Investigating the effect of NucleoSpin® Forensic Filters on DNA recovery from swabs

by

ALINA NDAHAFA HITEWA

HTWALI001

SUBMITTED TO THE UNIVERSITY OF CAPE TOWN

In partial fulfilment of the requirements for the degree

M.Phil. (Biomedical Forensic Science)

Division of Forensic Medicine and Toxicology

Faculty of Health Sciences

UNIVERSITY OF CAPE TOWN

Date of Submission: **10 February 2020**

Supervisor: **Dr Laura Heathfield**
Co-Supervisor(s): **Dr Andrea Gibbon**
Mr Calvin Mole

DECLARATION

I, Alina Ndahafa Hitewa, hereby declare that the work on which this dissertation is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being or is to be submitted for another degree in this or any other university. I authorise the university to reproduce for the purpose of research either the whole or any portion of the content.

This thesis/dissertation has been submitted to the Turnitin module (or equivalent similarity and originality checking software) and I confirm that my supervisor has seen my report and any concerns revealed by such have been resolved with my supervisors in any manner whatsoever.

Name: Alina Ndahafa Hitewa

Student number: HTWALI001

Signature: Signed by candidate

Date: 10 February 2020

ABSTRACT

The burden of unresolved crime in South Africa highlights the need to improve methods of identifying perpetrators of crimes. One globally accepted method for human identification is forensic DNA profiling. Since trace evidence is often retrieved in small amounts, the optimal recovery of DNA from these samples is crucial. Methods for the recovery of touch DNA from swabs typically make use of a spin basket or filter, combined with a centrifugation step, to enhance the release of cells from the swab prior to DNA extraction. The NucleoSpin® Forensic Filter (Macherey-Nagel, Düren) is one such example, but it has not been thoroughly assessed on touch DNA samples. This study aimed to assess if the inclusion of the NucleoSpin® Forensic Filter significantly improved DNA recovery and DNA profiling success from cotton and flocked swabs used to collect touch DNA and buccal cells (control). Buccal cells and touch DNA samples were collected from 25 volunteers using each swab type (cotton and flocked) in duplicate. DNA was extracted from the samples using the NucleoSpin® DNA Forensic kit, one set with, and the other set without, NucleoSpin® Forensic Filters. DNA concentration was assessed using Qubit™ fluorometry and qPCR, and DNA profiling was done using the PowerPlex® ESX 16 system. The inclusion of the NucleoSpin® Forensic Filters significantly improved DNA concentration in buccal cells collected using flocked swabs ($p = 0.035$). However, no significant differences were noted for touch DNA samples, for either swab type. There was also no significant difference in DNA profiling success when NucleoSpin® Forensic Filters were used, regardless of swab and sample type. These results suggest that the NucleoSpin® Forensic Filters should not be included in the DNA extraction workflow, particularly for touch DNA samples. With only 16 % of touch DNA samples yielding full DNA profiles, there is the need to improve DNA recovery. Factors such as swab type and swab preservation buffers, should be investigated in future research.

Acknowledgements

Firstly, I would like to thank the Lord Almighty for giving me strength and courage throughout the research project.

I wish to express my sincere gratitude to my supervisors; Dr Laura Heathfield, Dr Andrea Gibbon and Calvin Mole for their guidance and motivation during the project.

Special appreciation goes to my family for their comfort, support and motivation throughout. I would also like to thank the Division of Forensic Medicine and Toxicology in the Department of Pathology at the Health Sciences Faculty, University of Cape Town for offering me the opportunity to pursue my master's degree.

Finally, I wish to thank the Namibia Students Financial Assistance Fund for funding my research project.

Table of contents

List of tables

List of figures

Abbreviations and symbols

C_t ..... cycle threshold

CE ..... capillary electrophoresis

DNA ..... deoxyribonucleic acid

EPG ..... electropherogram

HREC ..... Human Research Ethics Committee

IPC ..... internal positive control

LCN ..... low copy number

MBG ..... molecular biology grade

NFDD ..... National Forensic DNA Database

ng ..... nanogram(s)

PCR ..... polymerase chain reaction

pg ..... picogram(s)

qPCR ..... real-time polymerase chain reaction

RFLP ..... restriction length polymorphisms

RFU ..... relative fluorescence units

rpm ..... revolutions per minute

SA ..... South Africa

SAPS ..... South African Police Services

SDS ..... sodium dodecyl sulphate

SPSS ..... statistical package for the social sciences

STRs ..... short tandem repeats

VNTRs ..... variable number tandem repeats

XS .....extra small

µL..... microlitre(s)

% .....percentage

˚C..... degree celsius

> .....greater than

< .....less than

*.....asterisk

Chapter 1: Introduction and Literature review

1.1. Background

Over the years, South Africa (SA) has experienced a considerable increase in crime. According to the Afrobarometer survey of 2016 ("Afrobarometer survey", 2016), crime is ranked amongst the most important challenges facing the country, which government needs to address. Africa Check (2018) reported on SA murder rates based on the South African Police Services (SAPS) annual crime report, indicating SA murder rates increased by 6.9 % between 2017 and 2018, with approximately 57 murders a day ("Africa Check", 2018).

When a crime is committed, perpetrators often leave behind traces of their biological evidence which may provide an investigative lead to who was present on the crime scene (Thompson and Black, 2006). Biological evidence in the form of biological samples, is then collected by forensic crime scene investigators and submitted to forensic science laboratories for different analyses. One such analysis is deoxyribonucleic acid (DNA) analysis, which involves the isolation of genetic material (*i.e.* DNA) from the biological samples to generate a 'DNA profile' (Budowle *et al.*, 2005). The resulting DNA profile is compared to DNA profiles generated from reference samples obtained from suspects, using a matching principle. Finding a match between these DNA profiles can ultimately lead to the identification of the perpetrators (Butler, 2015).

DNA is a molecule found in the cells of organisms, which contains the genetic information passed on from parents to offspring (Singh, 2011). In the cells, DNA can either be found in the nucleus (nuclear or genomic DNA) or in the mitochondria (Siegel and Saukko, 2012). DNA can be recovered from a variety of biological samples, including hard or soft tissues from individuals (Beckett *et al.*, 2008, Zgonjanin *et al.*, 2017) as well as skin epithelial cells recovered from surfaces that have been in contact with individuals, which is known as touch DNA (Van Oorschot and Jones, 1997, Martin *et al.*, 2018). Different types of devices are commercially available for the collection and recovery of DNA from biological samples, an example is that of cotton swabs, which are commonly used in the forensic setting (Brownlow *et al.*, 2012, O'Brien *et al.*, 2012, Bruijns *et al.*, 2018). These biological samples are often in small amounts (Irwin *et al.*, 2007) and recovery of DNA from these samples can be challenging.

This literature review will explain the methods used for DNA recovery from forensic samples, particularly from swabs. Emphasis will be on touch DNA samples, as well as buccal samples which are often collected from individuals as reference samples. Touch DNA samples are often compromised and only recovered in trace amounts, hence making the generation of usable profiles from these samples a challenge. The NucleoSpin® Forensic Filter is a relatively new product thought to improve DNA recovery from swabs, it has however not been thoroughly investigated on touch DNA samples. This literature review will also address this product by comparing it to other similar products that have been used in the field.

1.2. Forensic genetics and human identification

Forensic genetics involves the use of DNA in resolving criminal, civil and legalinvestigations (Arenas *et al.*, 2017). It involves the analysis of non-coding regions of DNA to determine familial relationships (particularly in kinship and paternity testing), as well as to convict and exonerate suspects (Butler, 2011). DNA analysis is important because of its ability to connect or dissociate individuals from criminal investigations (Butler, 2015).

Forensic DNA identification is achieved through a process called DNA profiling, which analyses DNA markers to generate a DNA profile (Singh, 2011, Puch-Solis *et al.*, 2013). DNA consists of microsatellite regions with short repeat units, having between two to seven bases (Romsos and Vallone, 2015). These repeat units are called short tandem repeats (STRs) and their order within a DNA sequence is different between individuals (Moretti *et al.*, 2001). A DNA profile thus is a combination of the variations at different non-coding DNA markers located throughout the genome (Carey and Mitnik, 2002).

DNA profiling relies on the ability to find a match between reference samples obtained from suspects or suspected biological relatives and unknown samples (Alonso *et al.*, 2005, Budowle *et al.*, 2005, Prinz *et al.*, 2007). If a match is found, statistical probabilities are calculated to determine the confidence of the match with a high discriminatory power (Parker *et al.*, 2013).

Forensic DNA analysis has become the gold standard for human identification globally (Butler, 2005), as well as locally, in South Africa (Criminal Law (Forensic Procedures) Amendment Act, 2013). DNA profiles generated from forensic DNA analysis are stored on a national DNA database which keeps record of reference DNA profiles generated from unidentified human

remains, missing persons, arrestees or convicted offenders. Storing DNA profiles on a database enables computerised searching and possibly matching of the unknown profiles to reference profiles (Machado and Silva, 2014). In SA, the 'DNA Act' is the regulating legislation which regulates the National Forensic DNA Database (NFDD) (Criminal Law (Forensic Procedures) Amendment Act, 2013).

