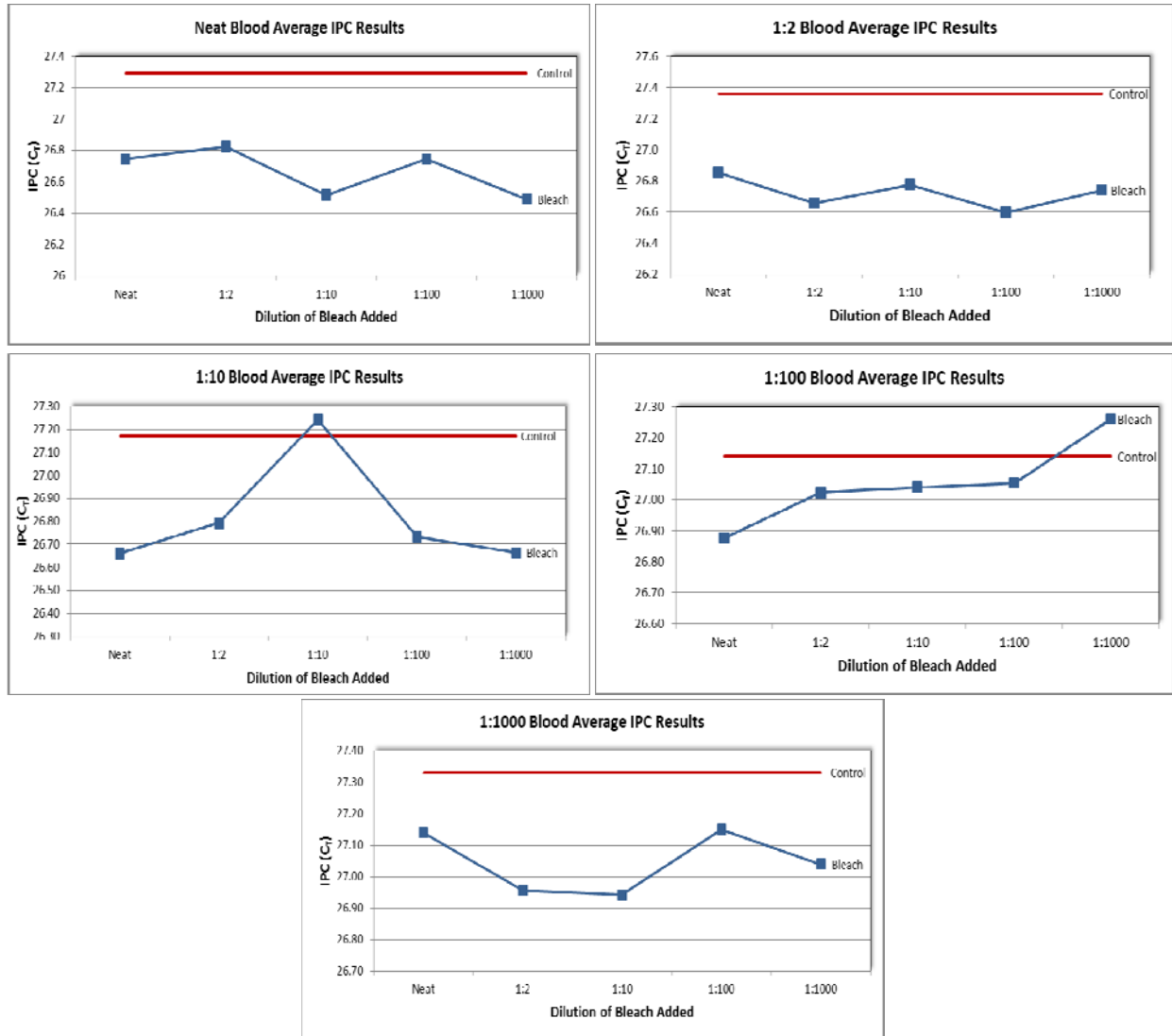
Figure 14. Average IPC C_t results for Sample Set 2 compared to the corresponding controls. Each graph displays the average IPC C_t results for each blood dilution. The red line on each graph illustrates the average IPC C_t value for the corresponding control blood dilution. The graphs show that the average IPC results reported for the treated samples in Set 2 were consistently lower than those of the controls across all samples, with the exception of 1:10 blood/1:10 bleach samples and 1:100 blood/1:1000 bleach samples.

The electropherograms generated during the final step of analysis for Sample Set 2 were each given two scores, just as the Set 1 samples, using the same scoring system described previously. These two sets of scores are reported for each individual sample in Appendix C, while the average number of alleles called and the average number of balanced heterozygous loci for each of the mixtures is reported in Table 7.

Table 7: Average Genetic Profile Scores for Sample Set 2

Sample	Description	Average Number of Alleles Present	Average Number of Heterozygous Loci Over 50%
2A	Neat Blood + Neat Bleach	28 ± 0	12 ± 0
2B	Neat Blood + 1:2 Bleach	28 ± 0	12 ± 0
2C	Neat Blood + 1:10 Bleach	28 ± 0	12 ± 0
2D	Neat Blood + 1:100 Bleach	28 ± 0	12 ± 0
2E	Neat Blood + 1:1000 Bleach	28 ± 0	12 ± 0
2F	1:2 Blood + Neat Bleach	0 ± 0	0 ± 0
2G	1:2 Blood + 1:2 Bleach	28 ± 0	12 ± 0
2H	1:2 Blood + 1:10 Bleach	28 ± 0	12 ± 0
2I	1:2 Blood + 1:100 Bleach	28 ± 0	12 ± 0
2J	1:2 Blood + 1:1000 Bleach	28 ± 0	12 ± 0
2K	1:10 Blood + Neat Bleach	0 ± 0	0 ± 0
2L	1:10 Blood + 1:2 Bleach	0 ± 0	0 ± 0
2M	1:10 Blood + 1:10 Bleach	0.33 ± 0.58	0 ± 0
2N	1:10 Blood + 1:100 Bleach	28 ± 0	12 ± 0
2O	1:10 Blood + 1:1000 Bleach	28 ± 0	12 ± 0
2P	1:100 Blood + Neat Bleach	0 ± 0	0 ± 0
2Q	1:100 Blood + 1:2 Bleach	0 ± 0	0 ± 0
2R	1:100 Blood + 1:10 Bleach	0 ± 0	0 ± 0
2S	1:100 Blood + 1:100 Bleach	0 ± 0	0 ± 0
2T	1:100 Blood + 1:1000 Bleach	28 ± 0	12 ± 0
2U	1:1000 Blood + Neat Bleach	0 ± 0	0 ± 0
2V	1:1000 Blood + 1:2 Bleach	0 ± 0	0 ± 0
2W	1:1000 Blood + 1:10 Bleach	0 ± 0	0 ± 0
2X	1:1000 Blood + 1:100 Bleach	0 ± 0	0 ± 0
2Y	1:1000 Blood + 1:1000 Bleach	0 ± 0	0 ± 0

These average results from the final genetic profiles generated from samples in Set 2 were

compared to the average values from the appropriate control samples. Those comparisons are

presented graphically in Figures 15 and 16.

Figure 15. Average number of alleles present in profiles for Sample Set 2 vs. controls. This graph illustrates the level of allelic dropout present in each blood/bleach combination in the dilution series. The corresponding control sample for each blood dilution is depicted in dark blue as the first bar at each blood dilution.
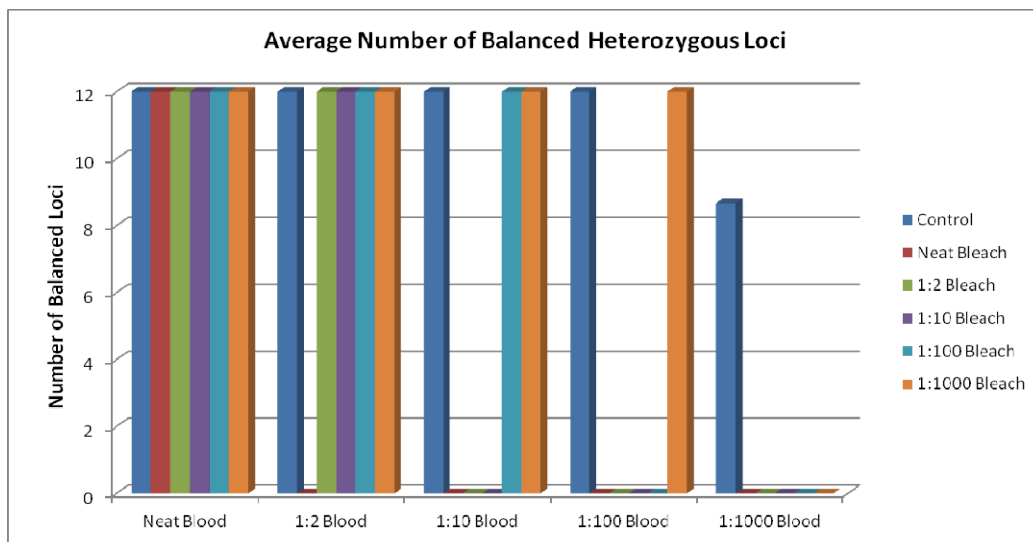
Figure 16. Average number of balanced heterozygous loci in profiles for Sample Set 2 vs. controls. This graph illustrates balance of peak height ratios present in each blood/bleach combination in the dilution series. The corresponding control sample for each blood dilution is depicted in dark blue as the first bar at each blood dilution.

It was also notable that for each of the three replicates (2A1, 2A2, and 2A3) of mixture 2A (neat blood + neat bleach), the electropherograms showed significant numbers of artifacts, such as pull-up and minus-A peaks. The peak heights for these three samples were also significantly higher than any of the other samples processed in Sample Set 2 or the control

samples of Set 1. This indicates that the amount of template DNA added at the beginning of the PCR process was too high, which leads to the conclusion that the quantification results reported for these three samples were lower than the amount of DNA actually present. This could be the result of DNA damage inflicted by the bleach that specifically affected the section of DNA amplified by the quantification kit, but did not affect the loci amplified by the Identifiler Plus® kit. All three replicates of the reagent blank for Sample Set 2 (RB2A, RB2B, and RB2C) performed as expected. The blanks showed no detectable quantification value and had no peaks above the analytical threshold on the electropherograms. Both the positive and negative amplification controls also performed as expected.

4.3 Samples Treated with Lysol® Multi-Surface All-Purpose Cleaner

Experimental Sample Set 3 contained samples of each of the five dilutions of blood combined with each one of the five dilutions of Lysol® Multi-Surface All-Purpose Cleaner. Each blood/Lysol® mixture was then extracted in triplicate resulting in 75 individual samples being analyzed. A reagent blank containing only undiluted Lysol® and ultrapure, deionized water was also extracted in triplicate alongside the Set 3 samples for a total of 78 samples. Based solely upon visual inspection, the samples containing both neat blood and 1:2 blood did not display any visible changes regardless of what dilution of Lysol® was added to the sample. However, in multiple samples containing more dilute blood, the liquid changed dramatically from a bright red, as in the control samples, to a dark green solution upon the addition of Lysol®. These differences can be seen Figure 17.

The quantification results from these samples were recorded and compared to the control samples for each blood dilution. The average quantification values for each Set 3 mixture, as well as the average values for each corresponding control sample, are reported below in Table 8, while the raw quantification data for each individual sample is located in Appendix B. The quantification values for the Lysol®-treated samples were consistently higher for every dilution with the exception of one sample, 3V, containing 1:1000 blood and 1:2 Lysol® (shown in red).

Figure 17. Examples of physical changes between control and Lysol®-treated samples. Control sample 1C (upper left)- 1:10 blood dilution vs. Sample 3K (upper right)- 1:10 blood mixed with neat Lysol®; Control sample 1D (lower left)- 1:100 blood dilution vs. Samples 3P and 3Q (lower right)- 1:100 blood mixed with neat Lysol® and 1:2 Lysol®, respectively.

Table 8: Average Quantification Values for Sample Set 3 vs. Control Samples

Sample	Description	Average Quantification Result (ng DNA/μL)	Average Control Quantification Result (ng DNA/μL)
3A	Neat Blood + Neat Lysol®	7.023 ± 0.333	
3B	Neat Blood + 1:2 Lysol®	6.767 ± 0.240	
3C	Neat Blood + 1:10 Lysol®	6.890 ± 0.204	5.840 ± 0.676 (Neat Blood)
3D	Neat Blood + 1:100 Lysol®	6.773 ± 0.721	
3E	Neat Blood + 1:1000 Lysol®	6.337 ± 1.315	
3F	1:2 Blood + Neat Lysol®	2.167 ± 0.042	
3G	1:2 Blood + 1:2 Lysol®	2.253 ± 0.035	
3H	1:2 Blood + 1:10 Lysol®	1.917 ± 0.544	1.703 ± 0.135 (1:2 Blood)
3I	1:2 Blood + 1:100 Lysol®	2.027 ± 0.075	
3J	1:2 Blood + 1:1000 Lysol®	2.130 ± 0.118	

Sample	Description	Average Quantification Result (ng DNA/µL)	Average Control Quantification Result (ng DNA/µL)
3K	1:10 Blood + Neat Lysol®	1.163 ± 0.937	0.401 ± 0.024 (1:10 Blood)
3L	1:10 Blood + 1:2 Lysol®	0.696 ± 0.030	
3M	1:10 Blood + 1:10 Lysol®	0.687 ± 0.024	
3N	1:10 Blood + 1:100 Lysol®	0.571 ± 0.020	
3O	1:10 Blood + 1:1000 Lysol®	0.608 ± 0.028	
3P	1:100 Blood + Neat Lysol®	0.101 ± 0.005	0.062 ± 0.008 (1:100 Blood)
3Q	1:100 Blood + 1:2 Lysol®	0.100 ± 0.015	
3R	1:100 Blood + 1:10 Lysol®	0.088 ± 0.028	
3S	1:100 Blood + 1:100 Lysol®	0.066 ± 0.011	
3T	1:100 Blood + 1:1000 Lysol®	0.063 ± 0.007	
3U	1:1000 Blood + Neat Lysol®	0.007 ± 0.004	0.003 ± 0.001 (1:1000 Blood)
3V	1:1000 Blood + 1:2 Lysol®	0.002 ± 0.001	
3W	1:1000 Blood + 1:10 Lysol®	0.006 ± 0.002	
3X	1:1000 Blood + 1:100 Lysol®	0.006 ± 0.003	
3Y	1:1000 Blood + 1:1000 Lysol®	0.007 ± 0.001	

The average quantification value for Sample 3K (1:10 Blood + 1:2 Lysol®) is considerably higher than the results for the other four Set 2 samples that contain 1:10 blood. This is due to one of the three replicates, sample 3K2, reporting a quantification value of 2.240. In an effort to determine if this abnormally high value was actually the correct DNA concentration for that sample, an additional replicate of sample 3K2, labeled as 3K2A, was amplified in the next step of analysis. This amount of this second replicate that was added to the amplification reaction was determined using an artificially calculated quantification value. This value, 0.625 ng DNA/µL, was obtained by averaging the quantification results of sample 3K1 and sample 3K3, which were both prepared from the same blood/Lysol® mixture as sample 3K2. Sample 3K2A, however, produced an electropherogram with multiple artifacts and extremely large peak heights, indicating that far too much DNA was added to the amplification reaction. This leads to the conclusion that the original elevated quantification value for sample 3K2 was accurate.

The C_t values for the Quantifiler® IPC for each of the samples in Set 3 were also recorded during the quantification process in order to monitor for the presence of inhibitors within the samples. These values can be found for each individual replicate in Appendix B with the rest of the Set 3 quantification data, while the averaged IPC C_t values for each of the blood/Lysol® mixtures were compared to the average IPC values for analogous control samples. These comparisons were used to generate the graphs seen in Figure 18. The IPC values for the treated samples were consistently lower than those for the control samples in every combination of blood and Lysol® dilutions.

Figure 18. Average IPC C_t results for Sample Set 3 compared to the corresponding controls. Each graph displays the average IPC C_t results for each blood dilution. The red line on each graph illustrates the average IPC C_t value for the corresponding control blood dilution. The graphs show that the average IPC results reported for the treated samples in Set 3 were consistently lower than those of the controls across all samples.

After the quantification data for Sample Set 3 was used to accurately amplify each of the samples, they were processed on a 3500 Series® Genetic Analyzer. The resulting data was generated in the form of electropherograms which were then scored based upon the completeness and quality of the genetic profiles. These two separate scores for each individual profile can be found in Appendix C, while the average scores for each mixture in each of these categories is listed below in Table 9.