DNA quantification by real-time polymerase chain reaction (qPCR) has become the standard way of measuring the amount of amplifiable DNA in a biological sample (Pineda *et al.*, 2014). Based on the amount of DNA present, DNA may be diluted and then subjected to a multiplex PCR (Nicklas *et al.*, 2003, Butler *et al.*, 2004). Capillary electrophoresis (CE) is then used to separate PCR products according to size, particularly because different STR alleles will vary in terms of PCR product size (Alaeddini *et al.*, 2010). The data generated by CE is displayed on a software program in the form of an electropherogram (EPG) (Puch-Solis *et al.*, 2013).

STR profiling permits the analysis of very small amounts of DNA (typically 0.5 ng - 1 ng) which are often encountered in forensic casework (Hughes-Stamm *et al.*, 2011). STR analysis becomes particularly important when dealing with compromised forensic samples such as touch DNA samples which are often of low copy number. Low copy number (LCN) DNA refers to the DNA recovered in trace amounts, often with a template value less than 200 pg threshold level (Caddy *et al.*, 2008). However, LCN DNA may refer to any sample thatshows stochastic effects such as allele drop-out, stutter peaks and peak imbalance during analysis (Puch-Solis *et al.*, 2009, Singh, 2011).

In addition to LCN DNA, forensic casework samples may also be degraded through exposure of samples to extreme environmental conditions. This results in cell rupture (caused by physical agents or chemical reactions), which leads to fragmentation of the DNA molecule (Alaeddini *et al.*, 2010, Frumkin *et al.*, 2011). DNA degradation and LCN DNA may introduce problems during downstream PCR amplification, as the DNA is not intact or it is insufficient for analysis (Budowle *et al.*, 2009).

Another problem encountered when working with compromised forensic samples is PCR inhibition, which is a common cause of PCR failure with LCN DNA samples (Alaeddini, 2012). Substances such as heme in blood (Akane *et al.*, 1994) and co-existing non-human DNA, that are present in the biological sample may interfere with the activity of DNA polymerase during PCR, leading to loss of amplification (Alaeddini *et al.*, 2010). Optimising

DNA analysis methods to improve DNA recovery from forensic samples will minimise problems encountered in the downstream processing of samples to generate DNA profiles.

1.3. Biological samples and swabs

Biological evidence left at crime scenes as well as those recovered from individuals contain DNA in their cells (Siegel and Saukko, 2012). These samples are processed using molecular techniques to recover DNA (Rudin and Inman, 2001, Butler, 2011). They exist in a variety of forms, including blood, semen, buccal cells, teeth, bone, fingernails, and epithelial cells shed from the skin onto surfaces, *i.e.* from touch. These are the biological samples commonly submitted to forensic DNA laboratories for DNA profiling (Lee and Ladd, 2001), and the type of samples submitted to these laboratories depend on the purpose the samples will serve *i.e.* in burnt body cases, bone or teeth are collected for DNA extraction, since buccal or blood samples are usually unavailable (Calacal *et al.*, 2015). Furthermore, sample collection also depends on the type of forensic case (Siriboonpiputtana *et al.*, 2018). The amount of DNA recovered from biological samples is greatly affected by the state of the sample (*i.e.* fresh or dry), the technology used for DNA analysis and the type of substrate from which the samples are collected (Brownlow *et al.*, 2012, Hess and Haas, 2017).

1.3.1. Buccal cells

As previously mentioned, forensic DNA profiling involves matching of unknown DNA profiles to reference DNA profiles. Buccal cells are often the sample of choice, collected to generate these reference DNA profiles. This is because the collection of buccal cells is non- invasive, feasible and generally painless (Beckett *et al.*, 2008, Lindstrom, 2012).

The collection of buccal cells is the gold standard for obtaining reference samples from individuals for forensic DNA profiling (Berger *et al.*, 2013). In SA, the collection of buccal cells is governed by the Criminal Law (Forensic Procedures) Amendment Act, 37 of 2013, which provides for trained police officers to collect reference buccal samples fromindividuals who are required to provide these samples. Buccal cell collection thus involves rubbing a swab inside an individual's cheeks for a short period of time, usually around 20 seconds to 30 seconds (Vance, 2005).

1.3.2. Touch DNA

The contact made between an individual and a surface leaves a trace of their biological material (Locard, 1930). The biological material left on a surface during contact is often in the form of skin epithelial cells which may be recovered for DNA analysis to determine the identity of the individual (van Oorschot *et al.*, 2010). The DNA recovered from these skin epithelial cells is known as touch DNA (Williamson, 2012). Touch DNA and LCN DNA have been used interchangeably (Gill *et al.*, 2000, Gill, 2001), however there is a clear distinction between the terms (van Oorschot *et al.*, 2010, Caragine *et al.*, 2013). Touch DNA refers to any sample that originates from epithelial cells from the action of touching, and may fall below recommended thresholds at any stage of the analysis, from collection to interpretation (van Oorschot *et al.*, 2010). In contrast, LCN DNA specifically refers to samples that contain small amounts of starting DNA, independent of their biological origin (Caragine *et al.*, 2013). Hence, touch DNA samples may also be LCN DNA samples, *i.e.* touch DNA is typically a form of LCN DNA.

Touch DNA is often below normal detection limits during analysis, usually with a DNA concentration of 100 pg/µL (0.1 ng/µL) or less (van Oorschot *et al.*, 2010). For this reason, forensic DNA laboratories seek to optimise touch DNA typing methods to improve the amount of DNA recovered from touch DNA samples (Lowe *et al.*, 2002, Butler, 2005).

To date, studies have obtained sufficient DNA yields from touch samples for DNA profiling (Phipps and Petricevic, 2007, Thomasma and Foran, 2013, Templeton *et al.*, 2015, Phetpeng *et al.*, 2015). The lowest DNA concentration to yield full DNA profiles was reported by van Oorschot *et al.* (2010) to be 0.025 ng/µL. This is in line with the 0.03 ng/µL suggested by Daly *et al.* (2012) as the cut-off DNA concentration for generating full DNA profiles.

1.3.2.1. Collection method

There are different methods used to recover touch DNA from handled items, which have been shown to affect the amount of usable DNA recovered for analysis (Stouder *et al.*, 2001, Pang and Cheung, 2007, Hess and Haas, 2017). One such method is swabbing, which involves rubbing and rotating the swab head onto a touched surface to recover the deposited sample (Sweet *et al.*, 1997, Pang and Cheung, 2007). Traditionally, studies have focused on the use of a single wet cotton swab for touch DNA collection (Lowe *et al.*, 2002, Carey and Mitnik, 2002),

although Van Oorschot and Jones (1997), Van Hoofstat *et al.* (1999) and Ladd *et al.* (1999) have previously found the amount of touch DNA collected by a single moistened cotton swab to be less than half of the deposited sample.

To improve touch DNA recovery, many studies have utilised two cotton swabs; rubbing the surface with a moistened swab, followed by a second dry swab (Van Oorschot *et al.*, 2003, Pang and Cheung, 2007, Castella *et al.*, 2006). These studies also found the second dry swab to recover more DNA than the initial moistened swab. This could be due to improved sample attachment onto the dry swab caused by hydration of the sample by the initial moistened swab (Pang and Cheung, 2007).

Sterile water is commonly used to moisten swabs prior to touch DNA collection (Wickenheiser, 2002, Pang and Cheung, 2007, Verdon *et al.*, 2014). However, using water as a swabmoistening agent may not recover much of the touch DNA deposited, due to no interaction between water and sample components (van Oorschot *et al.*, 2010). Although only a few studies have investigated swab moistening agents, they suggest that using detergents to moisten swabsmay improve sample recovery. For example, Thomasma and Foran (2013) compared different detergents and water as swabbing solutions and found that all detergents analysed outperformed water. This could be due to the amphiphilic nature of detergents, enabling them to solubilise cellular components (Templeton *et al.*, 2015), and because detergents improve cell lysis during DNA extraction (Norris *et al.*, 2007, Opel *et al.*, 2010).

1.3.2.2. Swab type

Previous studies into the comparison of the efficacy of different swab types in touch DNA recovery from surfaces reported that the design of the swab greatly affects DNA recovery (Templeton *et al.*, 2015, Verdon *et al.*, 2014). Although different swab types exist, only a few have been thoroughly assessed to determine their ability to collect touch DNA from different surfaces. These include cotton, nylon flocked and foam swabs (Phetpeng *et al.*, 2015).

Cotton swabs have heads with a mattress design that are glued and tightly wound onto the wooden shaft. These fibres are absorbent and easy to moisten, allowing for fast and easy processing (Bruijns *et al.*, 2018). The cotton swab fibres may, however, take a long time to dry after sample collection and they often detach from the shaft to accumulate in the sample mixture. This accumulation introduces problems such as PCR inhibition during downstream processing (Voorhees *et al.*, 2006, Brownlow *et al.*, 2012). Concomitantly, the design of the

cotton swab allows entrapment of a portion of the collected sample within the swab fibres, which potentially reduces sample release from the swab (Benschop *et al.*, 2010).

Flocked swabs on the other hand have hydrophilic fibres extending outward from a plastic shaft (Plaza *et al.*, 2016). These swabs provide an increased surface area of fibres to accommodate for maximal sample collection (Benschop *et al.*, 2010). Flocked swabs lack an absorbent inner core which facilitates sample entrapment, and for this reason, the manufacturer (COPAN, Brescia) claims that the swabs are effective at releasing samples, which may improve DNA recovery from the sample. In contrast, foam swabs have fibres that are not closely packed together but tightly wound to the shaft (Plaza *et al.*, 2016, Bruijns *et al.*, 2018).

Different studies have investigated the three swab types described above by assessing their ability to collect and release touch DNA from various surfaces. Hansson *et al.* (2009) compared cotton, flocked and foam swabs for touch DNA collection from absorbent and non-absorbent surfaces. They did not find significant differences in touch DNA recovery from absorbent surfaces. However, the foam swab outperformed cotton and flocked swabs in collecting touch DNA from non-absorbent surfaces, whereby the flocked swab performed the poorest.

Verdon *et al.* (2014) and Phetpeng *et al.* (2015) compared different swab types for touch DNA collection from different surfaces. Their results were similar to the findings of Hansson *et al.* (2009) in that nylon flocked swabs performed the poorest with all types of surfaces. The poor performance of flocked swabs could be due to the low absorbency of the swab, which makes moistening of the swab difficult (Phetpeng *et al.*, 2015), thus often requiring a larger volume of the swab moistening agent which dilutes the sample. Foam swabs yielded the highest DNA concentrations from porous surfaces *i.e.* wood (Verdon *et al.*, 2014), whereas cotton swabs yielded optimal results with nonporous surfaces (Phetpeng *et al.*, 2015).

Another study by Templeton *et al.* (2015) which also investigated the performance of cotton, flocked and foam swabs in touch DNA retrieval and release from plastic slides found contrasting results in that flocked swabs performed the best. Their results have shown that flocked swabs recovered considerable yields of DNA, generating full DNA profiles. It is noteworthy mentioning the difference in DNA analysis methods used by these studies, which may provide a reason for the optimal DNA recovery from flocked swabs. The studies described above have extracted DNA from the swabs prior to quantification, whereas Templeton *et al.* (2015) utilised the direct PCR method. Based on their results, Templeton *et al.* (2015) have suggested the use of direct PCR in touch DNA analysis.

While flocked swabs may not be good at touch DNA recovery and release, they may perform better with samples containing high amounts of starting DNA. Benschop *et al.* (2010) assessed the efficacy of cotton and flocked swabs in vaginal sampling. Their results showed that flocked swabs recovered approximately five to ten times more cells than cotton swabs. Moreover, flocked swabs were better at releasing vaginal cells during processing, with only 9 % of the collected sample retained on the swab when compared to cotton swabs which retained 30 % of the sample collected. These results correspond with findings of Dadhania *et al.* (2013) who found flocked swabs to perform better than cotton swabs at collecting bacteria and blood cells.

The contrasting results from these studies show that there are various factors that affect swab performance, including the type of sample collected. Overall, the studies show that foam swabs may perform better than cotton and flocked swabs at collecting touch DNA. In contrast, flocked swabs are more effective than cotton and foam swabs in sample collection and releasing when processing samples with large amounts of starting DNA, such as blood.

1.4. DNA analysis and assessment

1.4.1. DNA recovery from swabs

Collected samples should ideally be extracted from the swab as soon as possible after collection to minimise the chance of DNA fragmentation within the sample (Raymond *et al.*, 2009, Ottoni *et al.*, 2017). The storage method and conditions under which the swab is stored before DNA extraction greatly affects the amount of usable DNA recovered (Voorhees *et al.*, 2006, Brownlow *et al.*, 2012). Swabs may be air dried and stored in the freezer or they may be stored in preservation buffers after sample collection, which allows for long term storage (Beckett *et al.*, 2008). The swab preservation methods stabilise DNA for long periods of time, enabling future analyses to be carried out on the samples (Karlsson *et al.*, 2013).

Similarly, the method used to process swabs prior to DNA analysis may influence the amount of recovered DNA. Brownlow *et al.* (2012) claims that removing the swab fibres from the shaft before DNA extraction recovers higher amounts of DNA than processing the swab head with the shaft attached. The authors proposed that by removing the swab fibres from the shaft, a

greater surface area was obtained, which potentially aided the process of cell lysis by the components of the extraction buffers.

Studies have shown significant sample retention (over 50 %) onto the swab during processing (Windram *et al.*, 2005, Adamowicz *et al.*, 2014), indicating the need for improved sample release. Attempts to improve sample release from swabs have focused on utilising enzymes that fully digest swab fibres to increase the surface area, making cells in the sample more accessible during extraction (Voorhees *et al.*, 2006, Norris *et al.*, 2007). Benschop *et al.* (2010) and Dadhania *et al.* (2013) have suggested the use of swabs that are designed to allow for high sample release during processing such as nylon flocked swabs.

Many studies have looked into optimising DNA recovery from swabs during DNA extraction by targeting different stages of the extraction process. Nori *et al.* (2015) argued for the use of short incubation times during DNA extraction, whereas van Oorschot *et al.* (2010) suggested the elution of DNA in smaller volumes during DNA extraction. Adamowicz *et al.* (2014) investigated a swab re-suspension method. They removed the swabs from the extraction buffer and placed them into spin baskets suspended in liquid buccal cell suspensions every 20 minutes of the one-hour incubation. Their results showed a two-fold increase in DNA recovery.

Filter devices may improve DNA extraction due to high template binding onto the membrane filter during processing, as well as allowing for low elution volumes needed in forensic casework. However, only a few studies (Schiffner *et al.*, 2005, Hudlow *et al.*, 2011) have investigated the use of filter devices to clean up and concentrate DNA samples. Schiffner *et al.* (2005) assessed the efficacy of Microcon® 100 filter devices (Millipore, MA) in concentrating LCN DNA samples. They reported a significant increase in DNA recovery of LCN DNA samples utilising the filter device.

A study by Hudlow *et al.* (2011) evaluated the efficacy of the NucleoSpin® XS centrifugation filter (Macherey-Nagel, Düren) in the clean-up and concentration of phenol-chloroform extracted DNA from blood, saliva and semen. Their results showed improved DNA recovery, with 100 % detection of all expected alleles in the DNA profiling step. It should however be noted that these filter devices are post extraction filters that are used during the DNA elution step, for concentrating and purifying the recovered DNA.

NucleoSpin® Forensic Filters, however, are used to improve removal of cells from swabs during a centrifugation step during the first stage in DNA extraction. The manufacturerclaims that assessment of the filter has shown up to 60 % increase in DNA recovery from swabs, when

compared to their standard DNA extraction methods. This increase in DNA recovery resulted from the improved release of cells from substrates (*i.e.* swabs), during DNA a centrifugation step in the DNA extraction workflow. To date there are no known studies that have evaluated these NucleoSpin® Forensic Filters.

Although the NucleoSpin® Forensic Filter has not been investigated in depth, there are similar products from other companies that have been used in the field. One example is the Investigator® Lyse & Spin Basket from Qiagen (Hilden, Germany). In a validation study, Cole (2019) investigated the performance of the Investigator® Lyse & spin basket (Qiagen, Hilden) in DNA recovery from blood, saliva and touch DNA. They compared the Investigator® Lyse & spin basket to the basket validated for use in their laboratory. They did not find significant differences in DNA recovery from touch DNA samples, however the Investigator® Lyse & spin basket performed the poorest in DNA recovery from blood and saliva samples. The author attributes this poor performance to the design of the basket; having small openings and a membrane over the openings which retain a portion of the sample during processing.

A study by Ambers *et al.* (2018) incorporated Nucleic Acid Optimiser (NAO®) spin baskets (Copan, Brescia) in their DNA extraction protocol to extract DNA from blood and saliva samples and found no improvement in DNA recovery. Similarly, Adamowicz *et al.* (2014) obtained low DNA yields from buccal samples extracted using DNA IQ™ spin baskets (Promega, Madison).

Overall, these studies did not show improved DNA recovery from samples analysed. This necessitates the need to investigate NucleoSpin® Forensic Filters, to assess whether DNA recovery from touch surfaces using these filters can be improved.

1.5. Rationale

The SA crime rates increase on a day to day basis. One of the key investigative tools in forensic casework is DNA analysis, to aid in the identification of perpetrators. Due to the nature of forensic casework, evidentiary samples are often in trace amounts and DNA recovered from these samples is not always sufficient for downstream analyses.

It is thus imperative to optimise DNA extraction methods to improve DNA recovery from forensic samples. This is particularly important because LCN DNA is commonly encountered with touch DNA samples, which often leads to partial DNA profiles. The NucleoSpin® Forensic Filter is a relatively new product which has claimed to improve DNA recovery, however, this product has not been thoroughly studied.

If DNA recovery is optimised, there are greater chances that better quality DNA profiles will be generated and loaded onto the NFDD. Better quality profiles on the NFDD will improve the matching potential for reference profiles, thereby enabling identification of individuals who were on crime scenes. This is an important response to the high crime rates in SA and in the identification of potential suspects.

1.6. Aims and objectives

1.6.1. Aim

The aim of this study is to evaluate the effect of NucleoSpin® Forensic Filters on touch DNA recovery from cotton and flocked swabs.

1.6.2. Objectives

- To collect buccal cells (controls) and touch DNA from individuals using cotton and flocked swabs.
- To extract DNA from these cotton and flocked swabs with and without NucleoSpin® Forensic Filters.
- To compare the quantity of DNA obtained when NucleoSpin® Forensic Filters were used and not used, for the different sample types and swab types.