Table 9: Average Genetic Profile Scores for Sample Set 3

Sample	Description	Average Number of Alleles Present	Average Number of Heterozygous Loci Over 50%
3A	Neat Blood + Neat Lysol®	28 ± 0	12 ± 0
3B	Neat Blood + 1:2 Lysol®	28 ± 0	12 ± 0
3C	Neat Blood + 1:10 Lysol®	28 ± 0	12 ± 0
3D	Neat Blood + 1:100 Lysol®	28 ± 0	12 ± 0
3E	Neat Blood + 1:1000 Lysol®	28 ± 0	12 ± 0
3F	1:2 Blood + Neat Lysol®	28 ± 0	12 ± 0
3G	1:2 Blood + 1:2 Lysol®	28 ± 0	12 ± 0
3H	1:2 Blood + 1:10 Lysol®	28 ± 0	12 ± 0
3I	1:2 Blood + 1:100 Lysol®	28 ± 0	12 ± 0
3J	1:2 Blood + 1:1000 Lysol®	28 ± 0	12 ± 0
3K	1:10 Blood + Neat Lysol®	28 ± 0	12 ± 0
3L	1:10 Blood + 1:2 Lysol®	28 ± 0	12 ± 0
3M	1:10 Blood + 1:10 Lysol®	28 ± 0	12 ± 0
3N	1:10 Blood + 1:100 Lysol®	28 ± 0	12 ± 0
3O	1:10 Blood + 1:1000 Lysol®	28 ± 0	12 ± 0
3P	1:100 Blood + Neat Lysol®	28 ± 0	12 ± 0
3Q	1:100 Blood + 1:2 Lysol®	28 ± 0	12 ± 0
3R	1:100 Blood + 1:10 Lysol®	28 ± 0	12 ± 0
3S	1:100 Blood + 1:100 Lysol®	28 ± 0	12 ± 0
3T	1:100 Blood + 1:1000 Lysol®	28 ± 0	12 ± 0
3U	1:1000 Blood + Neat Lysol®	23 ± 2.65	7 ± 2.65
3V	1:1000 Blood + 1:2 Lysol®	19.67 ± 3.79	5.67 ± 2.08
3W	1:1000 Blood + 1:10 Lysol®	24.67 ± 1.53	8.67 ± 0.58
3X	1:1000 Blood + 1:100 Lysol®	22.33 ± 4.16	7 ± 3.61
3Y	1:1000 Blood + 1:1000 Lysol®	22.67 ± 2.08	6.33 ± 0.58

The results reported for the genetic profiles generated for Sample Set 3 were compared to

the results from the appropriate dilution of blood within the control sample set, and these

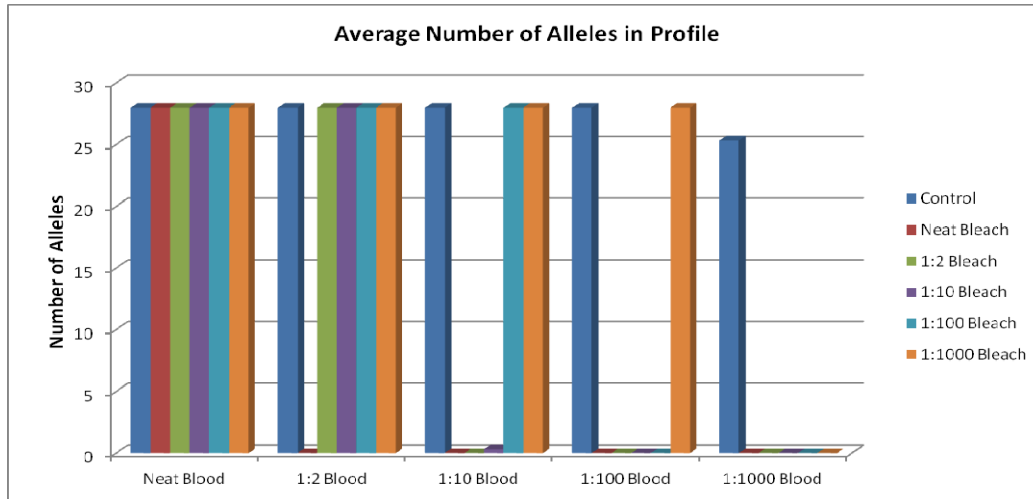comparisons are illustrated in Figures 19 and 20.

Figure 19. Average number of alleles present in profiles for Sample Set 3 vs. controls. This graph illustrates the level of allelic dropout present in each blood/Lysol® combination in the dilution series. The corresponding control sample for each blood dilution is depicted in dark blue as the first bar at each blood dilution. The treatment had no effect on the profile quality other than slightly lowered scores in the 1:1000 blood dilution when compared to the controls.

Figure 20. Average number of balanced heterozygous loci in profiles for Sample Set 3 vs. controls. This graph illustrates the level of peak height balance in each blood/Lysol® combination in the dilution series. The corresponding control sample for each blood dilution is depicted in dark blue as the first bar at each blood dilution. The graph shows a similar pattern to the one in Figure 19 with only the most diluted blood showing any differences from the controls.

Each of the three replicates of the reagent blank analyzed along with these samples showed no detectable quantification result and their respective electropherograms showed no visible peaks above the analytical threshold. Both the positive and negative amplification controls also displayed the expected results.

4.4 Samples Treated with Resolve® Carpet Cleaner

The final set of experimental samples, Sample Set 4, consisted of blood samples treated with various dilutions of Resolve® Carpet Cleaner. This sample set included 3 replicates of each of the 25 blood/Resolve® mixtures as well as 3 replicates of a reagent blank sample containing undiluted Resolve® Carpet Cleaner and ultrapure, deionized water. The samples in this set underwent dramatic changes when treated with various dilutions of Resolve®. The most notable change was the production of a significant amount of gas, evidenced by the generation of a great deal of foamy bubbles, as seen in Figure 21.
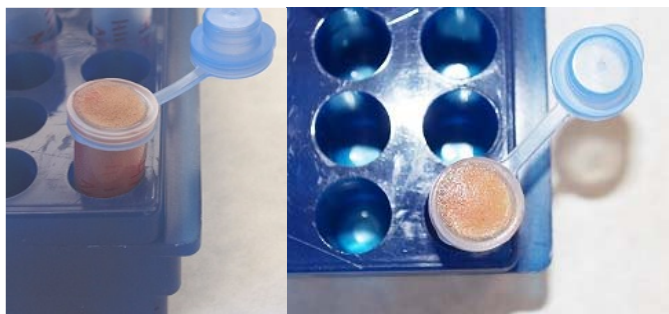
Figure 21. Reaction when neat Resolve® Carpet Cleaner is added to liquid whole blood. The addition of Resolve® to liquid blood resulted in an immediate reaction that filled the entire 1.5 mL tube with red/brown foam.

Several of the samples also showed a dramatic color change, in many cases going from a bright red liquid to a completely clear, foamy solution after the addition of the cleaner. Examples of these changes are illustrated in Figure 22.

Figure 22. Visible reactions between various dilutions of blood and Resolve® Carpet Cleaner. The red tubes contain a 1:2 blood dilution (top), a 1:10 blood dilution (center), and a 1:100 blood dilution (bottom) with no treatment. The blue tubes contain the same blood dilutions in each row with neat Resolve® in the first tube, 1:2 Resolve® in the second tube, and 1:10 Resolve in the third tube seen only in the last photograph.

As with the previously reported sample sets, the average quantification result for each blood/Resolve® mixture was compared each complementary control sample and is listed in Table 10. The complete quantification data for all replicates analyzed in Sample Set 4 is located in Appendix B.

Table 10: Average Quantification Values for Sample Set 4 vs. Control Samples

Sample	Description	Average Quantification Result (ng DNA/µL)	Average Control Quantification Result (ng DNA/µL)
4A	Neat Blood + Neat Resolve®	9.427 ± 1.736	
4B	Neat Blood + 1:2 Resolve®	9.630 ± 0.465	
4C	Neat Blood + 1:10 Resolve®	7.173 ± 0.440	5.840 ± 0.676 (Neat Blood)
4D	Neat Blood + 1:100 Resolve®	6.173 ± 0.454	
4E	Neat Blood + 1:1000 Resolve®	7.150 ± 0.114	

Sample	Description	Average Quantification Result (ng DNA/μL)	Average Control Quantification Result (ng DNA/μL)
4F	1:2 Blood + Neat Resolve[®]	3.187 ± 0.268	1.703 ± 0.135 (1:2 Blood)
4G	1:2 Blood + 1:2 Resolve[®]	3.027 ± 0.201	
4H	1:2 Blood + 1:10 Resolve[®]	2.327 ± 0.050	
4I	1:2 Blood + 1:100 Resolve[®]	1.807 ± 0.047	
4J	1:2 Blood + 1:1000 Resolve[®]	1.777 ± 0.106	
4K	1:10 Blood + Neat Resolve[®]	0.781 ± 0.145	0.401 ± 0.024 (1:10 Blood)
4L	1:10 Blood + 1:2 Resolve[®]	0.712 ± 0.052	
4M	1:10 Blood + 1:10 Resolve[®]	0.619 ± 0.052	
4N	1:10 Blood + 1:100 Resolve[®]	0.504 ± 0.045	
4O	1:10 Blood + 1:1000 Resolve[®]	0.492 ± 0.013	
4P	1:100 Blood + Neat Resolve[®]	0.066 ± 0.009	0.062 ± 0.008 (1:100 Blood)
4Q	1:100 Blood + 1:2 Resolve[®]	0.050 ± 0.021	
4R	1:100 Blood + 1:10 Resolve[®]	0.082 ± 0.004	
4S	1:100 Blood + 1:100 Resolve[®]	0.068 ± 0.002	
4T	1:100 Blood + 1:1000 Resolve[®]	0.053 ± 0.007	
4U	1:1000 Blood + Neat Resolve[®]	0.008 ± 0.007	0.003 ± 0.001 (1:1000 Blood)
4V	1:1000 Blood + 1:2 Resolve[®]	0.010 ± 0.003	
4W	1:1000 Blood + 1:10 Resolve[®]	0.011 ± 0.002	
4X	1:1000 Blood + 1:100 Resolve[®]	0.010 ± 0.004	
4Y	1:1000 Blood + 1:1000 Resolve[®]	0.008 ± 0.001	

The first three dilutions of blood within Set 4 (Neat, 1:2, and 1:10 blood) all follow a similar

pattern, in that, the samples with more concentrated Resolve[®] added (Neat, 1:2, and 1:10) all

showed considerably higher concentrations of DNA present after the extraction process. This

relationship is represented graphically in Figure 23.
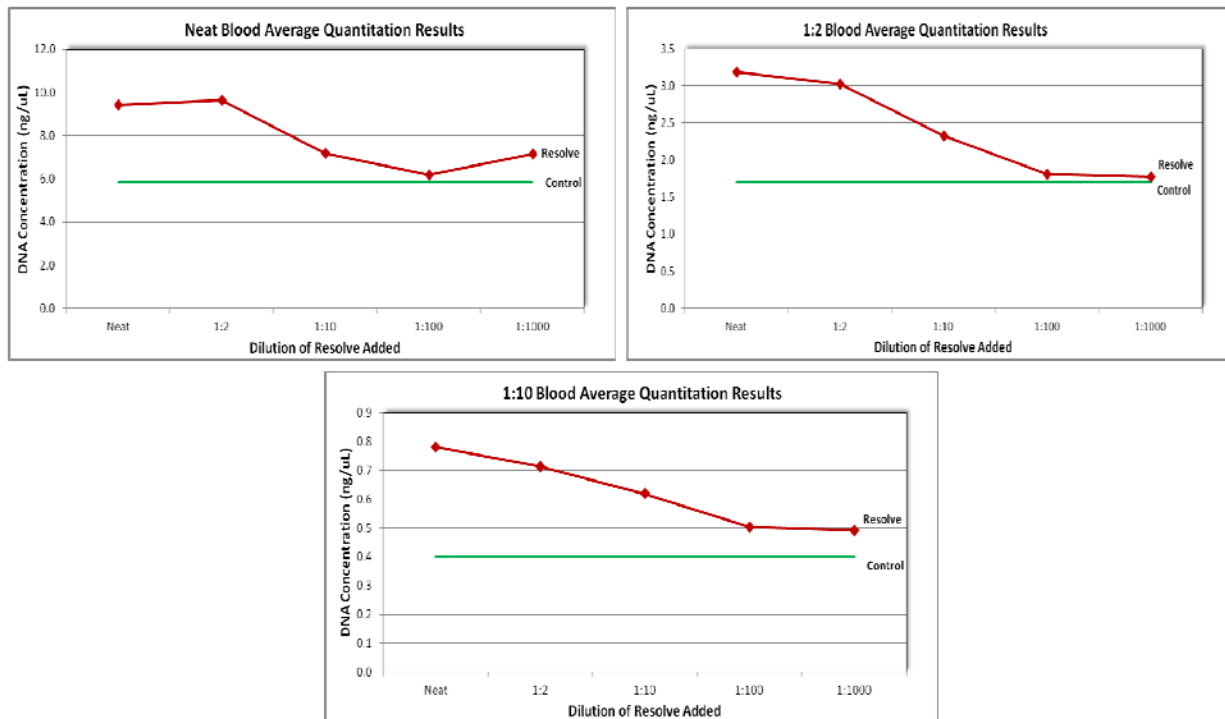
Figure 23. Average quantification results for the first 3 dilutions of Sample Set 4 vs. control samples. The graphs show the increased amount of DNA extracted from blood samples treated with Resolve® Carpet Cleaner (red) vs. blood only samples (green). As the concentration of Resolve® in the samples increased, the amount of DNA recovered increased as well.

Collected simultaneously with the quantification data was the C_t value for the Internal

PCR Control that was added to each sample during the quantification process to monitor for

inhibition. These individual values are reported out in the quantification data located in

Appendix B, while the average IPC C_t value for each blood dilution was compared to the average

IPC for the appropriate control sample to generate the graphs that are displayed in Figure 24. The

IPC C_t values do not appear to follow a discernable pattern within each dilution set; however, the

IPC result for every sample containing undiluted Resolve® was consistently higher than the

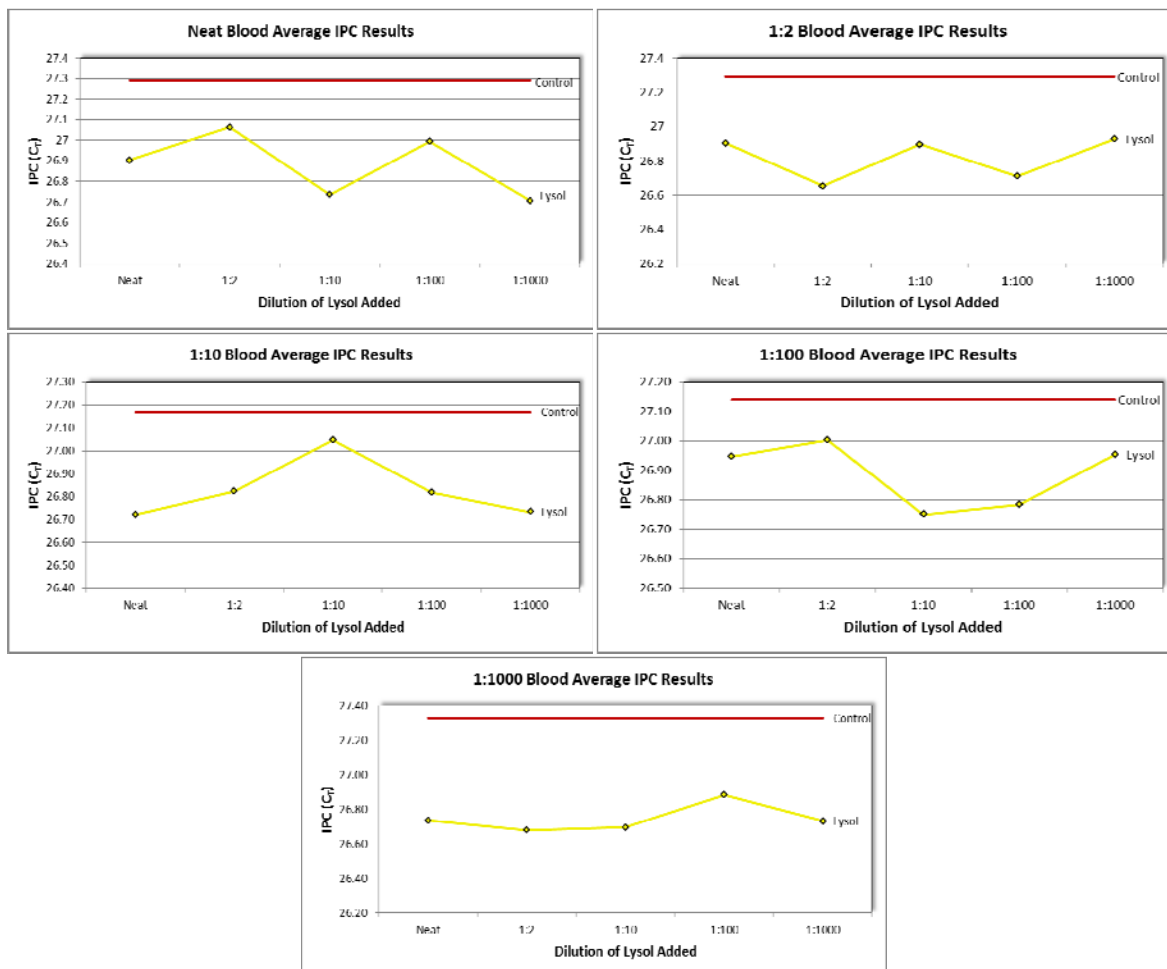corresponding control IPC value regardless of the dilution of blood within the sample.
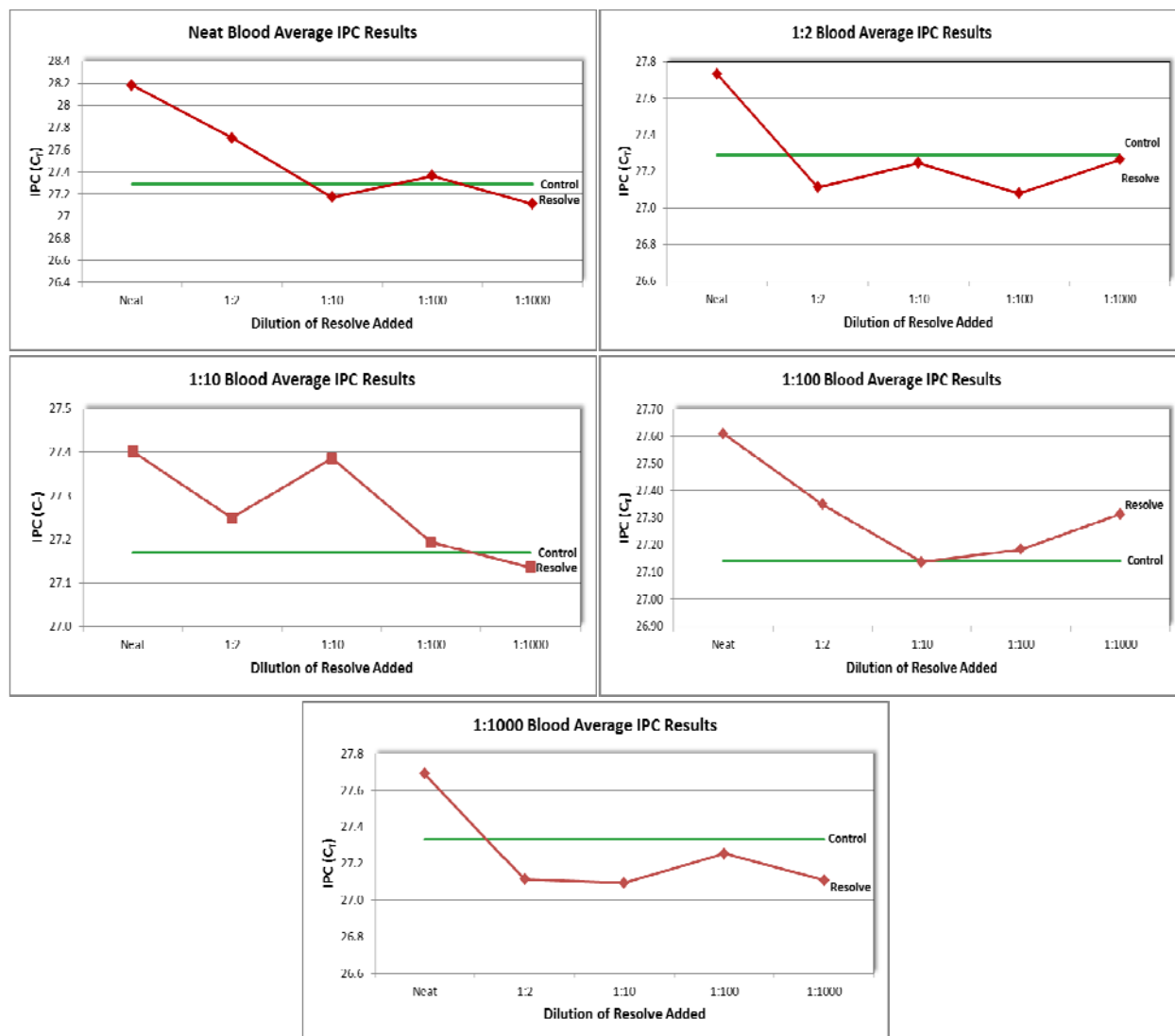
Figure 24. Average IPC C_t results for Sample Set 4 compared to the corresponding controls. Each graph displays the average IPC C_t results for each blood dilution. The green line on each graph illustrates the average IPC C_t value for the corresponding control blood dilution. The only discernable trend illustrated by the graphs is that every sample treated with undiluted Resolve® Carpet Cleaner reported an elevated IPC C_t value compared to the control, regardless of the dilution of blood in the sample.

The final product of analysis for each sample was an electropherogram displaying the genetic profile. The quality of each of these electropherograms was translated to quantitative data using the scoring system described previously to record the number of allelic peaks present in the profile as well as the number of balanced heterozygous loci. This quantitative data is listed in its entirety in Appendix C, while the average values are listed in the following table.

Table 11: Average Genetic Profile Scores for Sample Set 4

Sample	Description	Average Number of Alleles Present	Average Number of Heterozygous Loci Over 50%
4A	Neat Blood + Neat Resolve[®]	28 ± 0	12 ± 0
4B	Neat Blood + 1:2 Resolve[®]	28 ± 0	12 ± 0
4C	Neat Blood + 1:10 Resolve[®]	28 ± 0	12 ± 0
4D	Neat Blood + 1:100 Resolve[®]	28 ± 0	12 ± 0
4E	Neat Blood + 1:1000 Resolve[®]	28 ± 0	12 ± 0
4F	1:2 Blood + Neat Resolve[®]	28 ± 0	12 ± 0
4G	1:2 Blood + 1:2 Resolve[®]	28 ± 0	12 ± 0
4H	1:2 Blood + 1:10 Resolve[®]	28 ± 0	12 ± 0
4I	1:2 Blood + 1:100 Resolve[®]	28 ± 0	12 ± 0
4J	1:2 Blood + 1:1000 Resolve[®]	28 ± 0	12 ± 0
4K	1:10 Blood + Neat Resolve[®]	28 ± 0	12 ± 0
4L	1:10 Blood + 1:2 Resolve[®]	28 ± 0	12 ± 0
4M	1:10 Blood + 1:10 Resolve[®]	28 ± 0	12 ± 0
4N	1:10 Blood + 1:100 Resolve[®]	28 ± 0	12 ± 0
4O	1:10 Blood + 1:1000 Resolve[®]	28 ± 0	12 ± 0
4P	1:100 Blood + Neat Resolve[®]	0 ± 0	0 ± 0
4Q	1:100 Blood + 1:2 Resolve[®]	28 ± 0	11.33 ± 0.58
4R	1:100 Blood + 1:10 Resolve[®]	28 ± 0	12 ± 0
4S	1:100 Blood + 1:100 Resolve[®]	28 ± 0	12 ± 0
4T	1:100 Blood + 1:1000 Resolve[®]	28 ± 0	12 ± 0
4U	1:1000 Blood + Neat Resolve[®]	1.33 ±	0 ± 0
4V	1:1000 Blood + 1:2 Resolve[®]	18.33 ±	6.67 ± 0.58
4W	1:1000 Blood + 1:10 Resolve[®]	28 ± 0	11.33 ± 1.15
4X	1:1000 Blood + 1:100 Resolve[®]	28 ± 0	10.67 ± 1.15
4Y	1:1000 Blood + 1:1000 Resolve[®]	28 ± 0	11.33 ± 1.15

The data presented in Table 11 was compared to the corresponding data from the control samples. In order to visualize the differences present in the Resolve[®]-treated samples, the comparative data is presented graphically in Figures 25 and 26.
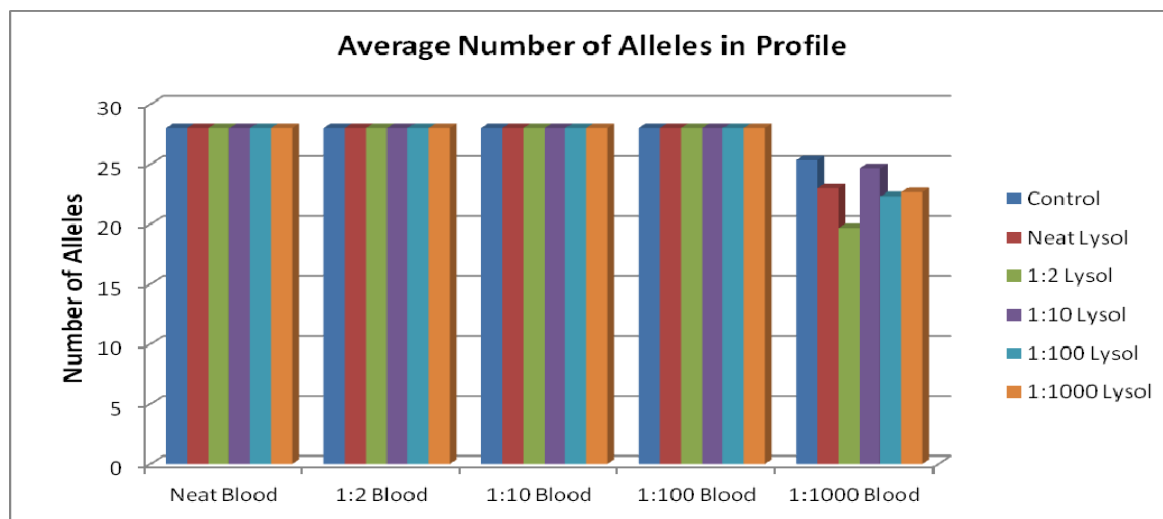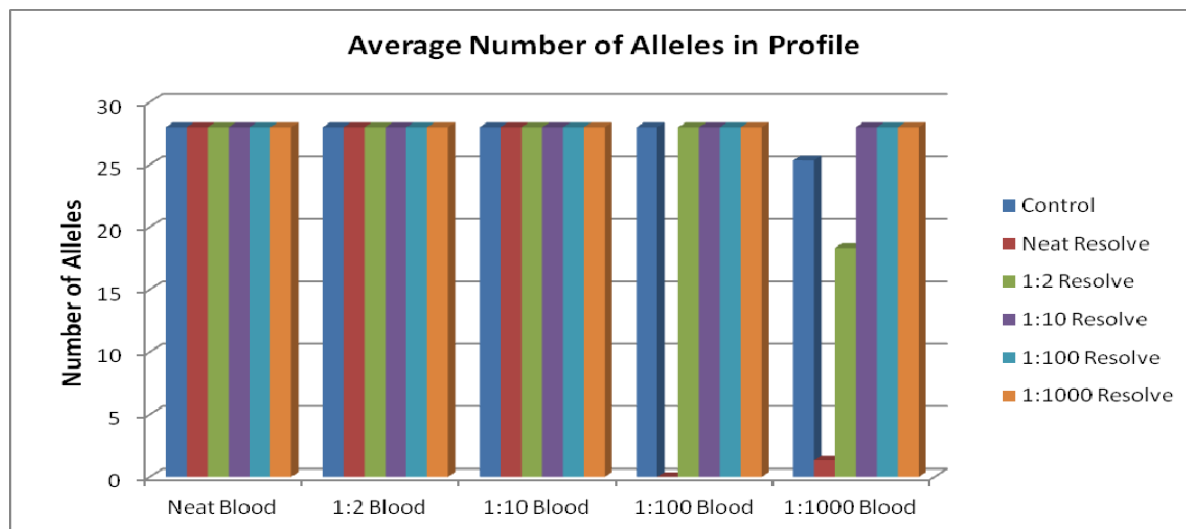
Figure 25. Average number of alleles present in profiles for Sample Set 4 vs. controls. This graph illustrates the level of allelic dropout present in each blood/Resolve® combination in the dilution series. The corresponding control sample for each blood dilution is depicted in dark blue as the first bar at each blood dilution. The 1:100 and 1:1000 blood dilutions show almost complete profile dropout when treated with neat Resolve® and the 1:1000 showed moderate dropout with 1:2 Resolve®.
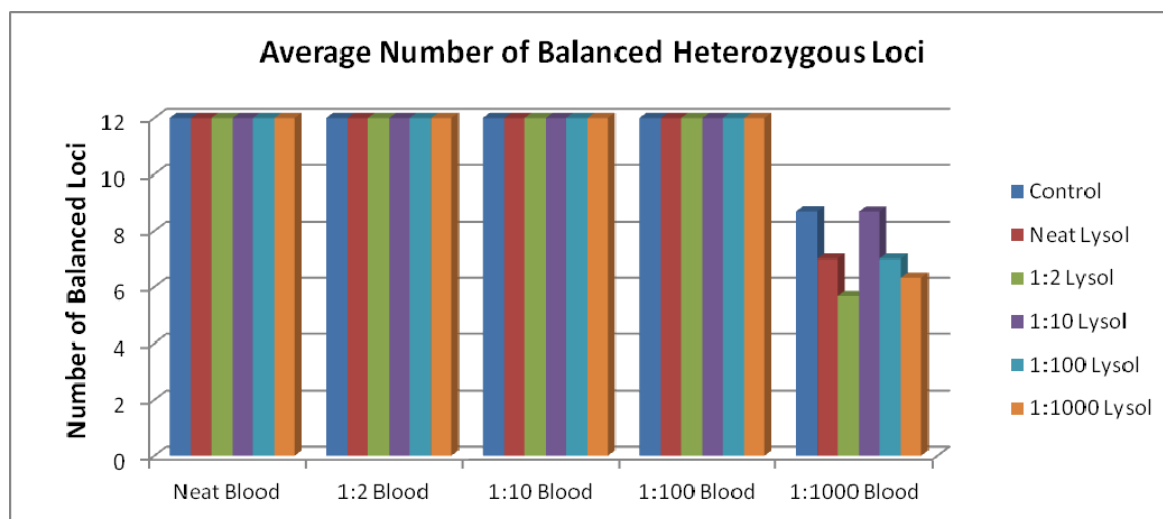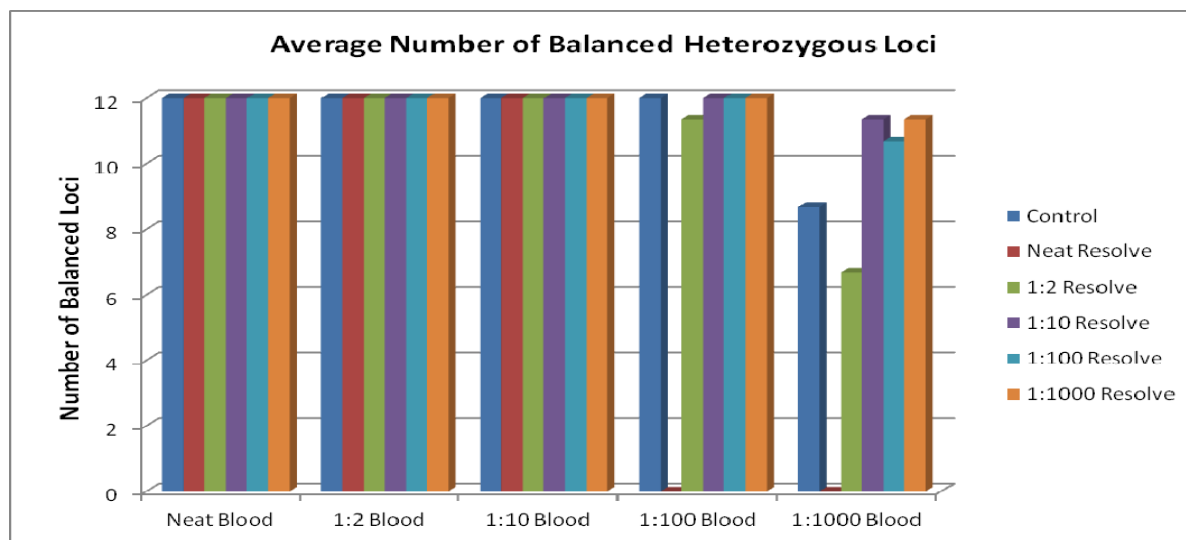

Figure 26. Average number of balanced heterozygous loci in profiles for Sample Set 4 vs. controls. This graph illustrates the level of peak height balance in each blood/Resolve® combination in the dilution series. The corresponding control sample for each blood dilution is depicted in dark blue as the first bar at each blood dilution. Given the level of allelic dropout seen in the 1:100 blood/neat Resolve® and 1:1000 blood/neat Resolve® samples, the average peak height balance score was 0 for both sample types.

All three of the reagent blank replicates performed as expected throughout the analysis process, displaying no quantification results and showing no peaks tall enough to be called by the analysis software on any of the three electropherograms. The positive amplification control also

displayed the expected results, while the negative control displayed no peaks, which indicates

that there was no contamination of the amplification reagents by extraneous DNA.

4. DISCUSSION AND CONCLUSIONS

4.1 DNA Quantification

After collecting and comparing data from the quantification of the DNA extracted from each of the samples, the relationships between the control and experimental data were used to evaluate the performance of the Automate *Express*™/Prepfiler *Express*™ extraction method under each of the three experimental conditions. The efficiency of the extraction system was evaluated by comparing the amount of DNA recovered from treated samples to that recovered from controls. The ability of this automated system to remove PCR inhibitors from extracted DNA was judged using the results from the Internal PCR Control contained in the quantification kit.

The samples from Set 2 that were treated with Clorox® Bleach showed the most dramatic difference in the amount of DNA extracted when compared to the control samples. The change in average quantification values across the entire data set is shown in Figure 27. The amount of DNA recovered from the samples decreases dramatically if treated with bleach at either full or half strength.
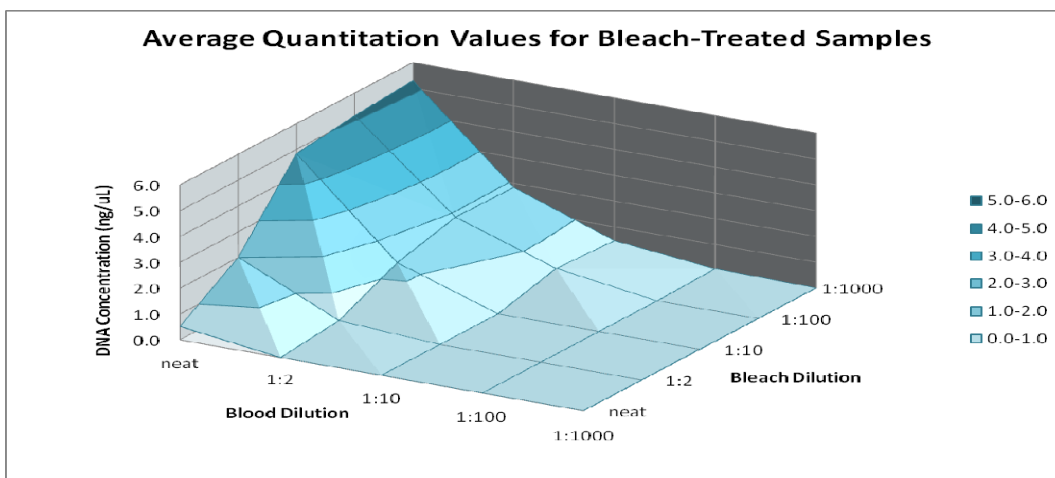
Figure 27. Average quantification values for the entirety of Sample Set 2. This graph is a 3-dimentional representation of the average amount of DNA recovered from each blood/bleach combination in set 3. It allows the visualization of the point in each dilution series when the most dramatic changes are seen.