1.7. Hypothesis

Literature has shown that the design of the flocked swab enables high sample recovery and release from the swab. Flocked swabs seem to release cells, better than cotton swabs, the latter retaining a portion of the collected sample within the swab fibres. Due to the theoretical better release of cells from flocked swabs, a considerable amount of the sample should be released by the swab without the aid of the filter. It is therefore hypothesised that the use of filters will not significantly increase DNA recovery from flocked swabs. However, we hypothesise that NucleoSpin® Forensic Filters will increase DNA recovery from cotton swabs. These hypotheses are independent of sample type.

Chapter 2: Materials and Methods

2.1. Study design

A prospective and experimental study was conducted to evaluate the effect of NucleoSpin® Forensic Filters (Macherey-Nagel, Düren) on DNA extraction yield from cotton and flocked swabs. This was achieved by assessing DNA concentration and purity (dependent variables), from two swab types (cotton and flocked swabs) from two sample types (buccal cells and touch DNA), when extracted with and without NucleoSpin® Forensic Filters (independent variables). The study received ethical approval, following review by the Human Research Ethics Committee of the University of Cape Town (HREC REF: 312/2019) (Appendix A).

2.2. Cohort

2.2.1. Study population

A total of 25 individuals from the Faculty of Health Sciences, University of Cape Town were recruited for the current study following voluntary participation, for which informed consent was given (Appendix B). All participants recruited in the study were 18 years or older at the time of sample collection.

2.3. Sample collection

Buccal cells, through the process of swabbing, were collected from 25 volunteers. Participants rinsed their mouths using tap water and refrained from eating and drinking for 30 minutes prior to providing a buccal sample. A total of four swabs; two cotton (Copan, Brescia) and two flocked (FLOQSwabs™, Copan Flock Technologies, Brescia) were used to collect buccal cells from each individual (Table 2.1). The collection procedure involved rubbing a dry swab against the inside of the individual's cheek for 30 seconds. The swabs were subsequently left to air dry

before being re-packaged into the swab tube. The participants collected their own buccal samples in a private space.

Touch DNA was also collected from the participants. This process involved swabbing a pre-cleaned bench surface for the presence of DNA left as a result of touching. Specifically, a laboratory bench was cleaned with 5 % bleach, followed by distilled water and lastly, 70 % ethanol. The participants were asked to rinse their hands with water and allow them to completely air dry to remove exogenous DNA. The participants were then instructed to rub their hands together for one minute and subsequently touch a designated area of the cleaned bench surface with back and forth motion and downward pressure. Four swabs (two cotton and two flocked) were each moistened with 2 µL of 2 % sodium dodecyl sulphate (SDS) (Sigma-Aldrich, St. Louis) and then used to swab different areas of the touched surface. This was done for each of the 25 participants (Table 2.1). The swabs were allowed to air-dry and then were placed back into the labelled swab tubes. The samples were assigned a unique alpha-numeric code to maintain confidentiality.

Table 2.1. Table showing the independent variables and how many swabs were collected from each of the eight combination of variables.

Sample type	**Swab type**	**DNA extraction without filter**	**DNA extraction with filter**	**Total**
Touch DNA	*Cotton swab*	n = 25	n = 25	n = 50
	Flocked swab	n = 25	n = 25	n = 50
Buccal cells (controls)	*Cotton swab*	n = 25	n = 25	n = 50
	Flocked swab	n = 25	n = 25	n = 50
Total		n = 100	n = 100	n = 200

2.4. Sample processing

2.4.1. DNA extraction

DNA was extracted from all swabs (n = 200) immediately after sample collection. The cotton swab fibres were completely removed from the wooden shafts as described by (Brownlow *et al.*, 2012) using a new, sterile scalpel blade (Hi-Care, Johannesburg) and inserted into 1.5 mL microcentrifuge tubes. The flocked swab heads were snapped off their shafts and were also placed into 1.5 mL microcentrifuge tubes. NucleoSpin® Forensic Filters were placed in tubes corresponding to one cotton and one flocked swab for every set of swabs from an individual.

DNA extraction was performed according to the procedures outlined in the NucleoSpin® DNA Forensic Kit user manual (Macherey-Nagel, Düren). Centrifugation steps occurred in the 5417C centrifuge (Eppendorf, Hamburg). For the incubation step, tubes were securely sealed with parafilm and incubated with shaking in a Thermomixer F2.0 (Eppendorf, Hamburg) for two hours at 56 º C and 500 rpm. DNA was eluted into 35 µL of FOE elution buffer (5 mM Tris/HCL, pH 8.5) which was preheated to 70 º C. The elution step was repeated with the same NucleoSpin® DNA Forensic Column using another 35 µL of the FOE elution buffer, resulting in two DNA eluates of 35 µL each per sample. The DNA samples were stored at 4 º C for the duration of the study.

2.4.2. DNA quantification

2.4.2.1. Qubit™ fluorometry

To assess the quantity of double-stranded DNA, samples were quantified using the Qubit™ 4 Fluorometer (Thermo Fisher Scientific, Waltham), and the accompanying Qubit™ dsDNA High Sensitivity (range: 0.01 ng/µL – 10 ng/µL) and Broad Range (range: 0.1 ng/µL – 100 ng/µL) Assay Kits (Invitrogen, Thermo Fisher Scientific, Waltham). DNA quantification using this system was performed in accordance with the protocol outlined in the Qubit™ 4 Fluorometer user manual (Thermo Fisher Scientific, Waltham) and the appropriate Qubit™ standards were used.

2.4.2.2. Real-time PCR

The 7500 Real-Time PCR instrument (Applied Biosystems, Foster) was used along with the Quantifiler® Human DNA Quantification Kit (Applied Biosystems, Foster) to quantify the amount of human DNA present in extracted DNA samples. A total of 2 µL of each sample and standard was quantified. If samples were > 50 ng/µL according to Qubit™ measurements, they were diluted with molecular biology grade (MBG) water (Lonza, Basel) prior to qPCR, in order to fit within the range of the qPCR standard curve (0.023 ng/µL – 50 ng/µL).

The Quantifiler® Human DNA Standard (200 ng/µL) (Applied Biosystems, Foster) was diluted in duplicate using a serial dilution of three, using MBG water (Lonza, Basel). The eight dilutions of known concentration (in duplicate) were used to generate a standard curve from which DNA concentrations were determined using the cycle threshold (C_t) values. A kit-specific internal PCR control (IPC) was included to assess PCR inhibition by determining whether the sample IPC C_t values fell within the range established by the manufacturer. A no template control (MBG water) was also included in the qPCR experiment.

2.4.3. DNA profiling

Touch DNA samples which had DNA concentrations greater than 0.000 ng/µL (as established by qPCR) were selected for DNA profiling. One sample from the buccal swabs per individual was also included for DNA profiling, which was used as a control to assess accordance of alleles from touch DNA samples. DNA (0.5 ng, or as much as possible up to 0.5 ng) was subjected to multiplex PCR on the T100™ Thermal Cycler (Bio-Rad, Hercules) using the PowerPlex® ESX 16 kit (Promega, Madison) which amplifies fifteen STR loci plus amelogenin.

The post-PCR products were then separated by capillary electrophoresis on the 3130*xl* Genetic Analyser (Applied Biosystems, Foster) coupled with the Gene Mapper 4.1 software (Applied Biosystems, Foster). The assay was performed according to the PowerPlex® ESX 16 kit user manual (Promega, Madison) and a 50 cm array POP-7 was used for the analysis. The capillary electrophoresis run was set for 1 800 seconds with an injection time of 16 seconds and injection voltage of 15.0 V. DNA profile success was assessed by counting the number of peaks that met

the analytical threshold (50 RFU). Samples were stored at - 20 °C after all laboratory analyses had been completed, in accordance with the internal standard operating procedure.

2.5. Data and statistical analysis

Statistical analysis was performed on the results to assess significant differences in DNA quality and quantity between different groups. The groups analysed were as follows: (i) sampletype (buccal versus touch) (ii) swab type (cotton versus flocked), and (iii) presence orabsence of filters. Shapiro Wilk tests were performed to determine normal distribution of the data, whereas the Wilcoxon sum rank test was performed to determine significant differences between the groups analysed. Furthermore, the Spearman's rank correlation coefficient was used to assess correlation between DNA concentration and DNA profiling success. All statistical analyses were performed using IBM SPSS version 25 (SPSS Inc., Chicago) and GraphPad Prism 8.3.1 (GraphPad Inc., San Diego). A *p*-value less than 0.05 was considered statistically significant.

Chapter 3: Results

3.1. Assessment of DNA quantity

Each of the 200 DNA samples was assessed in terms of concentration, using fluorometry and qPCR. In addition, DNA profiling success was measured in touch DNA samples where DNA was detectable during qPCR (n = 77). A full table of results is available in Appendix F. The relationship between each of the dependant variables was measured against each of the independent variables using statistical tests and is presented below. A table of *p*-values is presented in Appendix G.

3.1.1. DNA concentration

The use of a filter with flocked swabs resulted in significantly higher DNA concentrations from buccal samples ($p < 0.05$) (Figure 3.1). Similarly, when filters were used, DNA concentrations were higher for flocked swabs than cotton swabs for buccal samples ($p < 0.05$). No significant differences were seen for all other buccal samples combinations or touch DNA samples.