The variance between this data and the control set was tested for statistical significance using a single-factor ANOVA test. The ANOVA tests for the equality of means between two sets of data and reports a "p-value" which is either above or below the critical value (α) selected based upon the confidence level used. If the p-value is above the critical value, the means are statistically equal, and if the p-value is below α, the means are not equal (D'Agostino, Sullivan, & Beiser, 2006). The p-value for each of the blood/bleach combinations is presented below in Figure 28. The test applied a 95% confidence level and the individual p-values are labeled in red if they are below 0.05, indicating the treated sample differed significantly from the corresponding control with regards to DNA quantification results. The green labels are given to samples that have a p-value above 0.05, meaning the data from the treated sample is not statistically different from the control data. In order to more easily correlate these statistical results with the trends in quantification values for Sample Set 2, they are presented alongside the graph from Figure 27 that has been rotated in order to give an overhead view.
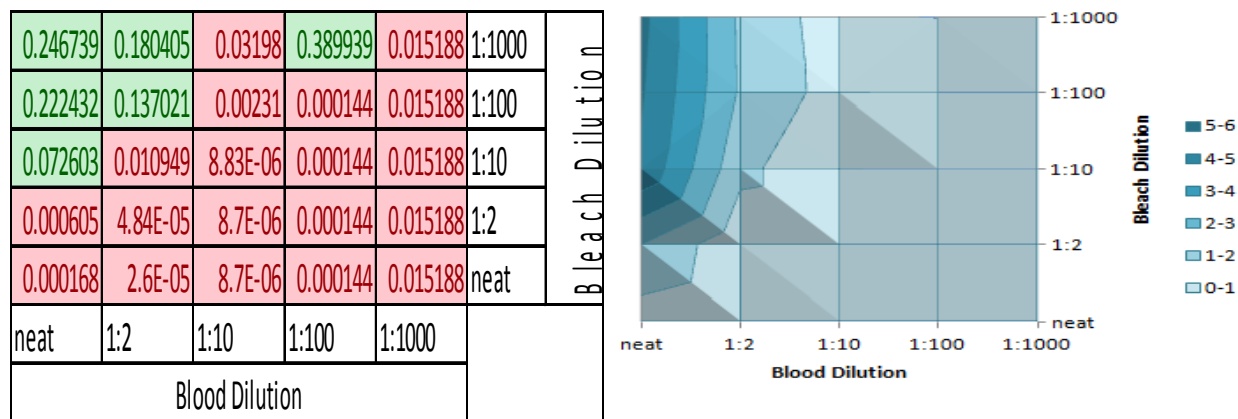
Figure 28. Statistical significance of variance of quantification data between control samples and bleach-treated samples. The chart on the left displays the p-value generated by conducting an ANOVA test between the control and the treated samples within each blood/bleach combination. The green squares indicate a p-value > 0.05 meaning the quantification values were not statistically different than the control data, while the red squares contain p-values < 0.05 meaning the treated samples are significantly different than the controls. This statistical data can then be compared to the changes in quantification values that are displayed in the graph to the right. Each red or green square corresponds with a data point on the graph where the blood dilution and bleach dilution lines intersect.

It is both visibly and statistically clear that the presence of bleach in a blood sample decreases the amount of amplifiable DNA that is recovered. This effect is more pronounced in the samples treated with more concentrated bleach. However, the current DNA quantification methods do not make a distinction between samples that do not contain DNA and samples that contain DNA that is degraded to the point that real-time PCR is not possible. Because of this limitation, the quantification data alone does not determine conclusively whether or not the Automate *Express™* system successfully extracted DNA from samples treated with bleach.

The data generated from the DNA quantification of both Sample Set 3, samples treated with Lysol®, and Sample Set 4, samples treated with Resolve®, display similar overall results in relation to the corresponding control data. Figure 29 below presents the average DNA concentration values for each data set as a whole.
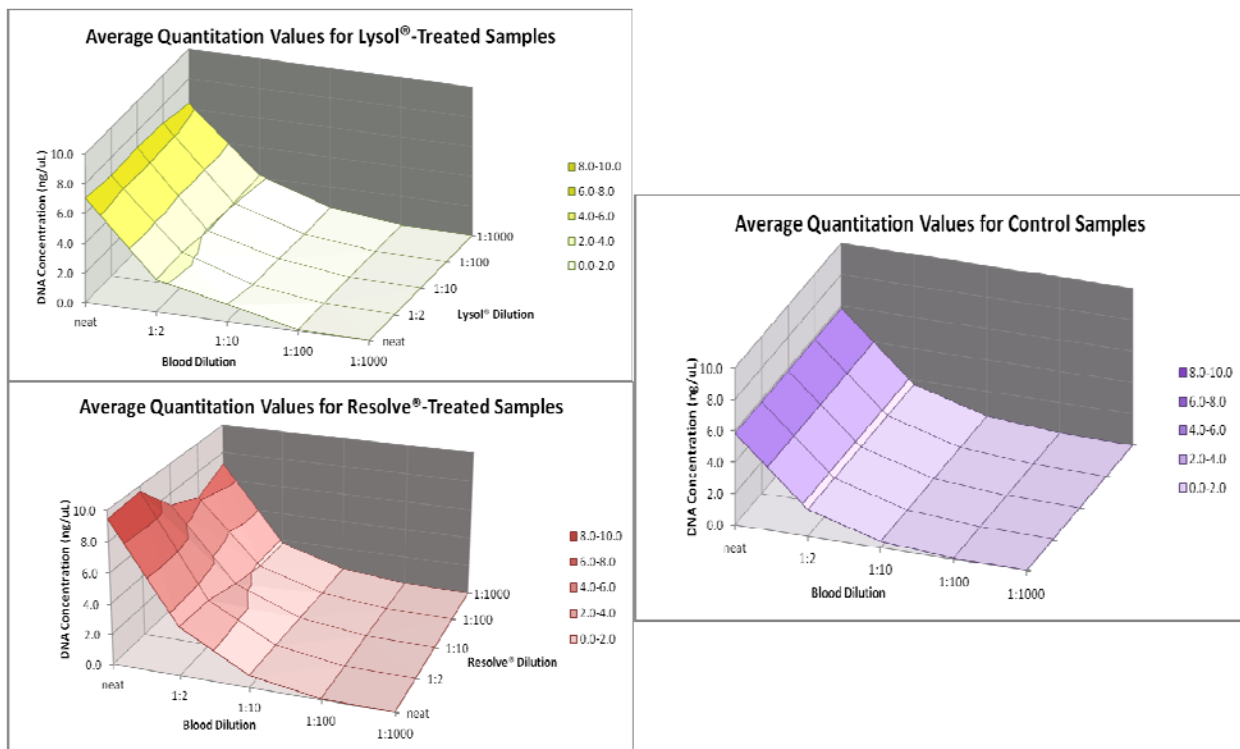
Figure 29. Average quantification values for Sample Sets 3 and 4 vs. control samples. These 3-D surface graphs display the changes in DNA concentration extracted across the entire sample set for Set 3 (top left), Set 4 (bottom left), and the control samples (right). Comparing each of the graphs on the left to the controls shows that both Set 3 and Set 4 follow a similar pattern to the controls, but Sample Set 4 shows an increased amount of DNA recovered from the samples containing high concentrations of both blood and Resolve®.

Graphically, the data from both Set 3 and Set 4 display little variance from the control data. The samples that show the most noticeable difference are those that contain a higher amount of both blood and each of the cleaners, particularly the neat blood/neat Resolve® samples. However, rather than showing a reduced amount of DNA extracted due to interference from the cleaning products as expected, these treated samples actually resulted in more DNA being isolated from the blood samples. With regards to the samples containing high amounts of hydrogen peroxide and blood, this is likely due to the heme compound in the red blood cells, a known PCR inhibitor, being catalyzed by the hydrogen peroxide. Quantitatively, both the samples treated with Lysol® and the samples treated with Resolve® reported higher concentrations of DNA extracted than the corresponding control samples at every blood/cleaner ratio with one exception in each set. For all of the ratios tested in both experimental sets, only two ratios had average DNA quantification values that were lower than the controls, 1:1000 blood/1:2 Lysol® and 1:100 blood/1:2 Resolve®. However, after applying a single-factor ANOVA test to compare these results with the control data, it was determined that the variance was not a statistically significant one. To summarize the quantification data as a whole for these two experimental sets, the treated samples resulted in a DNA concentration that was higher than that of the controls in the majority of samples, and even those samples that did not show an improvement in the amount of DNA extracted reported data that was statistically similar to the controls. Overall, this leads to the conclusion that with regards to amount of DNA recovered, the extraction methodology performs at least as well, if not more efficiently, under each of these two treatment conditions.

5.2 Internal PCR Control Results

The C_t results for the Internal PCR Control (IPC) included in each quantification reaction were used as an indicator of the presence of substances that inhibit the PCR process, as described in Section 4.1. The IPC results for the samples from Set 2 that were treated with Clorox® Regular Bleach were consistently lower for every blood/bleach ratio than they were for the corresponding untreated control sample with the exception of the two samples described in Section 4.2. As both of these samples displayed IPC results that were only slightly elevated and contained diluted bleach, it is more likely that these results were due to non-uniform sampling of the blood dilutions rather than the effects of the bleach treatment. These results indicate that not only is the Automate *Express*® system not negatively impacted by the addition of bleach to a blood sample, the presence of inhibitors is actually reduced after adding bleach. Unfortunately, the limits of the IPC technology do not allow one to determine if the inhibitors were removed more effectively by the extraction technology, or if the inhibiting substances were simply destroyed by the bleach prior to extraction. Regardless, the results do show definitively that bleach does not reduce the ability of the Automate *Express*™/Prepfiler *Express*™ system to remove inhibitors from a sample.

The IPC C_t results for the Lysol®-treated samples from Set 3 resembled those of Set 2; however, in this sample set the average IPC values were lower in the treated samples than the controls for every single sample without exception. This leads to a similar conclusion as that reached for Sample Set 2, that although the lowered IPC values cannot be attributed definitively to an increased effectiveness in the extraction system, the system is not negatively impacted in general by the presence of Lysol® in liquid blood samples.

The effects of Resolve[®] on the IPC C_t results for Sample Set 4 differ from those of the previous two sample sets. The average IPC values for samples containing neat Resolve[®] are all elevated when compared to the control data, regardless of the dilution of blood present in the sample. These results support the conclusions of Akane's study discussed in Section 2.3.3 which determined that hydrogen peroxide, which is the active ingredient in Resolve[®] Carpet Cleaner, can inhibit PCR in forensic samples (Akane, 1996). The majority of the samples containing dilutions of Resolve[®] have IPC values that are similar to the control samples, differing only slightly in either direction and without a discernable trend. This data indicates that diluted Resolve[®] has no observable negative impact upon the efficiency of the Automate *Express*[®] system; however, the system is unable to completely remove the inhibitory compounds present in full strength Resolve[®], regardless of the concentration of blood present in the sample.

5.3 Genetic Profile Quality

The evaluation of the quality of genetic profiles obtained from samples treated with Clorox[®] Bleach was completed by scoring the electropherograms generated with regards to the number of alleles present, with a maximum of 28, and the number of balanced heterozygous loci, with a maximum of 12. The analysis of the data from this sample set was relatively straightforward as each of the samples produced either a complete balanced profile, or they generated no useful profile whatsoever. The only exception to this was Sample 2M1 that generated an electropherogram with only one allele called. In order to compare the allelic dropout scores across the entire data set, the ratio of percent blood to percent bleach in each sample, which ranged from 0.001 to 1000, was converted to a logarithmic value and graphed in relation to each sample's dropout score, as seen in Figure 30.
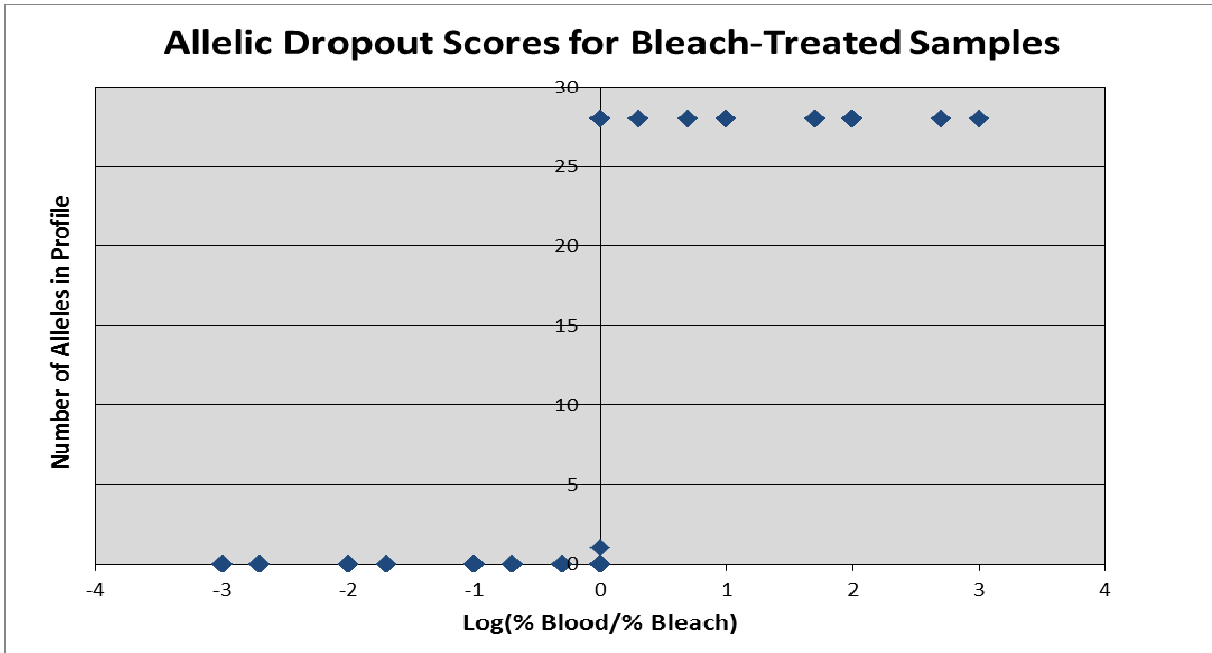
Figure 30. Allelic dropout scores for bleach-treated samples. This graph presents the average allelic dropout scores for samples treated with bleach based upon the (%blood)/(%bleach) ratio in each sample. When the ratios are converted to a logarithmic scale, the graph clearly depicts the critical point at which the samples go from a full profile (28 alleles) to profiles with no alleles called. This point is located directly at the vertical axis where the log(%blood/%bleach) = 0. This translates to a 1:1 blood:bleach ratio.

Figure 30 clearly illustrates the critical point at which the profiles changed from a complete profile to no useful profile recovered at all. This point falls directly on the y-axis in the graph which indicates a log(%blood/%bleach) value of 0. When this value is converted back to a ratio it is equal to 1:1 ratio of blood to bleach. This indicates that the concentration of blood must be higher than that of bleach in a sample in order to obtain a DNA profile. In order for this data trend to be useful to a crime scene agent, it can be correlated to the physical appearance of the samples. In general terms, the samples which produced full genetic profiles were those that were visibly red or dark brown in color, while the samples that turned completely clear or clear with a yellow precipitate produced no genetic information. It should be noted that, while it was not the topic of this research, these results indicate that the common decontamination procedures employed in forensic DNA laboratories, typically using 1:10 bleach dilutions, may not be sufficient to destroy DNA after handling liquid blood.

The genetic profiles generated from the Lysol®-treated samples in Sample Set 3 showed no signs of allelic dropout and were balanced at all 12 heterozygous loci in all replicates of samples containing neat, 1:2, 1:10, and 1:100 blood. The samples with the most diluted blood showed slight variation with each dilution of Lysol® added, however, all of the samples still generated partial profiles with anywhere from 18-27 alleles present and there was no discernable trend with regards to the strength of the Lysol® in each sample. This does not differ dramatically from the results seen in the 3 control sample replicates with 1:1000 blood, and with quantification levels ranging from only 0.002-0.011 ng/µL of DNA these somewhat variable results are not surprising. Overall, treatment with Lysol® did not have a measurable negative impact on genetic profile quality in any blood/cleaner ratio, even those that showed dramatic changes in physical appearance.

The quality scores for the genetic profiles produced from Sample Set 4, those treated with Resolve® Carpet Cleaner, give more evidence to the conclusion that the hydrogen peroxide is inhibiting PCR in these samples. The samples containing neat, 1:2, and 1:10 blood all generated full, balanced profiles with no dropout, however, the 1:100 and 1:1000 blood samples all showed a drastic difference from the controls when treated with neat Resolve®. All three of the 1:100 blood/neat Resolve® samples produced no profile whatsoever, and only one of the replicates of 1:1000 blood/neat Resolve® generated even a partial profile, that profile having only 4 alleles present. Given that the average quantification values for these samples were higher than the corresponding controls, it can be concluded that the inability to generate useful profiles from the samples is due to PCR inhibition caused by the hydrogen peroxide in Resolve®. Unfortunately, these results cannot be correlated directly to the physical appearance of the sample as several of the samples with more diluted Resolve® generated full genetic profiles, but looked very similar

to the completely inhibited samples. In crime scene situations dealing with minute amounts of blood, if crime scene personnel suspect that the scene was cleaned with a hydrogen peroxide-based cleaner, they should be prepared for possible negative consequences with downstream DNA analysis. Further research investigating this property of hydrogen peroxide with regards to the DNA present in other types of biological fluids would be beneficial to the forensic community.

5.4 Conclusions

The goals of this research were two-fold. The first was to evaluate the performance of the Automate *Express*™ Forensic DNA Extraction System and the Prepfiler *Express*™ Forensic DNA Extraction Kit when extracting DNA from blood samples treated with three different common cleaning products. The second goal was to evaluate the impact that these three types of cleaners have on the forensic DNA analysis process with regards to the quality of the genetic profile that is generated. With regards to Clorox® Regular Bleach-treated samples, the Automate *Express*™ system extracted lower amounts of DNA in the majority of samples than what was extracted from corresponding control samples, however, it is not possible to conclusively determine if this was due to lowered effectiveness of the extraction system or due to severe degradation of the DNA by the bleach to the point that it could not be quantified. The IPC results showed that the automated system was able to successfully remove any inhibitors that may have been present in the bleach-treated samples. In the case of the samples that were treated with Lysol® Multi-Surface All-Purpose Cleaner, the Automate *Express*™ system effectively extracted DNA in quantities similar to or greater than the untreated samples, and the DNA extracted was effectively purified by the system to remove PCR inhibitors. Finally, the automated system was able to extract high amounts of DNA from samples treated with Resolve® Carpet Cleaner, but it

was unable to completely remove the PCR inhibiting hydrogen peroxide from samples

containing full strength Resolve® which led to downstream amplification problems in more

diluted blood samples. Overall, the Automate *Express*™ Forensic DNA Extraction System and

the Prepfiler *Express*™ Forensic DNA Extraction Kit performed well in the presence of these

chemical contaminants with regards to the amount and quality of DNA extracted.

With regards to the impact that these cleaning chemicals have on the ability to generate a

useful genetic profile, bleach had by far the most detrimental impact on the profiles. If a sample

is collected that contains a higher concentration of bleach than blood, the likelihood of obtaining

any useful information at all is very remote. In physical terms, if the blood at a crime scene has

been cleaned with bleach to the point that it is no longer visible to the naked eye, it is unlikely

that investigators should expect a useable profile. In the other extreme, Lysol® Multi-Surface

All-Purpose Cleaner has no negative impact on the DNA in blood samples at all. If investigators

have reason to believe a crime scene has been cleaned with a product containing quaternary

ammonium compounds, they can still reasonably expect useful profiles to be obtained from

blood evidence present despite how drastically the physical appearance of said blood has been

altered. Lastly, the samples treated with the hydrogen peroxide-based Resolve® Carpet Cleaner

gave results indicating that larger amounts of blood should still produce full profiles even after

being cleaned with this product. Small amounts of blood, however, that are treated with larger

volumes of Resolve® could be severely inhibited to the point that no useful genetic information

can be obtained. Bearing this in mind, crime scene personnel should clearly communicate to the

laboratory analyst that a hydrogen peroxide-based product was used to clean blood evidence, so

that the analyst will be prepared to take additional steps to deal with possible PCR inhibition in

the sample. These results provide a scientific foundation that both crime scene and forensic

laboratory personnel can use to make decisions and manage expectations regarding the collection

and processing of evidence that has been contaminated with cleaning products. Further research

in this area should include other types of chemical contaminants that might be used in an attempt

to destroy DNA; it should also study other types of biological samples in addition to blood.

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APPENDIX A: Description of Samples

Sample Set 1: Control Samples

Sample (Replicates)	Description
1A (1A1, 1A2, 1A3)	Control- Neat Blood
1B (1B1, 1B2, 1B3)	Control- 1:2 Blood
1C (1C1, 1C2, 1C3)	Control- 1:10 Blood
1D (1D1, 1D2, 1D3)	Control- 1:100 Blood
1E (1E1, 1E2, 1E3)	Control- 1:1000 Blood
RB1	PBS + Ultrapure H_2O

Sample Set 2: Samples Treated with Clorox® Regular Bleach

Sample	Description
2A (2A1, 2A2, 2A3)	Neat Blood + Neat Bleach
2B (2B1, 2B2, 2B3)	Neat Blood + 1:2 Bleach
2C (2C1, 2C2, 2C3)	Neat Blood + 1:10 Bleach
2D (2D1, 2D2, 2D3)	Neat Blood + 1:100 Bleach
2E (2E1, 2E2, 2E3)	Neat Blood + 1:1000 Bleach
2F (2F1, 2F2, 2F3)	1:2 Blood + Neat Bleach
2G (2G1, 2G2, 2G3)	1:2 Blood + 1:2 Bleach
2H (2H1, 2H2, 2H3)	1:2 Blood + 1:10 Bleach
2I (2I1, 2I2, 2I3)	1:2 Blood + 1:100 Bleach
2J (2J1, 2J2, 2J3)	1:2 Blood + 1:1000 Bleach
2K (2K1, 2K2, 2K3)	1:10 Blood + Neat Bleach
2L (2L1, 2L2, 2L3)	1:10 Blood + 1:2 Bleach
2M (2M1, 2M2, 2M3)	1:10 Blood + 1:10 Bleach
2N (2N1, 2N2, 2N3)	1:10 Blood + 1:100 Bleach
2O (2O1, 2O2, 2O3)	1:10 Blood + 1:1000 Bleach
2P (2P1, 2P2, 2P3)	1:100 Blood + Neat Bleach
2Q (2Q1, 2Q2, 2Q3)	1:100 Blood + 1:2 Bleach
2R (2R1, 2R2, 2R3)	1:100 Blood + 1:10 Bleach
2S (2S1, 2S2, 2S3)	1:100 Blood + 1:100 Bleach
2T (2T1, 2T2, 2T3)	1:100 Blood + 1:1000 Bleach
2U (2U1, 2U2, 2U3)	1:1000 Blood + Neat Bleach
2V (2V1, 2V2, 2V3)	1:1000 Blood + 1:2 Bleach
2W (2W1, 2W2, 2W3)	1:1000 Blood + 1:10 Bleach
2X (2X1, 2X2, 2X3)	1:1000 Blood + 1:100 Bleach
2Y (2A1, 2A2, 2Y3)	1:1000 Blood + 1:1000 Bleach
RB2 (RB2A, RB2B, RB2C)	Neat Bleach + Ultrapure H_2O

Sample Set 3: Samples Treated with Lysol® Multi-Surface All-Purpose Cleaner

Sample (Replicates)	Description
3A (3A1, 3A2, 3A3)	Neat Blood + Neat Lysol
3B (3B1, 3B2, 3B3)	Neat Blood + 1:2 Lysol
3C (3C1, 3C2, 3C3)	Neat Blood + 1:10 Lysol
3D (3D1, 3D2, 3D3)	Neat Blood + 1:100 Lysol
3E (3E1, 3E2, 3E3)	Neat Blood + 1:1000 Lysol
3F (3F1, 3F2, 3F3)	1:2 Blood + Neat Lysol
3G (3G1, 3G2, 3G3)	1:2 Blood + 1:2 Lysol
3H (3H1, 3H2, 3H3)	1:2 Blood + 1:10 Lysol
3I (3I1, 3I2, 3I3)	1:2 Blood + 1:100 Lysol
3J (3J1, 3J2, 3J3)	1:2 Blood + 1:1000 Lysol
3K (3K1, 3K2, 3K3)	1:10 Blood + Neat Lysol
3L (3L1, 3L2, 3L3)	1:10 Blood + 1:2 Lysol
3M (3M1, 3M2, 3M3)	1:10 Blood + 1:10 Lysol
3N (3N1, 3N2, 3N3)	1:10 Blood + 1:100 Lysol
3O (3O1, 3O2, 3O3)	1:10 Blood + 1:1000 Lysol
3P (3P1, 3P2, 3P3)	1:100 Blood + Neat Lysol
3Q (3Q1, 3Q2, 3Q3)	1:100 Blood + 1:2 Lysol
3R (3R1, 3R2, 3R3)	1:100 Blood + 1:10 Lysol
3S (3S1, 3S2, 3S3)	1:100 Blood + 1:100 Lysol
3T (3T1, 3T2, 3T3)	1:100 Blood + 1:1000 Lysol
3U (3U1, 3U2, 3U3)	1:1000 Blood + Neat Lysol
3V (3V1, 3V2, 3V3)	1:1000 Blood + 1:2 Lysol
3W (3W1, 3W2, 3W3)	1:1000 Blood + 1:10 Lysol
3X (3X1, 3X2, 3X3)	1:1000 Blood + 1:100 Lysol
3Y (3A1, 3A2, 3Y3)	1:1000 Blood + 1:1000 Lysol
RB3 (RB3A, RB3B, RB3C)	Neat Lysol + Ultrapure H$_2$O

Sample Set 4: Samples Treated with Resolve$^{®}$ Carpet Cleaner

Sample (Replicates)	Description
4A (4A1, 4A2, 4A3)	Neat Blood + Neat Resolve
4B (4B1, 4B2, 4B3)	Neat Blood + 1:2 Resolve
4C (4C1, 4C2, 4C3)	Neat Blood + 1:10 Resolve
4D (4D1, 4D2, 4D3)	Neat Blood + 1:100 Resolve
4E (4E1, 4E2, 4E3)	Neat Blood + 1:1000 Resolve
4F (4F1, 4F2, 4F3)	1:2 Blood + Neat Resolve
4G (4G1, 4G2, 4G3)	1:2 Blood + 1:2 Resolve
4H (4H1, 4H2, 4H3)	1:2 Blood + 1:10 Resolve
4I (4I1, 4I2, 4I3)	1:2 Blood + 1:100 Resolve
4J (4J1, 4J2, 4J3)	1:2 Blood + 1:1000 Resolve
4K (4K1, 4K2, 4K3)	1:10 Blood + Neat Resolve
4L (4L1, 4L2, 4L3)	1:10 Blood + 1:2 Resolve
4M (4M1, 4M2, 4M3)	1:10 Blood + 1:10 Resolve
4N (4N1, 4N2, 4N3)	1:10 Blood + 1:100 Resolve
4O (4O1, 4O2, 4O3)	1:10 Blood + 1:1000 Resolve
4P (4P1, 4P2, 4P3)	1:100 Blood + Neat Resolve
4Q (4Q1, 4Q2, 4Q3)	1:100 Blood + 1:2 Resolve
4R (4R1, 4R2, 4R3)	1:100 Blood + 1:10 Resolve
4S (4S1, 4S2, 4S3)	1:100 Blood + 1:100 Resolve
4T (4T1, 4T2, 4T3)	1:100 Blood + 1:1000 Resolve
4U (4U1, 4U2, 4U3)	1:1000 Blood + Neat Resolve
4V (4V1, 4V2, 4V3)	1:1000 Blood + 1:2 Resolve
4W (4W1, 4W2, 4W3)	1:1000 Blood + 1:10 Resolve
4X (4X1, 4X2, 4X3)	1:1000 Blood + 1:100 Resolve
4Y (4A1, 4A2, 4Y3)	1:1000 Blood + 1:1000 Resolve
RB4 (RB4A, RB4B, RB4C)	Neat Resolve + Ultrapure H_2O

APPENDIX B: DNA Quantification Results

File:	ART Thesis_Controls_102814
Print Date:	Tuesday, October 28, 2014 16:37:38
User:	Allison.Taylor
Plate Type:	Absolute Quantification
PCR Volume:	25 μL

Document Information

Operator:	ART
Run Date:	Tuesday, October 28, 2014 12:11:44
Last Modified:	Tuesday, October 28, 2014 13:44:29
Instrument Type:	Applied Biosystems 7500 Real-Time PCR System

Comments

Comments:
ART Thesis_Controls_102814

Thermal Profile

Stage	Temperature	Time	Repeat	Ramp Rate	Auto Increment
1	95.0 °C	10:00	1	Auto	
2	95.0 °C	0:15	40	Auto	
	60.0 °C	1:00		Auto	

9600 Mode Emulation
Data Collection : Stage 2, Step 2

Analysis Methods:

Delta Rn

Detector Information

Detector Name		Reporter	Quencher	Threshold	Baseline Start	Baseline End
	Human Quant	FAM	(none)	0.200000	3	15
	IPC	VIC	(none)	0.200000	3	15

Well	Sample Name	Detector	Task	Ct	StdDev Ct	Qty
A1	Standard A	Human Quant	Standard	22.73		50.00
		IPC	Unknown	28.93		
A2	Standard B	Human Quant	Standard	24.35		16.70
		IPC	Unknown	27.48		
A3	Standard C	Human Quant	Standard	26.11		5.56
		IPC	Unknown	27.31		
A4	Standard D	Human Quant	Standard	27.70		1.85
		IPC	Unknown	27.40		
A5	Standard E	Human Quant	Standard	29.35		6.20e-001
		IPC	Unknown	27.45		
A6	Standard F	Human Quant	Standard	31.06		2.10e-001

		IPC	Unknown	27.58	
A7	Standard G	Human Quant	Standard	32.12	6.80e-002
		IPC	Unknown	27.62	
A8	Standard H	Human Quant	Standard	34.30	2.30e-002
		IPC	Unknown	27.52	
A9	QNC	Human Quant	Unknown	Undet.	
		IPC	Unknown	27.37	
A10	1A1	Human Quant	Unknown	26.06	5.47
		IPC	Unknown	27.37	
A11	1A1 (1:20)	Human Quant	Unknown	30.25	3.20e-001
		IPC	Unknown	27.18	
A12	1A2	Human Quant	Unknown	25.78	6.62
		IPC	Unknown	27.31	
B1	1A2 (1:20)	Human Quant	Unknown	30.63	2.48e-001
		IPC	Unknown	27.22	
B2	1A3	Human Quant	Unknown	26.07	5.43
		IPC	Unknown	27.18	
B3	1A3 (1:20)	Human Quant	Unknown	30.62	2.51e-001
		IPC	Unknown	27.20	
B4	1B1	Human Quant	Unknown	27.67	1.84
		IPC	Unknown	27.25	
B5	1B1 (1:10)	Human Quant	Unknown	31.31	1.57e-001
		IPC	Unknown	27.37	
B6	1B2	Human Quant	Unknown	27.91	1.57
		IPC	Unknown	27.48	
B7	1B2 (1:10)	Human Quant	Unknown	31.28	1.60e-001
		IPC	Unknown	27.46	
B8	1B3	Human Quant	Unknown	27.79	1.70
		IPC	Unknown	27.36	
B9	1B3 (1:10)	Human Quant	Unknown	31.13	1.77e-001
		IPC	Unknown	27.31	
B10	1C1	Human Quant	Unknown	29.93	3.98e-001
		IPC	Unknown	27.18	
B11	1C2	Human Quant	Unknown	29.83	4.27e-001
		IPC	Unknown	27.15	
B12	1C3	Human Quant	Unknown	30.00	3.79e-001
		IPC	Unknown	27.17	
C1	1D1	Human Quant	Unknown	32.49	7.05e-002
		IPC	Unknown	27.12	
C2	1D2	Human Quant	Unknown	32.82	5.63e-002
		IPC	Unknown	27.13	
C3	1D3	Human Quant	Unknown	32.75	5.89e-002
		IPC	Unknown	27.18	
C4	1E1	Human Quant	Unknown	37.42	2.52e-003
		IPC	Unknown	27.21	
C5	1E2	Human Quant	Unknown	36.40	5.00e-003
		IPC	Unknown	27.35	
C6	1E3	Human Quant	Unknown	37.40	2.54e-003
		IPC	Unknown	27.44	
C7	RB1 102714	Human Quant	Unknown	Undet.	
		IPC	Unknown	27.42	

File:	ART Thesis_Bleach 1_110314
Print Date:	Friday, March 13, 2015 11:26:15
User:	Allison.Taylor
Plate Type:	Absolute Quantification
PCR Volume:	25 µL

Document Information

Operator:	ART
Run Date:	Monday, November 03, 2014 18:01:37
Last Modified:	Tuesday, November 04, 2014 11:33:32
Instrument Type:	Applied Biosystems 7500 Real-Time PCR System

Comments

Comments:
ART Thesis_Bleach 1_110314

Thermal Profile

Stage	Temperature	Time	Repeat	Ramp Rate	Auto Increment
1	95.0 °C	10:00	1	Auto	
2	95.0 °C	0:15	40	Auto	
	60.0 °C	1:00		Auto	

9600 Mode Emulation
Data Collection : Stage 2, Step 2

Analysis Methods:

Delta Rn

Detector Information

Detector Name	Reporter	Quencher	Threshold	Baseline Start	Baseline End
Human Quant	FAM	(none)	0.200000	3	15
IPC	VIC	(none)	0.200000	3	15

Well	Sample Name	Detector	Task	Ct	Qty
A1	Standard A	Human Quant	Standard	22.39	50.00
		IPC	Unknown	27.73	
A2	Standard B	Human Quant	Standard	24.16	16.70
		IPC	Unknown	26.60	
A3	Standard C	Human Quant	Standard	25.95	5.56
		IPC	Unknown	26.67	
A4	Standard D	Human Quant	Standard	27.43	1.85
		IPC	Unknown	26.90	
A5	Standard E	Human Quant	Standard	29.04	6.20e-001
		IPC	Unknown	26.93	
A6	Standard F	Human Quant	Standard	30.52	2.10e-001

		IPC	Unknown	27.07	
A7	Standard G	Human Quant	Standard	32.06	6.80e-002
		IPC	Unknown	27.13	
A8	Standard H	Human Quant	Standard	34.12	2.30e-002
		IPC	Unknown	26.94	
A9	QNC	Human Quant	Unknown	Undet.	
		IPC	Unknown	26.76	
A10	2A1	Human Quant	Unknown	29.37	4.92e-001
		IPC	Unknown	26.73	
A11	2A1 (1:20)	Human Quant	Unknown	33.78	2.53e-002
		IPC	Unknown	26.65	
A12	2A2	Human Quant	Unknown	29.35	4.97e-001
		IPC	Unknown	26.82	
B1	2A2 (1:20)	Human Quant	Unknown	34.09	2.04e-002
		IPC	Unknown	26.67	
B2	2A3	Human Quant	Unknown	29.16	5.65e-001
		IPC	Unknown	26.68	
B3	2A3 (1:20)	Human Quant	Unknown	33.55	2.95e-002
		IPC	Unknown	26.70	
B4	2B1	Human Quant	Unknown	27.26	2.03
		IPC	Unknown	26.79	
B5	2B1 (1:20)	Human Quant	Unknown	31.32	1.32e-001
		IPC	Unknown	26.86	
B6	2B2	Human Quant	Unknown	27.29	2.00
		IPC	Unknown	26.89	
B7	2B2 (1:20)	Human Quant	Unknown	31.51	1.16e-001
		IPC	Unknown	26.94	
B8	2B3	Human Quant	Unknown	27.29	2.00
		IPC	Unknown	26.79	
B9	2B3 (1:20)	Human Quant	Unknown	31.28	1.35e-001
		IPC	Unknown	26.74	
B10	2C1	Human Quant	Unknown	26.02	4.70
		IPC	Unknown	26.51	
B11	2C1 (1:20)	Human Quant	Unknown	30.18	2.85e-001
		IPC	Unknown	26.55	
B12	2C2	Human Quant	Unknown	25.89	5.11
		IPC	Unknown	26.53	
C1	2C2 (1:20)	Human Quant	Unknown	30.14	2.92e-001
		IPC	Unknown	26.65	
C2	2C3	Human Quant	Unknown	26.01	4.71
		IPC	Unknown	26.50	
C3	2C3 (1:20)	Human Quant	Unknown	30.25	2.71e-001
		IPC	Unknown	26.69	
C4	2D1	Human Quant	Unknown	25.80	5.46
		IPC	Unknown	26.71	
C5	2D1 (1:20)	Human Quant	Unknown	30.09	3.03e-001
		IPC	Unknown	26.85	
C6	2D2	Human Quant	Unknown	25.81	5.40
		IPC	Unknown	26.84	
C7	2D2 (1:20)	Human Quant	Unknown	30.03	3.15e-001
		IPC	Unknown	26.84	
C8	2D3	Human Quant	Unknown	26.08	4.51
		IPC	Unknown	26.68	
C9	2D3 (1:20)	Human Quant	Unknown	30.24	2.73e-001
		IPC	Unknown	26.71	
C10	2E1	Human Quant	Unknown	25.90	5.09
		IPC	Unknown	26.47	