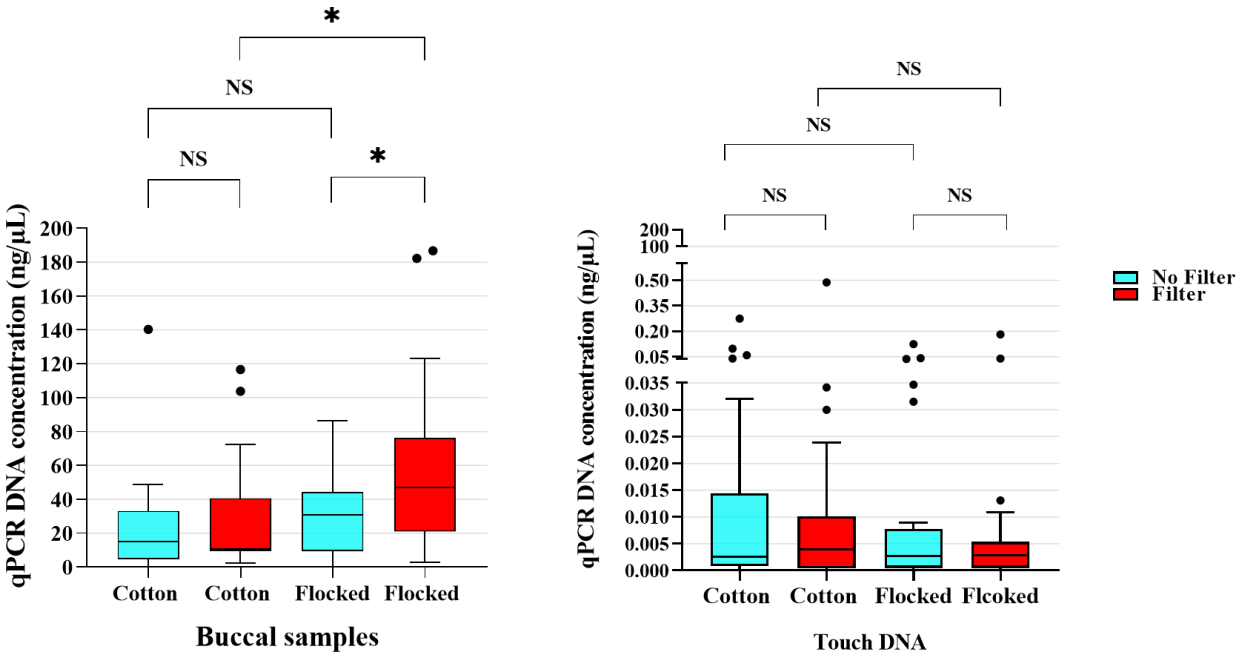

Figure 3.1. DNA concentrations of buccal and touch DNA samples measured using qPCR. The asterisks (*****) denote significant differences at p < 0.05. The abbreviation NS indicates no significant differences in DNA concentration.

The DNA concentrations produced by quantification on qPCR were plotted against a subset of the DNA concentrations recorded by Qubit™ fluorometry to determine the proportion of human DNA (qPCR DNA concentrations) relative to double stranded DNA (Qubit™ DNA concentration) for touch DNA samples and buccal samples (Figure 3.2).

Flocked swabs recovered higher human DNA yields and total DNA yields than cotton swabs from buccal samples. The use of filters resulted in lower human DNA yields and total DNA yields from buccal samples. In contrast, flocked swabs had lower total DNA yields from touch DNA samples, however the proportion of human DNA was greater compared to those obtained using cotton swabs. As opposed to buccal samples, the use of filters improved the proportion of human DNA yields from touch DNA samples (Figure 3.2)

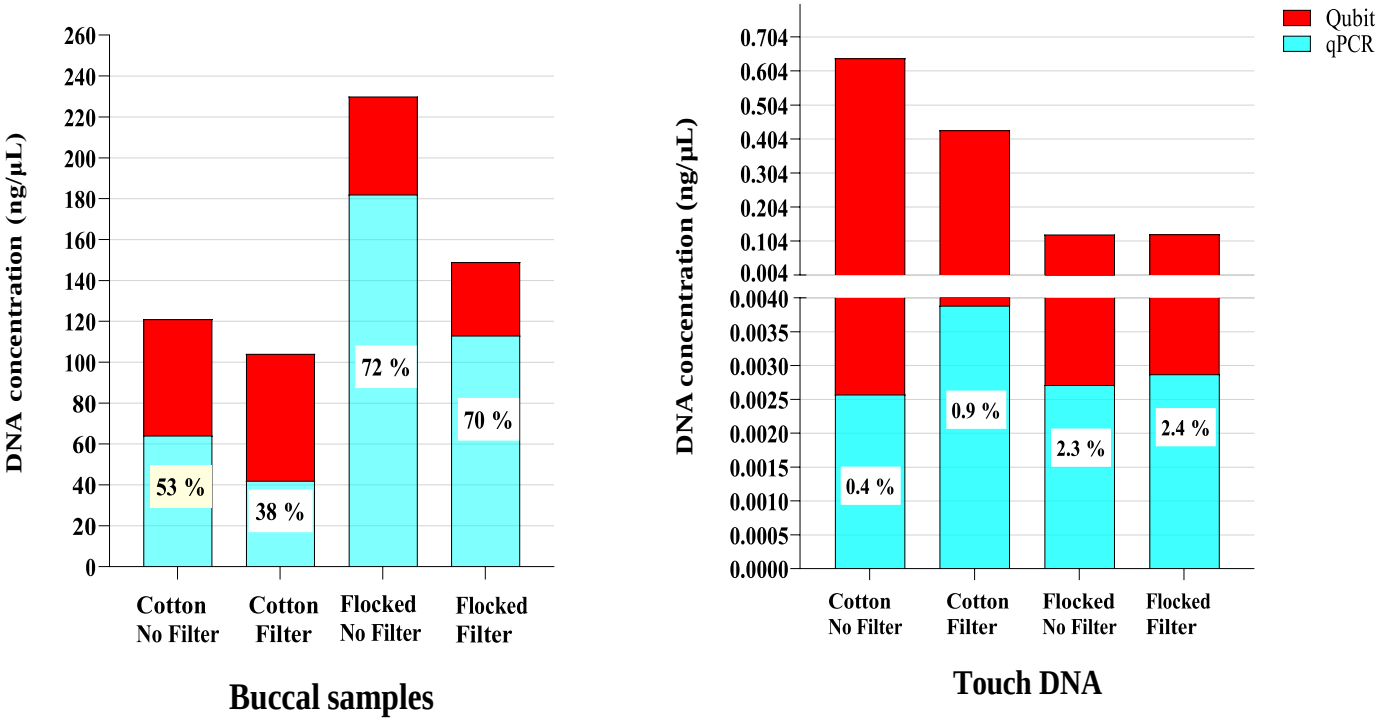

Figure 3.2. The percentage of human DNA (qPCR DNA concentration) out of double stranded DNA (Qubit™ DNA concentration). The red bars represent concentrations of DNA measured by Qubit™ fluorometry and the blue bars indicate qPCR DNA concentrations.

3.2. DNA profiling

3.2.1. DNA profiling success

DNA profiles were analysed by grouping results into three different categories based on the number of STR markers called; fail (0 – 3.5 markers), partial (4 – 11.5 markers) and full (12 – 15 markers) (Figure 3.3). Sixteen touch DNA samples yielded full DNA profiles (16 %), partialDNA profiles were recovered from 28 touch DNA samples (28 %) and 56 touch DNA samplesgenerated failed DNA profiles (56 %).

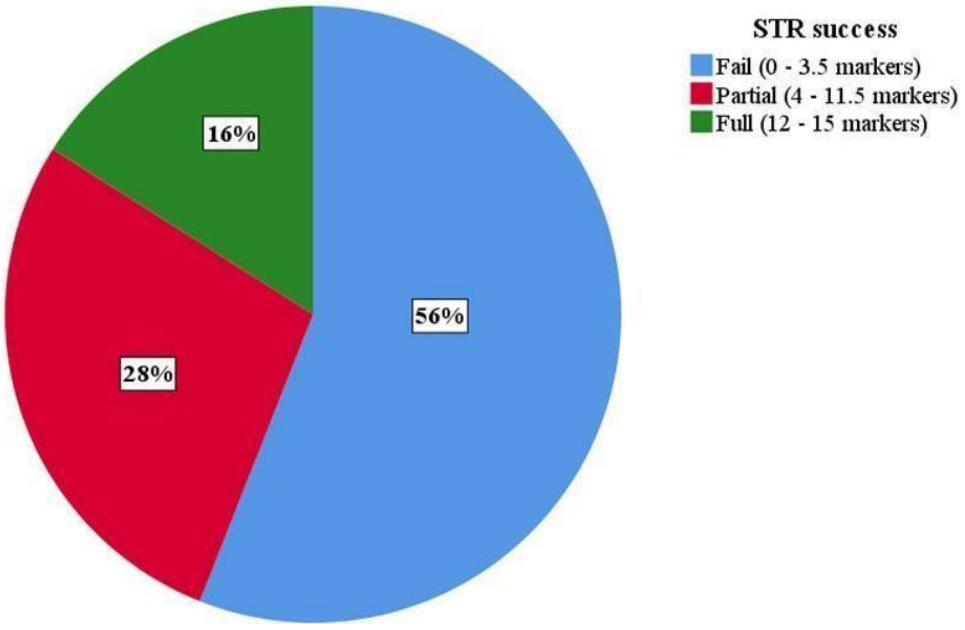

Figure 3.3. Pie chart showing DNA profiling success of 100 touch DNA samples. The blue, red and green colours denote the percentage of failed, partial and full DNA profiles respectively.