C11	2E1 (1:20)	Human Quant	Unknown	30.12	2.96e-001
		IPC	Unknown	26.56	
C12	2E2	Human Quant	Unknown	25.82	5.38
		IPC	Unknown	26.49	
D1	2E2 (1:20)	Human Quant	Unknown	29.97	3.27e-001
		IPC	Unknown	26.56	
D2	2E3	Human Quant	Unknown	25.81	5.41
		IPC	Unknown	26.50	
D3	2E3 (1:20)	Human Quant	Unknown	30.15	2.91e-001
		IPC	Unknown	26.63	
D4	2F1	Human Quant	Unknown	Undet.	
		IPC	Unknown	26.80	
D5	2F1 (1:10)	Human Quant	Unknown	Undet.	
		IPC	Unknown	26.83	
D6	2F2	Human Quant	Unknown	Undet.	
		IPC	Unknown	26.95	
D7	2F2 (1:10)	Human Quant	Unknown	Undet.	
		IPC	Unknown	26.92	
D8	2F3	Human Quant	Unknown	Undet.	
		IPC	Unknown	26.81	
D9	2F3 (1:10)	Human Quant	Unknown	Undet.	
		IPC	Unknown	26.62	
D10	2G1	Human Quant	Unknown	30.34	2.55e-001
		IPC	Unknown	26.67	
D11	2G1 (1:10)	Human Quant	Unknown	33.62	2.81e-002
		IPC	Unknown	26.59	
D12	2G2	Human Quant	Unknown	30.49	2.32e-001
		IPC	Unknown	26.64	
E1	2G2 (1:10)	Human Quant	Unknown	33.58	2.89e-002
		IPC	Unknown	26.66	
E2	2G3	Human Quant	Unknown	30.44	2.38e-001
		IPC	Unknown	26.66	
E3	2G3 (1:10)	Human Quant	Unknown	33.67	2.71e-002
		IPC	Unknown	26.72	
E4	2H1	Human Quant	Unknown	27.83	1.38
		IPC	Unknown	26.70	
E5	2H1 (1:10)	Human Quant	Unknown	31.14	1.49e-001
		IPC	Unknown	26.79	
E6	2H2	Human Quant	Unknown	28.02	1.22
		IPC	Unknown	26.87	
E7	2H2 (1:10)	Human Quant	Unknown	31.40	1.25e-001
		IPC	Unknown	26.90	
E8	2H3	Human Quant	Unknown	27.94	1.29
		IPC	Unknown	26.75	
E9	2H3 (1:10)	Human Quant	Unknown	31.22	1.41e-001
		IPC	Unknown	26.67	
E10	2I1	Human Quant	Unknown	27.36	1.90
		IPC	Unknown	26.54	
E11	2I1 (1:10)	Human Quant	Unknown	30.65	2.07e-001
		IPC	Unknown	26.57	
E12	RB2A 103014	Human Quant	Unknown	Undet.	
		IPC	Unknown	26.65	
F1	2I2	Human Quant	Unknown	27.33	1.94
		IPC	Unknown	26.62	
F2	2I2 (1:10)	Human Quant	Unknown	30.87	1.79e-001
		IPC	Unknown	26.66	
F3	2I3	Human Quant	Unknown	27.46	1.78

		IPC	Unknown	26.63	
F4	2I3 (1:10)	Human Quant	Unknown	30.87	1.79e-001
		IPC	Unknown	26.78	
F5	2J1	Human Quant	Unknown	27.42	1.83
		IPC	Unknown	26.81	
F6	2J1 (1:10)	Human Quant	Unknown	30.69	2.02e-001
		IPC	Unknown	26.92	
F7	2J2	Human Quant	Unknown	27.45	1.79
		IPC	Unknown	26.81	
F8	2J2 (1:10)	Human Quant	Unknown	30.55	2.22e-001
		IPC	Unknown	26.78	
F9	2J3	Human Quant	Unknown	27.36	1.90
		IPC	Unknown	26.60	
F10	2J3 (1:10)	Human Quant	Unknown	30.68	2.04e-001
		IPC	Unknown	26.61	
F11	2K1	Human Quant	Unknown	Undet.	
		IPC	Unknown	26.60	
F12	2K2	Human Quant	Unknown	Undet.	
		IPC	Unknown	26.65	
G1	2K3	Human Quant	Unknown	Undet.	
		IPC	Unknown	26.73	
G2	2L1	Human Quant	Unknown	Undet.	
		IPC	Unknown	26.76	
G3	2L2	Human Quant	Unknown	Undet.	
		IPC	Unknown	26.79	
G4	2L3	Human Quant	Unknown	Undet.	
		IPC	Unknown	26.83	
G5	2M1	Human Quant	Unknown	Undet.	
		IPC	Unknown	27.01	
G6	2M2	Human Quant	Unknown	37.31	2.34e-003
		IPC	Unknown	27.68	
G7	2M3	Human Quant	Unknown	Undet.	
		IPC	Unknown	27.04	
G8	2N1	Human Quant	Unknown	29.24	5.35e-001
		IPC	Unknown	26.85	
G9	2N2	Human Quant	Unknown	29.14	5.73e-001
		IPC	Unknown	26.69	
G10	2N3	Human Quant	Unknown	29.06	6.05e-001
		IPC	Unknown	26.66	
G11	2O1	Human Quant	Unknown	29.50	4.51e-001
		IPC	Unknown	26.60	
G12	2O2	Human Quant	Unknown	29.40	4.81e-001
		IPC	Unknown	26.62	
H1	2O3	Human Quant	Unknown	29.52	4.45e-001
		IPC	Unknown	26.77	
H2	2P1	Human Quant	Unknown	Undet.	
		IPC	Unknown	26.88	
H3	2P2	Human Quant	Unknown	Undet.	
		IPC	Unknown	26.82	
H4	2P3	Human Quant	Unknown	Undet.	
		IPC	Unknown	26.93	
H5	2Q1	Human Quant	Unknown	Undet.	
		IPC	Unknown	26.98	
H6	2Q2	Human Quant	Unknown	Undet.	
		IPC	Unknown	27.02	
H7	RB2B 103014	Human Quant	Unknown	Undet.	
		IPC	Unknown	27.02	

File:	ART Thesis_Bleach 2_110314
Print Date:	Friday, March 13, 2015 11:32:31
User:	Allison.Taylor
Plate Type:	Absolute Quantification
PCR Volume:	25 µL

Document Information

Operator:	ART
Run Date:	Monday, November 03, 2014 18:04:33
Last Modified:	Tuesday, November 04, 2014 11:36:48
Instrument Type:	Applied Biosystems 7500 Real-Time PCR System

Comments

Comments:
ART Thesis_Bleach 2_110314

Thermal Profile

Stage	Temperature	Time	Repeat	Ramp Rate	Auto Increment
1	95.0 °C	10:00	1	Auto	
2	95.0 °C	0:15	40	Auto	
	60.0 °C	1:00		Auto	

9600 Mode Emulation
Data Collection : Stage 2, Step 2

Analysis Methods:

Delta Rn

Detector Information

Detector Name	Reporter	Quencher	Threshold	Baseline Start	Baseline End
Human Quant	FAM	(none)	0.200000	3	15
IPC	VIC	(none)	0.200000	3	15

Well	Sample Name	Detector	Task	Ct	Qty
A1	Standard A	Human Quant	Standard	22.58	50.00
		IPC	Unknown	28.41	
A2	Standard B	Human Quant	Standard	24.31	16.70
		IPC	Unknown	27.11	
A3	Standard C	Human Quant	Standard	26.10	5.56
		IPC	Unknown	27.03	
A4	Standard D	Human Quant	Standard	27.69	1.85
		IPC	Unknown	27.14	
A5	Standard E	Human Quant	Standard	29.13	6.20e-001
		IPC	Unknown	27.22	
A6	Standard F	Human Quant	Standard	30.80	2.10e-001

		IPC	Unknown	27.33	
A7	Standard G	Human Quant	Standard	32.21	6.80e-002
		IPC	Unknown	27.36	
A8	Standard H	Human Quant	Standard	34.11	2.30e-002
		IPC	Unknown	27.30	
A9	QNC	Human Quant	Unknown	Undet.	
		IPC	Unknown	27.16	
A10	2Q3	Human Quant	Unknown	Undet.	
		IPC	Unknown	27.07	
A11	2R1	Human Quant	Unknown	Undet.	
		IPC	Unknown	27.02	
A12	2R2	Human Quant	Unknown	Undet.	
		IPC	Unknown	27.10	
B1	2R3	Human Quant	Unknown	Undet.	
		IPC	Unknown	27.00	
B2	2S1	Human Quant	Unknown	Undet.	
		IPC	Unknown	26.99	
B3	2S2	Human Quant	Unknown	Undet.	
		IPC	Unknown	27.04	
B4	2S3	Human Quant	Unknown	Undet.	
		IPC	Unknown	27.13	
B5	2T1	Human Quant	Unknown	32.48	6.55e-002
		IPC	Unknown	27.19	
B6	2T2	Human Quant	Unknown	33.07	4.39e-002
		IPC	Unknown	27.30	
B7	2T3	Human Quant	Unknown	32.76	5.43e-002
		IPC	Unknown	27.29	
B8	2U1	Human Quant	Unknown	Undet.	
		IPC	Unknown	27.23	
B9	2U2	Human Quant	Unknown	Undet.	
		IPC	Unknown	27.15	
B10	2U3	Human Quant	Unknown	Undet.	
		IPC	Unknown	27.04	
B11	2V1	Human Quant	Unknown	Undet.	
		IPC	Unknown	26.98	
B12	2V2	Human Quant	Unknown	Undet.	
		IPC	Unknown	27.01	
C1	2V3	Human Quant	Unknown	Undet.	
		IPC	Unknown	26.88	
C2	2W1	Human Quant	Unknown	Undet.	
		IPC	Unknown	26.90	
C3	2W2	Human Quant	Unknown	Undet.	
		IPC	Unknown	26.93	
C4	2W3	Human Quant	Unknown	Undet.	
		IPC	Unknown	27.00	
C5	2X1	Human Quant	Unknown	Undet.	
		IPC	Unknown	27.10	
C6	2X2	Human Quant	Unknown	Undet.	
		IPC	Unknown	27.20	
C7	2X3	Human Quant	Unknown	Undet.	
		IPC	Unknown	27.15	
C8	2Y1	Human Quant	Unknown	Undet.	
		IPC	Unknown	27.16	
C9	2Y2	Human Quant	Unknown	Undet.	
		IPC	Unknown	26.98	
C10	2Y3	Human Quant	Unknown	Undet.	
		IPC	Unknown	26.98	

ART Thesis_Bleach 2_110314   Page 2 of 3 (03/13/15 11:32:31)

C11	RB2C 103014	Human Quant	Unknown	Undet.	
		IPC	Unknown	26.90	

File: ART Thesis_Lysol 1_110714
Print Date: Friday, March 13, 2015 11:45:02
User: Allison.Taylor
Plate Type: Absolute Quantification
PCR Volume: 25 µL

Document Information

Operator: ART
Run Date: Friday, November 07, 2014 15:12:43
Last Modified: Monday, November 10, 2014 09:51:30
Instrument Type: Applied Biosystems 7500 Real-Time PCR System

Comments

Comments:
ART Thesis_Lysol 1_110714

Thermal Profile

Stage	Temperature	Time	Repeat	Ramp Rate	Auto Increment
1	95.0 °C	10:00	1	Auto	
2	95.0 °C	0:15	40	Auto	
	60.0 °C	1:00		Auto	

9600 Mode Emulation
Data Collection : Stage 2, Step 2

Analysis Methods:

Delta Rn

Detector Information

Detector Name	Reporter	Quencher	Threshold	Baseline Start	Baseline End
Human Quant	FAM	(none)	0.200000	3	15
IPC	VIC	(none)	0.200000	3	15

Well	Sample Name	Detector	Task	Ct	Qty
A1	Standard A	Human Quant	Standard	22.40	50.00
		IPC	Unknown	28.27	
A2	Standard B	Human Quant	Standard	24.10	16.70
		IPC	Unknown	26.99	
A3	Standard C	Human Quant	Standard	25.86	5.56
		IPC	Unknown	26.94	
A4	Standard D	Human Quant	Standard	27.51	1.85
		IPC	Unknown	27.07	
A5	Standard E	Human Quant	Standard	29.14	6.20e-001
		IPC	Unknown	27.23	
A6	Standard F	Human Quant	Standard	30.88	2.10e-001

		IPC	Unknown	27.40	
A7	Standard G	Human Quant	Standard	32.32	6.80e-002
		IPC	Unknown	27.24	
A8	Standard H	Human Quant	Standard	34.75	2.30e-002
		IPC	Unknown	27.15	
A9	QNC	Human Quant	Unknown	Undet.	
		IPC	Unknown	27.00	
A10	3A1	Human Quant	Unknown	25.46	6.90
		IPC	Unknown	26.93	
A11	3A1 (1:20)	Human Quant	Unknown	29.63	4.78e-001
		IPC	Unknown	26.96	
A12	3A2	Human Quant	Unknown	25.49	6.77
		IPC	Unknown	26.95	
B1	3A2 (1:20)	Human Quant	Unknown	29.79	4.34e-001
		IPC	Unknown	26.92	
B2	3A3	Human Quant	Unknown	25.35	7.40
		IPC	Unknown	26.83	
B3	3A3 (1:20)	Human Quant	Unknown	29.49	5.23e-001
		IPC	Unknown	26.88	
B4	3B1	Human Quant	Unknown	25.46	6.89
		IPC	Unknown	27.08	
B5	3B1 (1:20)	Human Quant	Unknown	29.85	4.17e-001
		IPC	Unknown	27.13	
B6	3B2	Human Quant	Unknown	25.55	6.49
		IPC	Unknown	27.13	
B7	3B2 (1:20)	Human Quant	Unknown	29.78	4.35e-001
		IPC	Unknown	27.08	
B8	3B3	Human Quant	Unknown	25.45	6.92
		IPC	Unknown	26.98	
B9	3B3 (1:20)	Human Quant	Unknown	29.88	4.08e-001
		IPC	Unknown	26.98	
B10	3C1	Human Quant	Unknown	25.43	7.05
		IPC	Unknown	26.80	
B11	3C1 (1:20)	Human Quant	Unknown	29.70	4.59e-001
		IPC	Unknown	26.82	
B12	3C2	Human Quant	Unknown	25.51	6.66
		IPC	Unknown	26.77	
C1	3C2 (1:20)	Human Quant	Unknown	29.62	4.81e-001
		IPC	Unknown	26.83	
C2	3C3	Human Quant	Unknown	25.44	6.96
		IPC	Unknown	26.64	
C3	3C3 (1:20)	Human Quant	Unknown	29.86	4.13e-001
		IPC	Unknown	26.90	
C4	3D1	Human Quant	Unknown	25.35	7.39
		IPC	Unknown	26.86	
C5	3D1 (1:20)	Human Quant	Unknown	29.76	4.40e-001
		IPC	Unknown	27.00	
C6	3D2	Human Quant	Unknown	25.68	5.98
		IPC	Unknown	27.08	
C7	3D2 (1:20)	Human Quant	Unknown	29.85	4.16e-001
		IPC	Unknown	27.06	
C8	3D3	Human Quant	Unknown	25.45	6.95
		IPC	Unknown	27.04	
C9	3D3 (1:20)	Human Quant	Unknown	29.75	4.42e-001
		IPC	Unknown	26.86	
C10	3E1	Human Quant	Unknown	26.02	4.82
		IPC	Unknown	26.73	

C11	3E1 (1:20)	Human Quant	Unknown	30.03	3.71e-001
		IPC	Unknown	26.68	
C12	3E2	Human Quant	Unknown	25.43	7.03
		IPC	Unknown	26.72	
D1	3E2 (1:20)	Human Quant	Unknown	29.65	4.73e-001
		IPC	Unknown	26.71	
D2	3E3	Human Quant	Unknown	25.40	7.16
		IPC	Unknown	26.67	
D3	3E3 (1:20)	Human Quant	Unknown	29.68	4.64e-001
		IPC	Unknown	26.83	
D4	3F1	Human Quant	Unknown	27.24	2.20
		IPC	Unknown	26.78	
D5	3F1 (1:10)	Human Quant	Unknown	30.35	3.02e-001
		IPC	Unknown	26.95	
D6	3F2	Human Quant	Unknown	27.31	2.12
		IPC	Unknown	27.01	
D7	3F2 (1:10)	Human Quant	Unknown	30.44	2.86e-001
		IPC	Unknown	27.14	
D8	3F3	Human Quant	Unknown	27.26	2.18
		IPC	Unknown	26.92	
D9	3F3 (1:10)	Human Quant	Unknown	30.38	2.97e-001
		IPC	Unknown	26.91	
D10	3G1	Human Quant	Unknown	27.21	2.25
		IPC	Unknown	26.64	
D11	3G1 (1:10)	Human Quant	Unknown	30.48	2.77e-001
		IPC	Unknown	26.75	
D12	3G2	Human Quant	Unknown	27.23	2.22
		IPC	Unknown	26.69	
E1	3G2 (1:10)	Human Quant	Unknown	30.46	2.81e-001
		IPC	Unknown	26.80	
E2	3G3	Human Quant	Unknown	27.18	2.29
		IPC	Unknown	26.63	
E3	3G3 (1:10)	Human Quant	Unknown	30.52	2.71e-001
		IPC	Unknown	26.74	
E4	3H1	Human Quant	Unknown	27.19	2.27
		IPC	Unknown	26.81	
E5	3H1 (1:10)	Human Quant	Unknown	30.53	2.69e-001
		IPC	Unknown	26.93	
E6	3H2	Human Quant	Unknown	27.25	2.19
		IPC	Unknown	26.91	
E7	3H2 (1:10)	Human Quant	Unknown	30.59	2.59e-001
		IPC	Unknown	27.03	
E8	3H3	Human Quant	Unknown	28.08	1.29
		IPC	Unknown	26.97	
E9	3H3 (1:10)	Human Quant	Unknown	31.33	1.61e-001
		IPC	Unknown	26.86	
E10	3I1	Human Quant	Unknown	27.43	1.95
		IPC	Unknown	26.70	
E11	3I1 (1:10)	Human Quant	Unknown	30.63	2.53e-001
		IPC	Unknown	26.75	
E12	RB3A 110614	Human Quant	Unknown	Undet.	
		IPC	Unknown	26.80	
F1	3I2	Human Quant	Unknown	27.32	2.10
		IPC	Unknown	26.71	
F2	3I2 (1:10)	Human Quant	Unknown	30.49	2.77e-001
		IPC	Unknown	26.76	
F3	3I3	Human Quant	Unknown	27.37	2.03

		IPC	Unknown	26.72	
F4	3I3 (1:10)	Human Quant	Unknown	31.01	1.98e-001
		IPC	Unknown	26.82	
F5	3J1	Human Quant	Unknown	27.32	2.10
		IPC	Unknown	27.02	
F6	3J1 (1:10)	Human Quant	Unknown	30.66	2.49e-001
		IPC	Unknown	26.99	
F7	3J2	Human Quant	Unknown	27.37	2.03
		IPC	Unknown	27.00	
F8	3J2 (1:10)	Human Quant	Unknown	30.91	2.11e-001
		IPC	Unknown	26.93	
F9	3J3	Human Quant	Unknown	27.20	2.26
		IPC	Unknown	26.76	
F10	3J3 (1:10)	Human Quant	Unknown	30.65	2.50e-001
		IPC	Unknown	26.80	
F11	3K1	Human Quant	Unknown	29.01	7.13e-001
		IPC	Unknown	26.72	
F12	3K2	Human Quant	Unknown	27.22	2.24
		IPC	Unknown	26.63	
G1	3K3	Human Quant	Unknown	29.45	5.36e-001
		IPC	Unknown	26.81	
G2	3L1	Human Quant	Unknown	29.00	7.16e-001
		IPC	Unknown	26.79	
G3	3L2	Human Quant	Unknown	29.01	7.10e-001
		IPC	Unknown	26.84	
G4	3L3	Human Quant	Unknown	29.13	6.61e-001
		IPC	Unknown	26.84	
G5	3M1	Human Quant	Unknown	29.00	7.15e-001
		IPC	Unknown	27.00	
G6	3M2	Human Quant	Unknown	29.09	6.75e-001
		IPC	Unknown	27.03	
G7	3M3	Human Quant	Unknown	29.10	6.71e-001
		IPC	Unknown	27.11	
G8	3N1	Human Quant	Unknown	29.30	5.93e-001
		IPC	Unknown	26.81	
G9	3N2	Human Quant	Unknown	29.37	5.65e-001
		IPC	Unknown	26.88	
G10	3N3	Human Quant	Unknown	29.40	5.54e-001
		IPC	Unknown	26.76	
G11	3O1	Human Quant	Unknown	29.19	6.33e-001
		IPC	Unknown	26.69	
G12	3O2	Human Quant	Unknown	29.34	5.78e-001
		IPC	Unknown	26.68	
H1	3O3	Human Quant	Unknown	29.25	6.12e-001
		IPC	Unknown	26.83	
H2	3P1	Human Quant	Unknown	31.98	1.06e-001
		IPC	Unknown	26.94	
H3	3P2	Human Quant	Unknown	32.11	9.81e-002
		IPC	Unknown	26.92	
H4	3P3	Human Quant	Unknown	32.12	9.76e-002
		IPC	Unknown	26.98	
H5	3Q1	Human Quant	Unknown	32.11	9.79e-002
		IPC	Unknown	27.07	
H6	3Q2	Human Quant	Unknown	31.85	1.16e-001
		IPC	Unknown	27.08	
H7	RB3B 110614	Human Quant	Unknown	Undet.	
		IPC	Unknown	27.11	

File:	ART Thesis_Lysol 2_110714
Print Date:	Friday, March 13, 2015 11:48:51
User:	Allison.Taylor
Plate Type:	Absolute Quantification
PCR Volume:	25 μL

Document Information

Operator:	ART
Run Date:	Friday, November 07, 2014 15:14:56
Last Modified:	Friday, November 07, 2014 17:47:49
Instrument Type:	Applied Biosystems 7500 Real-Time PCR System

Comments

Comments:
ART Thesis_Lysol 2_110714

Thermal Profile

Stage	Temperature	Time	Repeat	Ramp Rate	Auto Increment
1	95.0 °C	10:00	1	Auto	
2	95.0 °C	0:15	40	Auto	
	60.0 °C	1:00		Auto	

9600 Mode Emulation
Data Collection : Stage 2, Step 2

Analysis Methods:

Delta Rn

Detector Information

Detector Name	Reporter	Quencher	Threshold	Baseline Start	Baseline End
Human Quant	FAM	(none)	0.200000	3	15
IPC	VIC	(none)	0.200000	3	15

Well	Sample Name	Detector	Task	Ct	Qty
A1	Standard A	Human Quant	Standard	22.33	50.00
		IPC	Unknown	27.81	
A2	Standard B	Human Quant	Standard	24.17	16.70
		IPC	Unknown	26.68	
A3	Standard C	Human Quant	Standard	25.92	5.56
		IPC	Unknown	26.71	
A4	Standard D	Human Quant	Standard	27.47	1.85
		IPC	Unknown	26.98	
A5	Standard E	Human Quant	Standard	29.15	6.20e-001
		IPC	Unknown	27.00	
A6	Standard F	Human Quant	Standard	30.70	2.10e-001

		IPC	Unknown	27.09	
A7	Standard G	Human Quant	Standard	32.54	6.80e-002
		IPC	Unknown	27.15	
A8	Standard H	Human Quant	Standard	33.70	2.30e-002
		IPC	Unknown	26.98	
A9	QNC	Human Quant	Unknown	Undet.	
		IPC	Unknown	26.77	
A10	3Q3	Human Quant	Unknown	32.02	8.55e-002
		IPC	Unknown	26.86	
A11	3R1	Human Quant	Unknown	32.49	6.21e-002
		IPC	Unknown	26.71	
A12	3R2	Human Quant	Unknown	32.04	8.42e-002
		IPC	Unknown	26.82	
B1	3R3	Human Quant	Unknown	31.54	1.18e-001
		IPC	Unknown	26.72	
B2	3S1	Human Quant	Unknown	32.14	7.85e-002
		IPC	Unknown	26.71	
B3	3S2	Human Quant	Unknown	32.47	6.32e-002
		IPC	Unknown	26.76	
B4	3S3	Human Quant	Unknown	32.63	5.68e-002
		IPC	Unknown	26.88	
B5	3T1	Human Quant	Unknown	32.31	7.02e-002
		IPC	Unknown	26.92	
B6	3T2	Human Quant	Unknown	32.66	5.57e-002
		IPC	Unknown	26.98	
B7	3T3	Human Quant	Unknown	32.48	6.25e-002
		IPC	Unknown	26.96	
B8	3U1	Human Quant	Unknown	35.04	1.13e-002
		IPC	Unknown	26.90	
B9	3U2	Human Quant	Unknown	36.29	4.87e-003
		IPC	Unknown	26.63	
B10	3U3	Human Quant	Unknown	36.67	3.77e-003
		IPC	Unknown	26.68	
B11	3V1	Human Quant	Unknown	37.10	2.84e-003
		IPC	Unknown	26.68	
B12	3V2	Human Quant	Unknown	37.82	1.75e-003
		IPC	Unknown	26.69	
C1	3V3	Human Quant	Unknown	38.01	1.54e-003
		IPC	Unknown	26.67	
C2	3W1	Human Quant	Unknown	36.59	3.99e-003
		IPC	Unknown	26.62	
C3	3W2	Human Quant	Unknown	35.90	6.33e-003
		IPC	Unknown	26.69	
C4	3W3	Human Quant	Unknown	35.79	6.82e-003
		IPC	Unknown	26.78	
C5	3X1	Human Quant	Unknown	37.05	2.92e-003
		IPC	Unknown	26.85	
C6	3X2	Human Quant	Unknown	36.15	5.33e-003
		IPC	Unknown	26.92	
C7	3X3	Human Quant	Unknown	35.36	9.07e-003
		IPC	Unknown	26.88	
C8	3Y1	Human Quant	Unknown	35.73	7.10e-003
		IPC	Unknown	26.83	
C9	3Y2	Human Quant	Unknown	36.13	5.44e-003
		IPC	Unknown	26.74	
C10	3Y3	Human Quant	Unknown	35.75	7.00e-003
		IPC	Unknown	26.63	

C11	RB3C 110714	Human Quant	Unknown	Undet.	
		IPC	Unknown	26.60	

File:	Quant Data_Resolve 1_111214
Print Date:	Friday, March 13, 2015 11:51:53
User:	Allison.Taylor
Plate Type:	Absolute Quantification
PCR Volume:	25 µL

Document Information

Operator:	ART
Run Date:	Wednesday, November 12, 2014 12:40:03
Last Modified:	Thursday, November 13, 2014 09:51:04
Instrument Type:	Applied Biosystems 7500 Real-Time PCR System

Comments

Comments:
ART Thesis_Resolve 1_111214

Thermal Profile

Stage	Temperature	Time	Repeat	Ramp Rate	Auto Increment
1	95.0 °C	10:00	1	Auto	
2	95.0 °C	0:15	40	Auto	
	60.0 °C	1:00		Auto	

9600 Mode Emulation
Data Collection : Stage 2, Step 2

Analysis Methods:

Delta Rn

Detector Information

Detector Name	Reporter	Quencher	Threshold	Baseline Start	Baseline End
Human Quant	FAM	(none)	0.200000	3	15
IPC	VIC	(none)	0.200000	3	15

Well	Sample Name	Detector	Task	Ct	Qty
A1	Standard A	Human Quant	Standard	22.41	50.00
		IPC	Unknown	29.07	
A2	Standard B	Human Quant	Standard	24.16	16.70
		IPC	Unknown	27.44	
A3	Standard C	Human Quant	Standard	25.98	5.56
		IPC	Unknown	27.26	
A4	Standard D	Human Quant	Standard	27.53	1.85
		IPC	Unknown	27.41	
A5	Standard E	Human Quant	Standard	29.15	6.20e-001
		IPC	Unknown	27.51	
A6	Standard F	Human Quant	Standard	30.87	2.10e-001

		IPC	Unknown	27.74	
A7	Standard G	Human Quant	Standard	32.21	6.80e-002
		IPC	Unknown	27.59	
A8	Standard H	Human Quant	Standard	33.59	2.30e-002
		IPC	Unknown	27.48	
A9	QNC	Human Quant	Unknown	Undet.	
		IPC	Unknown	27.34	
A10	4A1	Human Quant	Unknown	25.03	9.65
		IPC	Unknown	28.33	
A11	4A1 (1:20)	Human Quant	Unknown	29.12	5.84e-001
		IPC	Unknown	27.31	
A12	4A2	Human Quant	Unknown	24.83	11.04
		IPC	Unknown	28.40	
B1	4A2 (1:20)	Human Quant	Unknown	29.18	5.64e-001
		IPC	Unknown	27.19	
B2	4A3	Human Quant	Unknown	25.38	7.59
		IPC	Unknown	27.82	
B3	4A3 (1:20)	Human Quant	Unknown	29.75	3.80e-001
		IPC	Unknown	27.22	
B4	4B1	Human Quant	Unknown	25.06	9.44
		IPC	Unknown	27.72	
B5	4B1 (1:20)	Human Quant	Unknown	29.46	4.64e-001
		IPC	Unknown	27.42	
B6	4B2	Human Quant	Unknown	25.08	9.29
		IPC	Unknown	27.80	
B7	4B2 (1:20)	Human Quant	Unknown	29.18	5.62e-001
		IPC	Unknown	27.38	
B8	4B3	Human Quant	Unknown	24.95	10.16
		IPC	Unknown	27.60	
B9	4B3 (1:20)	Human Quant	Unknown	29.31	5.15e-001
		IPC	Unknown	27.26	
B10	4C1	Human Quant	Unknown	25.36	7.68
		IPC	Unknown	27.25	
B11	4C1 (1:20)	Human Quant	Unknown	29.58	4.26e-001
		IPC	Unknown	27.19	
B12	4C2	Human Quant	Unknown	25.52	6.89
		IPC	Unknown	27.22	
C1	4C2 (1:20)	Human Quant	Unknown	29.59	4.25e-001
		IPC	Unknown	27.14	
C2	4C3	Human Quant	Unknown	25.51	6.95
		IPC	Unknown	27.04	
C3	4C3 (1:20)	Human Quant	Unknown	30.02	3.17e-001
		IPC	Unknown	27.23	
C4	4D1	Human Quant	Unknown	25.60	6.52
		IPC	Unknown	27.22	
C5	4D1 (1:20)	Human Quant	Unknown	29.80	3.69e-001
		IPC	Unknown	27.32	
C6	4D2	Human Quant	Unknown	25.80	5.66
		IPC	Unknown	27.48	
C7	4D2 (1:20)	Human Quant	Unknown	30.09	3.02e-001
		IPC	Unknown	27.41	
C8	4D3	Human Quant	Unknown	25.64	6.34
		IPC	Unknown	27.39	
C9	4D3 (1:20)	Human Quant	Unknown	29.86	3.53e-001
		IPC	Unknown	27.23	
C10	4E1	Human Quant	Unknown	25.47	7.10
		IPC	Unknown	27.16	

C11	4E1 (1:20)	Human Quant	Unknown	29.62	4.17e-001
		IPC	Unknown	27.05	
C12	4E2	Human Quant	Unknown	25.44	7.28
		IPC	Unknown	27.14	
D1	4E2 (1:20)	Human Quant	Unknown	29.61	4.19e-001
		IPC	Unknown	27.12	
D2	4E3	Human Quant	Unknown	25.48	7.07
		IPC	Unknown	27.03	
D3	4E3 (1:20)	Human Quant	Unknown	29.82	3.63e-001
		IPC	Unknown	27.19	
D4	4F1	Human Quant	Unknown	26.54	3.43
		IPC	Unknown	27.52	
D5	4F1 (1:10)	Human Quant	Unknown	29.82	3.62e-001
		IPC	Unknown	27.27	
D6	4F2	Human Quant	Unknown	26.63	3.23
		IPC	Unknown	27.96	
D7	4F2 (1:10)	Human Quant	Unknown	30.15	2.89e-001
		IPC	Unknown	27.47	
D8	4F3	Human Quant	Unknown	26.78	2.90
		IPC	Unknown	27.72	
D9	4F3 (1:10)	Human Quant	Unknown	30.15	2.89e-001
		IPC	Unknown	27.30	
D10	4G1	Human Quant	Unknown	26.80	2.86
		IPC	Unknown	27.08	
D11	4G1 (1:10)	Human Quant	Unknown	30.15	2.89e-001
		IPC	Unknown	27.14	
D12	4G2	Human Quant	Unknown	26.75	2.97
		IPC	Unknown	27.17	
E1	4G2 (1:10)	Human Quant	Unknown	30.22	2.76e-001
		IPC	Unknown	27.15	
E2	4G3	Human Quant	Unknown	26.61	3.25
		IPC	Unknown	27.09	
E3	4G3 (1:10)	Human Quant	Unknown	30.11	2.98e-001
		IPC	Unknown	27.15	
E4	4H1	Human Quant	Unknown	27.11	2.32
		IPC	Unknown	27.20	
E5	4H1 (1:10)	Human Quant	Unknown	30.36	2.51e-001
		IPC	Unknown	27.29	
E6	4H2	Human Quant	Unknown	27.13	2.28
		IPC	Unknown	27.28	
E7	4H2 (1:10)	Human Quant	Unknown	30.40	2.43e-001
		IPC	Unknown	27.40	
E8	4H3	Human Quant	Unknown	27.07	2.38
		IPC	Unknown	27.26	
E9	4H3 (1:10)	Human Quant	Unknown	30.27	2.67e-001
		IPC	Unknown	27.22	
E10	4I1	Human Quant	Unknown	27.50	1.77
		IPC	Unknown	27.06	
E11	4I1 (1:10)	Human Quant	Unknown	30.95	1.67e-001
		IPC	Unknown	27.19	
E12	RB4A 111114	Human Quant	Unknown	Undet.	
		IPC	Unknown	27.74	
F1	4I2	Human Quant	Unknown	27.43	1.86
		IPC	Unknown	27.09	
F2	4I2 (1:10)	Human Quant	Unknown	30.93	1.70e-001
		IPC	Unknown	27.15	
F3	4I3	Human Quant	Unknown	27.49	1.79