The Spearman's rank correlation coefficient test showed a slight positive correlation between DNA concentration and the number of STR markers recovered per sample ($r^2 = 0.57$, $p < 0.0001$). (Figure 3.4).

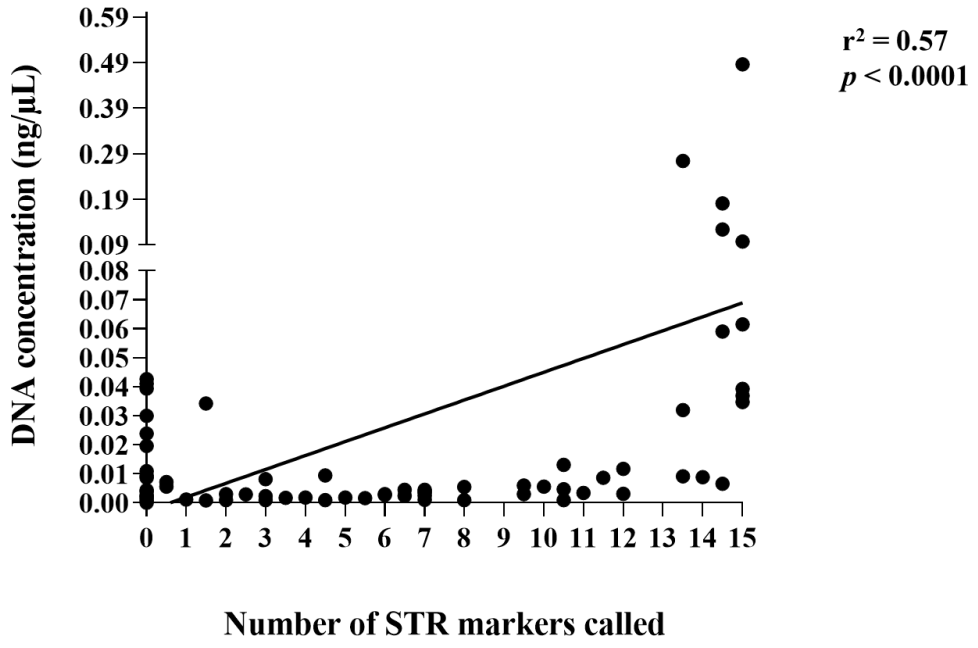

Figure 3.4. Scatter plot showing a slight correlation between qPCR DNA concentrations and the number of STR markers called during DNA profiling of 100 touch DNA samples. The r^2 value is 0.57.

Only 77 of the 100 touch DNA samples showed any amplifiable DNA on qPCR, and these were subjected to DNA profiling. The samples where no DNA was detected using qPCR were automatically interpreted as 'failed' DNA profiles with 0 peaks present. No significant differences were seen in the number of STR markers yielded by touch DNA samples for both swab types when filters were used and not used (Figure 3.5). However, the median number of STR markers was slightly higher when filters were not used, for both swab types.

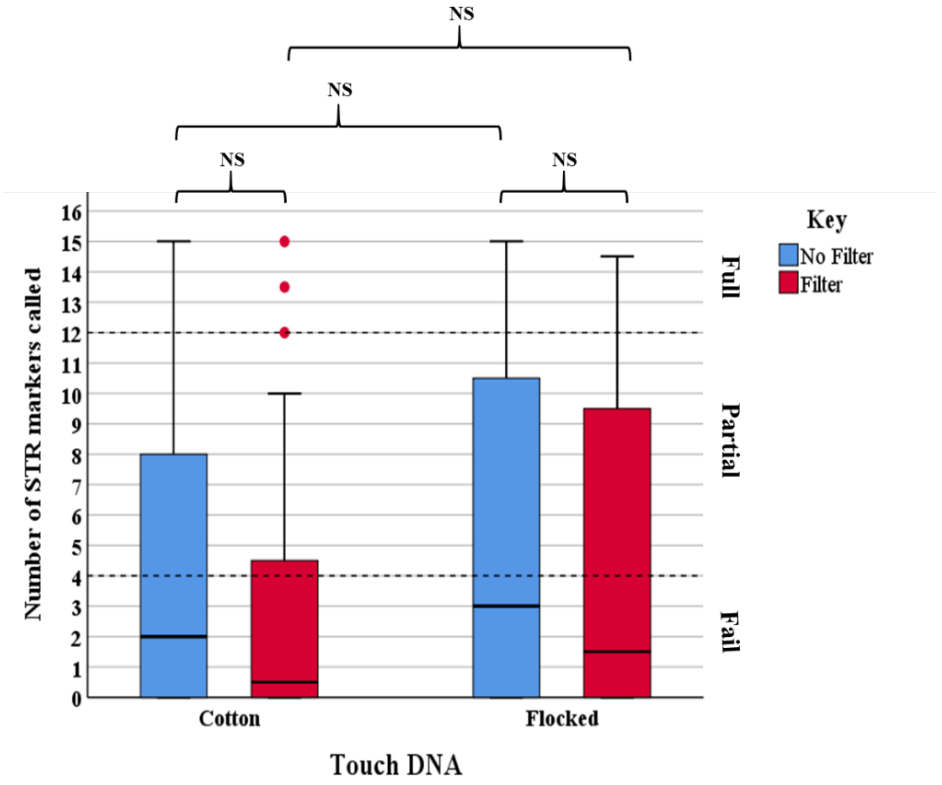

Figure 3.5. Number of STR markers recovered during DNA profiling of touch DNA samples. The abbreviation NS indicates no significant differences in DNA concentration.

.

Chapter 4: Discussion and conclusion

The increasing crime rates in SA have highlighted the importance to improve methods of identifying perpetrators of crimes. The application of DNA analysis to SA forensic investigations has enabled identification of biological evidence left by perpetrators at scenes of crime (Goodwin and Meintjes-Van Der Walt, 1997). Due to low DNA recovery from touch DNA samples, however, there is scope to improve methods that recover DNA from touch samples.

This study aimed at assessing the effect of NucleoSpin® Forensic Filters on touch DNA recovery from cotton and flocked swabs. DNA recovery was assessed by measuring concentration of different targets of DNA (double stranded DNA and human DNA) using two different DNA quantification methods; Qubit™ fluorometry and qPCR respectively. Since human DNA is the target of interest, analysis of DNA concentration data was primarily based on qPCR results, and Qubit™ results provided a means to assess the proportion of human DNA within the total DNA extracted. DNA profiling was performed to assess filter performance on DNA profiling success on touch DNA samples. Buccal samples were included as control samples.

4.1. Does the NucleoSpin® Forensic Filter improve DNA recovery from swabs?

The reviewed literature has shown that, although cotton swabs may be effective at collecting samples from surfaces, they may not be best at sample release during DNA extraction (Dadhania *et al.*, 2013, Bruijns *et al.*, 2018). The argument of poor sample release from cotton swabs can be explained by the nature of the cotton swab fibres which retain a portion of the collected sample during processing (Benschop *et al.*, 2010). It was therefore hypothesized that NucleoSpin® Forensic Filters would improve DNA recovery from cotton swabs, regardless of sample type. Contrary to the hypothesis, the use of filters did not improve DNA recovery from cotton swabs for both sample types (Figure 3.1).

Research has shown high sample release and DNA recovery from flocked swabs, owing to the design of the swab (Templeton *et al.*, 2015, Bruijns *et al.*, 2018). However, studies have attributed the high recovery of DNA from flocked swabs to samples containing high amounts of starting DNA (Benschop *et al.*, 2010, Dadhania *et al.*, 2013). It was hypothesised that NucleoSpin® Forensic Filters would not improve DNA recovery from flocked swabs as much as it would for cotton swabs. It was therefore not surprising, that the filters did not significantly improve DNA yield from flocked swabs used to collect touch DNA (Figure 3.1).

However, the combination of flocked swabs with filters produced significantly higher DNA concentrations than flocked swabs without filters from buccal samples ($p < 0.05$) (Figure 3.1). This observation may be attributed to compatibility of NucleoSpin® Forensic Filters with flocked swabs in situations of high cell content. However, this was the only situation where NucleoSpin® Forensic Filters improved DNA recovery from swabs and supported the manufacturer's claim.

Moreover, flocked swabs also yielded higher DNA yields than cotton swabs when filters were used, with significant differences at $p < 0.05$ (Figure 3.1). The higher DNA yields produced by flocked swabs could be explained by the better performance of flocked swabs in DNA recovery from samples containing high amounts of starting DNA (Dadhania *et al.*, 2013). As shown by the reviewed literature, studies have obtained higher DNA yields from flocked swabs when compared to cotton swabs (Benschop *et al.*, 2010, Bruijns *et al.*, 2018). The authors attribute these higher DNA yields to the loosely packed design of the flocked swab fibres as well as lack of an absorbent internal core, which allows for maximal release of samples during processing.

The use of filters with touch DNA samples did not result in significant differences between DNA concentrations recovered from both swab types (Figure 3.1). One reason for this observation could be that the filters are not effective at releasing trace samples (*i.e.* touch DNA) from swabs, regardless of swab type. The poor performance of filters could also be explained by the design of the filter as Cole (2019) previously described, where filters with small pores prevented flow through of the cells.

Similarly, Ambers *et al.* (2018) and Cole (2019) did not find significant differences in touch DNA concentrations recovered by the Investigator® Lyse & Spin basket, when compared with touch DNA recovery by spin baskets from other companies.