		IPC	Unknown	27.09	
F4	4I3 (1:10)	Human Quant	Unknown	31.07	1.54e-001
		IPC	Unknown	27.22	
F5	4J1	Human Quant	Unknown	27.51	1.76
		IPC	Unknown	27.31	
F6	4J1 (1:10)	Human Quant	Unknown	30.70	1.99e-001
		IPC	Unknown	27.34	
F7	4J2	Human Quant	Unknown	27.58	1.68
		IPC	Unknown	27.38	
F8	4J2 (1:10)	Human Quant	Unknown	30.88	1.76e-001
		IPC	Unknown	27.30	
F9	4J3	Human Quant	Unknown	27.41	1.89
		IPC	Unknown	27.11	
F10	4J3 (1:10)	Human Quant	Unknown	30.72	1.96e-001
		IPC	Unknown	27.16	
F11	4K1	Human Quant	Unknown	28.49	9.03e-001
		IPC	Unknown	27.30	
F12	4K2	Human Quant	Unknown	29.03	6.21e-001
		IPC	Unknown	27.38	
G1	4K3	Human Quant	Unknown	28.63	8.19e-001
		IPC	Unknown	27.53	
G2	4L1	Human Quant	Unknown	28.72	7.70e-001
		IPC	Unknown	27.21	
G3	4L2	Human Quant	Unknown	28.86	6.99e-001
		IPC	Unknown	27.26	
G4	4L3	Human Quant	Unknown	28.93	6.68e-001
		IPC	Unknown	27.28	
G5	4M1	Human Quant	Unknown	28.91	6.78e-001
		IPC	Unknown	27.32	
G6	4M2	Human Quant	Unknown	29.09	5.99e-001
		IPC	Unknown	27.40	
G7	4M3	Human Quant	Unknown	29.14	5.79e-001
		IPC	Unknown	27.44	
G8	4N1	Human Quant	Unknown	29.20	5.55e-001
		IPC	Unknown	27.22	
G9	4N2	Human Quant	Unknown	29.39	4.86e-001
		IPC	Unknown	27.23	
G10	4N3	Human Quant	Unknown	29.44	4.71e-001
		IPC	Unknown	27.13	
G11	4O1	Human Quant	Unknown	29.41	4.79e-001
		IPC	Unknown	27.11	
G12	4O2	Human Quant	Unknown	29.34	5.05e-001
		IPC	Unknown	27.10	
H1	4O3	Human Quant	Unknown	29.38	4.91e-001
		IPC	Unknown	27.20	
H2	4P1	Human Quant	Unknown	32.46	5.96e-002
		IPC	Unknown	27.62	
H3	4P2	Human Quant	Unknown	32.38	6.27e-002
		IPC	Unknown	27.54	
H4	4P3	Human Quant	Unknown	32.08	7.71e-002
		IPC	Unknown	27.67	
H5	4Q1	Human Quant	Unknown	33.28	3.41e-002
		IPC	Unknown	27.43	
H6	4Q2	Human Quant	Unknown	32.99	4.14e-002
		IPC	Unknown	27.44	
H7	RB4B 111114	Human Quant	Unknown	Undet.	
		IPC	Unknown	28.42	

File:	Quant Data_Resolve 2_111214
Print Date:	Friday, March 13, 2015 11:53:18
User:	Allison.Taylor
Plate Type:	Absolute Quantification
PCR Volume:	25 µL

Document Information

Operator:	ART
Run Date:	Wednesday, November 12, 2014 12:40:48
Last Modified:	Thursday, November 13, 2014 09:58:20
Instrument Type:	Applied Biosystems 7500 Real-Time PCR System

Comments

Comments:
ART Thesis_Resolve 2_111214

Thermal Profile

Stage	Temperature	Time	Repeat	Ramp Rate	Auto Increment
1	95.0 °C	10:00	1	Auto	
2	95.0 °C	0:15	40	Auto	
	60.0 °C	1:00		Auto	

9600 Mode Emulation
Data Collection : Stage 2, Step 2

Analysis Methods:

Delta Rn

Detector Information

Detector Name	Reporter	Quencher	Threshold	Baseline Start	Baseline End
Human Quant	FAM	(none)	0.200000	3	15
IPC	VIC	(none)	0.200000	3	15

Well	Sample Name	Detector	Task	Ct	Qty
A1	Standard A	Human Quant	Standard	22.38	50.00
		IPC	Unknown	28.72	
A2	Standard B	Human Quant	Standard	24.13	16.70
		IPC	Unknown	27.19	
A3	Standard C	Human Quant	Standard	25.94	5.56
		IPC	Unknown	27.14	
A4	Standard D	Human Quant	Standard	27.48	1.85
		IPC	Unknown	27.27	
A5	Standard E	Human Quant	Standard	29.08	6.20e-001
		IPC	Unknown	27.32	
A6	Standard F	Human Quant	Standard	30.87	2.10e-001

		IPC	Unknown	27.38	
A7	Standard G	Human Quant	Standard	32.34	6.80e-002
		IPC	Unknown	27.50	
A8	Standard H	Human Quant	Standard	33.18	2.30e-002
		IPC	Unknown	27.34	
A9	QNC	Human Quant	Unknown	Undet.	
		IPC	Unknown	27.20	
A10	4Q3	Human Quant	Unknown	32.03	7.42e-002
		IPC	Unknown	27.18	
A11	4R1	Human Quant	Unknown	31.89	8.18e-002
		IPC	Unknown	27.12	
A12	4R2	Human Quant	Unknown	31.81	8.64e-002
		IPC	Unknown	27.20	
B1	4R3	Human Quant	Unknown	31.94	7.89e-002
		IPC	Unknown	27.09	
B2	4S1	Human Quant	Unknown	32.20	6.56e-002
		IPC	Unknown	27.11	
B3	4S2	Human Quant	Unknown	32.13	6.90e-002
		IPC	Unknown	27.17	
B4	4S3	Human Quant	Unknown	32.11	6.99e-002
		IPC	Unknown	27.27	
B5	4T1	Human Quant	Unknown	32.39	5.75e-002
		IPC	Unknown	27.29	
B6	4T2	Human Quant	Unknown	32.44	5.57e-002
		IPC	Unknown	27.31	
B7	4T3	Human Quant	Unknown	32.76	4.45e-002
		IPC	Unknown	27.34	
B8	4U1	Human Quant	Unknown	38.11	1.09e-003
		IPC	Unknown	27.38	
B9	4U2	Human Quant	Unknown	35.59	6.29e-003
		IPC	Unknown	27.93	
B10	4U3	Human Quant	Unknown	34.27	1.57e-002
		IPC	Unknown	27.76	
B11	4V1	Human Quant	Unknown	35.52	6.58e-003
		IPC	Unknown	27.11	
B12	4V2	Human Quant	Unknown	34.73	1.14e-002
		IPC	Unknown	27.15	
C1	4V3	Human Quant	Unknown	34.76	1.12e-002
		IPC	Unknown	27.08	
C2	4W1	Human Quant	Unknown	35.17	8.37e-003
		IPC	Unknown	27.07	
C3	4W2	Human Quant	Unknown	34.58	1.27e-002
		IPC	Unknown	27.08	
C4	4W3	Human Quant	Unknown	34.73	1.14e-002
		IPC	Unknown	27.13	
C5	4X1	Human Quant	Unknown	35.53	6.53e-003
		IPC	Unknown	27.23	
C6	4X2	Human Quant	Unknown	35.12	8.71e-003
		IPC	Unknown	27.30	
C7	4X3	Human Quant	Unknown	34.50	1.34e-002
		IPC	Unknown	27.23	
C8	4Y1	Human Quant	Unknown	35.14	8.55e-003
		IPC	Unknown	27.15	
C9	4Y2	Human Quant	Unknown	35.47	6.82e-003
		IPC	Unknown	27.12	
C10	4Y3	Human Quant	Unknown	35.11	8.77e-003
		IPC	Unknown	27.05	

C11	RB4C 111114	Human Quant	Unknown	Undet.	
		IPC	Unknown	27.58	

APPENDIX C: Genetic Profile Quality Scores

Sample Set 1

Sample Name	# of Peaks	# of HZ Loci over 50%	Notes
1A1	28	12	Pull-up in TPOX and D18.
1A2	28	12	
1A3	28	12	
1B1	28	12	OL allele at FGA. 511 RFU.
1B2	28	12	Pull-up in D18.
1B3	28	12	
1C1	28	12	
1C2	28	12	
1C3	28	12	
1D1	28	12	
1D2	28	12	
1D3	28	12	
1E1	27	9	D3: 43.7%, D2: 50.0%, FGA: 22.3 drop out
1E2	25	8	D13: 44.1%, D18: 13.2 drop out, Amel: Y drop out, D5: 14 drop out
1E3	24	9	Amel: X and Y drop out, D5: 12 drop out, FGA: 23 drop out
RB1	0	0	

Sample Set 2

Sample Name	# of Peaks	# of HZ Loci over 50%	Notes
2A1	28	12	Multiple artifacts (pull-up, minus-a, etc.) due to excess DNA.
2A2	28	12	Multiple artifacts (pull-up, minus-a, etc.) due to excess DNA.
2A3	28	12	Multiple artifacts (pull-up, minus-a, etc.) due to excess DNA.
2B1	28	12	Pull-up at FGA.
2B2	28	12	Pull-up at D19, TPOX, D18, and FGA.
2B3	28	12	Pull-up at TPOX, D18, and FGA.
2C1	28	12	Pull-up at FGA.
2C2	28	12	Pull-up at D19, TPOX, D18, and FGA.
2C3	28	12	Pull-up at D19, TPOX, D18, and FGA.

Sample Name	# of Peaks	# of HZ Loci over 50%	Notes
2D1	28	12	Pull-up at D19, TPOX, D18, and FGA.
2D2	28	12	
2D3	28	12	Pull-up at FGA.
2E1	28	12	Pull-up at D19, TPOX, D18, and FGA.
2E2	28	12	Pull-up at D19, vWA, TPOX, D18, and FGA.
2E3	28	12	Pull-up at D19, D18, D5, and FGA.
2F1	0	0	Spike at 285.50 bp in red and blue channels.
2F2	0	0	Spike at 130.92 bp in blue, green, and red channels.
2F3	0	0	
2G1	28	12	
2G2	28	12	Pull-up at FGA.
2G3	28	12	Pull-up at D19, TPOX, D18, and FGA.
2H1	28	12	Pull-up at TPOX.
2H2	28	12	
2H3	28	12	Spike in all channels at 176.91 bp.
2I1	28	12	Pull-up at D19, TPOX, and D18.
2I2	28	12	
2I3	28	12	Pull-up at D19, TPOX, D18, and FGA.
2J1	28	12	Pull-up at D19, vWA, TPOX, D18, and FGA.
2J2	28	12	Pull-up at D19, TPOX, D18, and FGA.
2J3	28	12	Pull-up at D19, TPOX, D18, and FGA.
2K1	0	0	
2K2	0	0	
2K3	0	0	Spike at 205.73.
2L1	0	0	
2L2	0	0	
2L3	0	0	
2M1	1	0	Y allele at Amel only.

Sample Name	# of Peaks	# of HZ Loci over 50%	Notes
2M2	0	0	
2M3	0	0	
2N1	28	12	Pull-up at D19, TPOX, D18, and FGA.
2N2	28	12	Pull-up at D19, TPOX, D18, and FGA.
2N3	28	12	
2O1	28	12	Pull-up at D19, D18, and FGA.
2O2	28	12	Pull-up at D19, TPOX, D18, and FGA.
2O3	28	12	Pull-up at D19 and D18.
2P1	0	0	
2P2	0	0	
2P3	0	0	
2Q1	0	0	
2Q2	0	0	
2Q3	0	0	Spike at ~263 bp in red and yellow channels.
2R1	0	0	
2R2	0	0	
2R3	0	0	
2S1	0	0	
2S2	0	0	Spike at 242.56 bp in blue, green and red channels.
2S3	0	0	
2T1	28	12	Spikes at 192.5 bp in red and ~247.8 bp in blue, green, and red channels.
2T2	28	12	Pull-up at D19 and D18.
2T3	28	12	
2U1	0	0	
2U2	0	0	
2U3	0	0	
2V1	0	0	
2V2	0	0	

Sample Name	# of Peaks	# of HZ Loci over 50%	Notes
2V3	0	0	
2W1	0	0	
2W2	0	0	
2W3	0	0	
2X1	0	0	
2X2	0	0	
2X3	0	0	
2Y1	0	0	
2Y2	0	0	
2Y3	0	0	
RB2A	0	0	
RB2B	0	0	
RB2C	0	0	

Sample Set 3

Sample Name	# of Peaks	# of HZ Loci over 50%	Notes
3A1	28	12	
3A2	28	12	
3A3	28	12	
3B1	28	12	
3B2	28	12	Pull-up at D5.
3B3	28	12	
3C1	28	12	
3C2	28	12	
3C3	28	12	Elevated baseline at Amel.
3D1	28	12	
3D2	28	12	
3D3	28	12	
3E1	28	12	Pull-up at D5.
3E2	28	12	
3E3	28	12	

Sample Name	# of Peaks	# of HZ Loci over 50%	Notes
3F1	28	12	
3F2	28	12	
3F3	28	12	
3G1	28	12	
3G2	28	12	
3G3	28	12	
3H1	28	12	
3H2	28	12	
3H3	28	12	
3I1	28	12	
3I2	28	12	
3I3	28	12	Elevated baseline at D5.
3J1	28	12	
3J2	28	12	
3J3	28	12	
3K1	28	12	Stutter at D3.
3K2	28	12	
3K2A	28	12	Multiple artifacts in various loci due to excess DNA in the sample.
3K3	28	12	
3L1	28	12	
3L2	28	12	Pull-up at D5.
3L3	28	12	
3M1	28	12	
3M2	28	12	
3M3	28	12	
3N1	28	12	
3N2	28	12	
3N3	28	12	
3O1	28	12	Pull-up at D5.
3O2	28	12	
3O3	28	12	
3P1	28	12	
3P2	28	12	
3P3	28	12	
3Q1	28	12	
3Q2	28	12	
3Q3	28	12	
3R1	28	12	

Sample Name	# of Peaks	# of HZ Loci over 50%	Notes
3R2	28	12	
3R3	28	12	
3S1	28	12	
3S2	28	12	
3S3	28	12	
3T1	28	12	
3T2	28	12	
3T3	28	12	
3U1	21	5	D7: 41%, D16: 49%, drop-out at D2, D18, Amel, D5, and FGA.
3U2	26	10	D7: 43%, FGA drop-out
3U3	22	6	D13: 30%, D16: 48%, drop-out at vWA, Amel, D5, and FGA.
3V1	17	5	Drop-out at D7, D2, D19, vWA, D18, D5, and FGA.
3V2	24	8	Drop-out at D3, D18, D5, and FGA.
3V3	18	4	Drop-out at D7, D13, D19, vWA, D18, Amel, D5, and FGA.
3W1	25	9	Drop-out at D7, Amel, and D5.
3W2	23	8	Drop-out at D7, Amel, D5 and FGA.
3W3	26	9	D16: 35%, drop-out at D5 and FGA.
3X1	27	11	D3: 37%, drop-out at D18.
3X2	19	4	D16: 49%, drop-out at D7, D2, vWA, D18, Amel, D5, and FGA.
3X3	21	6	Drop-out at D7, D16, D2, D18, D5, and FGA.
3Y1	22	6	D3: 30%, drop-out at D13, D2, D18, D5, and FGA.
3Y2	21	6	Drop-out at D13, D2, D18, Amel, D5, and FGA.
3Y3	25	7	D3: 49%, D16: 48%, drop-out at D7, D18, and D5.
RB3A	0	0	
RB3B	0	0	
RB3C	0	0	

Sample Set 4

Sample Name	# of Peaks	# of HZ Loci over 50%	Notes
4A1	28	12	
4A2	28	12	
4A3	28	12	
4B1	28	12	
4B2	28	12	Pull-up at TPOX and D5.

Sample Name	# of Peaks	# of HZ Loci over 50%	Notes
4B3	28	12	
4C1	28	12	
4C2	28	12	
4C3	28	12	
4D1	28	12	
4D2	28	12	
4D3	28	12	
4E1	28	12	Pull-up at TPOX, D18, and D5.
4E2	28	12	
4E3	28	12	
4F1	28	12	
4F2	28	12	
4F3	28	12	
4G1	28	12	
4G2	28	12	
4G3	28	12	Pull-up at Amel and D5.
4H1	28	12	Artifact present in multiple channels at ~278 bp.
4H2	28	12	
4H3	28	12	
4I1	28	12	Various artifacts and bad peak shapes due to bad injection.
4I2	28	12	
4I3	28	12	
4J1	28	12	Pull-up at D5.
4J2	28	12	
4J3	28	12	
4K1	28	12	
4K2	28	12	
4K3	28	12	
4L1	28	12	
4L2	28	12	
4L3	28	12	Pull-up at Amel and D5.
4M1	28	12	
4M2	28	12	
4M3	28	12	
4N1	28	12	
4N2	28	12	
4N3	28	12	
4O1	28	12	

Sample Name	# of Peaks	# of HZ Loci over 50%	Notes
4O2	28	12	Pull-up at D18.
4O3	28	12	
4P1	0	0	
4P2	0	0	
4P3	0	0	Incorrect allele called at FGA (353 RFU). Artifact?
4Q1	28	12	Elevated stutter at D3.
4Q2	28	11	D7: 34%
4Q3	28	11	D7: 48%
4R1	28	12	
4R2	28	12	
4R3	28	12	
4S1	28	12	
4S2	28	12	Elevated stutter at D3.
4S3	28	12	
4T1	28	12	
4T2	28	12	
4T3	28	12	
4U1	4	0	Drop-out everywhere except D8 and CSF1PO.
4U2	0	0	
4U3	0	0	Artifact visible in blue and red channels.
4V1	19	7	Drop-out at D7, D13, D2, D18, and FGA
4V2	17	6	Drop-out at D7, D13, D18, Amel, D5 and FGA
4V3	19	7	Drop-out at D7, D13, D18, D5, and FGA.
4W1	28	12	
4W2	28	12	
4W3	28	10	D18: 43%, FGA: 49%
4X1	28	10	D16: 47%, D18: 48%
4X2	28	10	D13: 44%, FGA: 40%
4X3	28	12	
4Y1	28	12	
4Y2	28	12	
4Y3	28	10	TH01: 42%, D19: 43%
RB4A	0	0	
RB4B	0	0	
RB4C	0	0	