DNA was only recovered from 77 touch DNA samples. The remaining 23 samples had no amplifiable DNA (n = 0.00 ng/µL) when quantified by qPCR.

Only 16 % (n = 16) of the touch DNA samples produced full DNA profiles. The flocked swab without a filter combination contributed the largest number, having six samples out the sixteen samples that produced full DNA profiles. The cotton swab without a filter, cotton swab with a filter and the flocked swab with a filter combinations comprised of five, three and two samples respectively. Partial DNA profiles were recovered from 28 % (n = 28) of the samples. The remaining 56 % of the samples constituted of failed DNA profiles.

The use of filters did not show significant differences in the number of STR markers recovered from cotton and flocked swabs for touch DNA samples. Although not significantly different, the number of STR markers were more when filters were not used for both swab types. This shows that the filters did not improve the success of DNA profiles.

Amongst the 77 touch DNA samples there were almost equal numbers of samples from each of the four categories; *i.e.* cotton swabs without filters (n = 20), cotton swabs without filters (n = 19), flocked swabs with filters (n = 19) and flocked swabs without filters (n = 19). The samples which had no amplifiable DNA were mostly from the same six individuals. This may be explained by many factors, including the shedder status (Lowe *et al.*, 2002), the type of hand used to deposit touch DNA (Daly *et al.*, 2012), the type of substrate (i.e. porous versus non-porous or absorbent versus non-absorbent) (Wickenheiser, 2002), as well as the activities of the donor prior to touch DNA transfer, such as handwashing (Phipps and Petricevic, 2007).

The majority of above-mentioned variables were controlled in the current study, however, shedding could not be controlled. In a study aiming to improve touch DNA collection from surfaces, Pang and Cheung (2007) found that it may be impossible for individuals to deposit a controlled number of cells onto surfaces. The amount of epithelial cells transferred to handled items differ between individuals, as well as between repeated trials from a single individual, showing interindividual variability (Phipps and Petricevic, 2007). This uncontrolled shedding of cells can be attributed to the differences in DNA concentrations between individuals seen in this study.

In the current study for example, the highest touch DNA concentrations were all recovered from the four swabs collected from the same individual (Appendix F). The lowest of these DNA concentrations was from a flocked swab extracted without a filter (0.12 ng/µL), followedby flocked swab with a filter (0.18 ng/µL), cotton swab without a filter (0.27 ng/µL) and cotton

swab with a filter yielded the highest DNA concentration of 0.49 ng/µL. DNA concentrations recovered from all other samples were below 0.10 ng/µL. The high DNA concentrations recovered from the same individual highlights the differences in the propensity of individuals to transfer touch DNA to surfaces. This means that, although there were no apparent differences in touch DNA concentrations when the filters were used, there seems to be a difference in DNA recovery per individual.

Other studies have also obtained varied DNA concentrations, with the highest concentrations recovered from samples deposited by the same individual on all surfaces analysed (Lowe *et al.*, 2002, Phipps and Petricevic, 2007, Goray *et al.*, 2016). In a different study, Phipps and Petricevic (2007) investigated the shedder status of individuals. Their results showed that DNA profiles of varying completeness were generated from the same individual at different times of the day, for all individuals. They concluded that classifying individuals as bad or good shedders may be misleading, since the type of DNA profile (*i.e.* fail, partial or full) generated from each individual at different times of the day was not same. Another factor that affect DNA recovery is the type of substrate from which touch DNA is collected. Porous and absorbent surfaces entrap a portion of the sample, hence resulting in low DNA recovery (Wickenheiser, 2002, Hansson *et al.*, 2009).

The type of hand (Wickenheiser, 2002) and/or finger (Thomasma and Foran, 2013) used to deposit touch DNA to surfaces has also been shown to affect shedding. The dominant hand and fingers of an individual are used the most, for example touching the face or other body parts, as a result, they often have larger amounts of epithelial cells. Although dominant hands and fingers shed the highest amounts of epithelial cells, Stanciu *et al.* (2015) reported that the number of epithelial cell transferred cannot be considered a reliable indicator of touch DNA recovery, since they did not find correlation between the number of cells collected and touch DNA recovery.

Overall, the NucleoSpin® Forensic Filters did not improve DNA recovery or DNA profiling success from touch DNA samples. The results of this study do not support incorporation if these filters into the forensic DNA extraction workflow. The only instance filters yielded higher DNA concentrations was when flocked swabs collected buccal samples. However, DNA from buccal samples can be recovered in high amounts sufficient for generating full DNA profiles, without the aid of filters. The additional cost of the filters is therefore not warranted for touch DNA or buccal samples, from either swab type.

4.2. How does DNA recovery and DNA profiling success compare between protocols using the NucleoSpin® Forensic Filter and filters by other manufacturers?

In evaluating the performance of NucleoSpin® Forensic Filters, the results of this study were compared to the results obtained from similar products from other companies

Adamowicz *et al.* (2014) used DNA IQ™ spin baskets (Promega, Madison) to extract buccal cells from cotton swabs and also recovered low DNA yields. Their results showed yields of DNA even lower than the DNA yields recovered from the equivalent sample and swab type in the current study. DNA concentrations reported by Ambers *et al.* (2018) blood and saliva samples extracted using Nucleic Acid Optimiser (NAO®) spin baskets (Copan, Brescia) were also lower than those obtained in this study.

Recovery of touch DNA from swabs is consistently low, even when attempts have been made to improve DNA recovery (Lowe *et al.*, 2002, Pang and Cheung, 2007, van Oorschot *et al.*, 2010). Stanciu *et al.* (2015) extracted touch DNA from different swabs using the Investigator® Lyse & Spin basket (Qiagen, Hilden) and recovered low DNA concentrations. They showed that only 18.7 % of the samples analysed contained amplifiable DNA during DNA quantification. Again, this was lower than the 77 % of touch DNA observed in the current study, which suggests that the methods used in this study were perhaps better optimised than those of Stanciu *et al.* (2015).

While DNA recovery seemed to be better in this study, it did not translate to better DNA profile success compared to other studies. Daly *et al.* (2012) obtained full DNA profiles from 36 % of the touch DNA samples analysed (n = 300) and Templeton *et al.* (2013) obtained full DNA profiles from all touch DNA samples analysed (n = 3) by direct PCR. These results were both superior to the 16 % of full DNA profiles from touch DNA samples reported in the current study.

In this study, a full DNA profile could be obtained from a DNA concentration as low as 0.0032 ng/µL, corresponding to a flocked swab sample with a filter. This touch DNA concentration was lower than the cut-off DNA concentration for producing DNA profiles (0.03 ng/µL)

suggested by van Oorschot *et al.* (2010) and Daly *et al.* (2012). Another study by Templeton *et al.* (2015) that collected touch DNA from different surfaces only obtained full DNA profiles from porous surfaces from DNA concentrations of 0.5 ng/µL.

The authors, van Oorschot *et al.* (2010) correlate generation of full DNA profiles from touch DNA samples to DNA recovery. To assess correlation between DNA concentration and DNA profiling success (*i.e.* number of STR markers called), the Spearman's rank correlation coefficient test was performed, which showed an r^2 value of 0.57. This shows that there was only a slight positive correlation between DNA concentration and the number of markers called (Figure 3.5). These results are perhaps in line with the findings of Goray *et al.* (2016) who found a poor correlation between DNA concentration and the number STR of alleles. Therefore, while efforts to improve DNA recovery from touch DNA samples is one aspect, efforts to improve the purity and integrity of touch DNA samples should also be considered.

4.3. Improving touch DNA recovery and DNA profiling success

The fact that DNA could not be obtained from all 100 touch DNA samples highlightsroom for improvement in the overall DNA profiling workflow. These results show that there is scope to optimise touch DNA recovery and analysis, by targeting different stages of the analysis workflow. The sampling method utilised in the current study involved moistening a swab with a detergent (2 % SDS) and collecting touch DNA from a porous surface (*i.e.* laboratory bench) through swabbing. This method was developed in accordance with the findings of a study by Thomasma and Foran (2013) who compared the efficacy of different swab moistening solutions in DNA recovery and obtained optimal results from 2 % SDS.

The literature has shown that using foam swabs in touch DNA collection may be a better option, since they seem to perform better than the widely used cotton and flocked swabs (Hansson *et al.*, 2009, Verdon *et al.*, 2014, Phetpeng *et al.*, 2015). Another approach to possibly improve touch DNA collection involves the use of adhesive tapes to lift epithelial cells from surfaces. Tape lifting recovers higher amounts of epithelial cells than swabbing, due to the sticky surfaces of the adhesive tapes which allows for higher attachment of cells onto the tape (Gunnarsson *et al.*, 2010, Hess and Haas, 2017).

However, studies have also found no correlation between the number of epithelial cells collected and the amount of touch DNA recovered (Stanciu *et al.*, 2015). Rather, emphasis should be placed on the preservation and storage of collected epithelial cells, as well as improving the recovery of touch DNA from these epithelial cells.

In the current study, DNA was extracted on the same day of collection to prevent degradation. However, some studies have made use of different swab preservation methods which stabilise DNA in the sample until analysis (Beckett *et al.*, 2008).

For example, swabs can be air-dried at room temperature or stored in specialised swab collection tubes containing a drying agent that facilitates drying of the swab, an example is the forensiX evidence collection tube (Prionics AG, Schlieren-Zürich). These swabs can then be stored in the freezer at – 20 °C for long term storage, while maintaining stability of the DNA in the sample (Garvin *et al.*, 2013). Alternatively, swabs may be preserved in buffers which inhibit bacterial growth and prevent fragmentation of DNA within the sample, such as DNA/RNA Shield™ (Zymo Research, Irvine). Buffer preserved swabs can be stored for long periods until analysis (Burgos *et al.*, 2017, Menke *et al.*, 2017).

One commonly used method that has been shown to generate higher DNA profiling success from touch samples is direct PCR. This method eliminates the DNA extraction and quantification steps because the swabs used for direct amplification are treated with a buffer which facilitates lysis of the sample on the swab fibres (Cavanaugh and Bathrick, 2018). Consequently, the swabs are subjected directly to PCR. Direct PCR minimises the chances of cross contamination and sample loss by eliminating DNA extraction which often involves sample transfer between tubes. The design of these swabs allows for the consumption of only small amounts of the sample, enabling future reanalyses which are often needed in LCN analyses. An example is the microFLOQ® direct swab from Copan (Brescia) (Templeton *et al.*, 2013, Ambers *et al.*, 2018).

Templeton *et al.* (2015) compared touch DNA recovery between direct PCR and a column-based DNA extraction method. Their results showed improved DNA yields from direct PCR, and improved DNA profiling success. Similar results were shown by Templeton *et al.* (2013) and Ottens *et al.* (2013), in that they obtained higher touch DNA yields and full DNA profiles from all samples analysed from direct PCR samples, when compared to extracted samples.

4.4. Limitations

Some of the limitations of the study have been already discussed in the chapter, such as variables (*e.g.* foam swabs) which could have been assessed alongside this study. While these were beyond the scope of this minor dissertation, these are important next steps in future.

Although this study was a preliminary study and the first step in assessing the effect of NucleoSpin® Forensic Filters on touch DNA recovery, the sample size (n = 25) of study relatively small. Due to the different shedder statuses of participants, DNA was not recovered from most samples provided by six individuals. The effect of the NucleoSpin® Forensic Filters was therefore essentially only assessed on samples from 19 individuals. Furthermore, small sample sizes are prone to false positive results. Since the amount of DNA recovered from different individuals can never be controlled for, it is important to increase the sample in order to produce highly representative results.

4.5. Conclusion

NucleoSpin® Forensic Filters did not improve DNA yield or DNA profiling success from cotton swabs for both sample types and flocked swabs from touch DNA samples. The filters may have been effective with buccal samples from flocked swabs, but buccal samples can yield high amounts of DNA, yielding full DNA profiles without the aid of filters. Therefore, NucleoSpin® Forensic Filters should not be incorporated in the DNA extraction workflow. Further, only 16 % of samples recovered full DNA profiles, which highlights the need to optimise touch DNA analysis methods, for human identification applications. Future work should focus on increasing sample size as well as improving other steps in the collection and the downstream analyses of touch DNA samples.

5. References

Adamowicz, M. S., Stasulli, D. M., Sobestanovich, E. M. & Bille, T. W. 2014. Evaluation of methods to improve the extraction and recovery of DNA from cotton swabs for forensic analysis. *Plos One.* 9(12):116-131.

Afrobarometer South Africa, round six survey of 2016. Available: https://afrobarometer.org/publications/south-africa-round-6-summary-results [2021, January, 17].

Akane, A., Matsubara, K., Nakamura, H., Takahashi, S. & Kimura, K. 1994. Identification of the heme compound copurified with deoxyribonucleic acid (DNA) from bloodstains, a major inhibitor of polymerase chain reaction (PCR) amplification. *Journal of Forensic Sciences.* 39(2):362-372.

Alaeddini, R., Walsh, S. J. & Abbas, A. 2010. Forensic implications of genetic analyses from degraded DNA—a review. *Forensic Science International: Genetics.* 4(3):148-157.

Alaeddini, R. 2012. Forensic implications of PCR inhibition—a review. *Forensic Science International: Genetics.* 6(3):297-305.

Alonso, A., Martín, P., Albarrán, C., Garcí, P., Simón, D., Fernández, L., Iturralde, M. J., Fernández-Rodríguez, A., Atienza, I. & Capilla, J. 2005. Challenges of DNA profiling in mass disaster investigations. *Croatian Medical Journal.* 46(4):1-5.

Ambers, A., Wiley, R., Novroski, N. & Budowle, B. 2018. Direct PCR amplification of DNA from human bloodstains, saliva, and touch samples collected with microFLOQ® swabs. *Forensic Science International: Genetics*. 32(3):80-87.

Arenas, M., Pereira, F., Oliveira, M., Pinto, N., Lopes, A. M., Gomes, V., Carracedo, A. & Amorim, A. 2017. Forensic genetics and genomics: much more than just a human affair. *Plos Genetics*.13(9):10-16.

Beckett, S. M., Laughton, S. J., Pozza, L. D., Mccowage, G. B., Marshall, G., Cohn, R. J., Milne, E. & Ashton, L. 2008. Buccal swabs and treated cards: methodological considerations for molecular epidemiologic studies examining pediatric populations. *American Journal of Epidemiology*. 167(2):1260-1267.

Benschop, C. C., Wiebosch, D. C., Kloosterman, A. D. & Sijen, T. 2010. Post-coital vaginal sampling with nylon flocked swabs improves DNA typing. *Forensic Science International: Gentics.* 4(2):115-121.

Berger, B., Parson, R., Clausen, J., Berger, C., Nachbaur, D. & Parson, W. 2013. Chimerism in DNA of buccal swabs from recipients after allogeneic hematopoietic stem cell transplantations: implications for forensic DNA testing. *International Journal of Legal Medicine.* 127(1): 49-54.

Brownlow, R. J., Dagnall, K. E. & Ames, C. E. 2012. A comparison of DNA collection and retrieval from two swab types (cotton and nylon flocked swab) when processed using three QIAGEN extraction methods. *Journal of Forensic Sciences.* 57(3):713-717.

Bruijns, B. B., Tiggelaar, R. M. & Gardeniers, H. 2018. The Extraction and RecoveryEfficiency of Pure DNA for Different Types of Swabs. *Journal of Forensic Sciences.* 63(5):1492-1499.

Budowle, B., Bieber, F. R. & Eisenberg, A. 2005. Forensic aspects of mass disasters: strategic considerations for DNA-based human identification. *Legal Medicine.* 7(4):230-243.

Budowle, B., Eisenberg, A. J. & Daal, A. V. 2009. Validity of low copy number typing and applications to forensic science. *Croatian Medical Journal*. 50(3):207-217.

Burgos, G., Ormasa, D., Camargo, M., Iturralde, G., Granda, J., Posada, Y., Sanchez, M., Tejera, E. & Calvopiña, M. 2017. Assessment of method for buccal swab samples preservation in extreme environmental conditions for population genetics and forensic purposes. *Forensic Science Internal: Genetics Supplement Series.* 6(20):460-462.

Butler, J. M. 2005. *Forensic DNA typing: biology, technology, and genetics of STR markers*. Elsevier.

Butler, J. M. 2011. *Advanced topics in forensic DNA typing: methodology*. Academic Press.

Butler, J. M., Buel, E., Crivellente, F. & Mccord, B. R. 2004. Forensic DNA typing by capillary electrophoresis using the ABI Prism 310 and 3100 genetic analyzers for STR analysis. *Electrophoresis.* 25(10):1397-1412.

Butler, J. M. 2015. The future of forensic DNA analysis. *Philosophical Transactions of the Royal Society: Biological Sciences.* 370(2):20-24.

Caddy, B., Taylor, G. & Linacre, A. 2008. *A review of the science of low template DNA analysis.* UK Home Office Report.

Calacal, G. C., Apaga, D. L. T., Salvador, J. M., Jimenez, J. A. D., Lagat, L. J., Villacorta, R. P. F., Lim, M. C. F., Dr Fortun, R., Datar, F. A. & De Ungria, M. C. A. 2015.Comparing different post-mortem human samples as DNA sources for downstream genotyping and identification. *Forensic Science International: Genetics*. 19(3):212-220.

Caragine, T., Currie, K., O'connor, C. & Budimlija, Z. 2013. Low-Template DNA Testing. *Journal of Forensic Sciences.* 4(3):252-256.

Carey, L. & Mitnik, L. J. E. 2002. Trends in DNA forensic analysis. *Journal of Forensic Sciences.* 23(3):1386-1397.

Castella, V., Dimo-Simonin, N., Brandt-Casadevall, C. & Mangin, P. 2006. Forensic evaluation of the QIAshredder/QIAamp DNA extraction procedure. *Forensic Science International*. 156(1):70-73.

Cavanaugh, S. E. & Bathrick, A. S. 2018. Direct PCR amplification of forensic touch and other challenging DNA samples: a review. *Forensic Science International: Genetics.* 32(1):40-49.

Cole, K. A. 2019. Validation of a DNA Extraction Method Using Diluted ATL as an Extraction

Buffer with the Qiagen Lyse&Spin Basket Kit. Masters Thesis. Boston University.

Criminal Law (Forensic Procedures) Amendment Act, No. 37 of 2013. 2013. Available: http://www.justice.gov.za/legislation/notices/2015/20150313-gg38561_r10387_gon207-act2013-037.pdf [2021, January 17].

Dadhania, A., Nelson, M., Caves, G., Santiago, R. & Podini, D. 2013. Evaluation of Copan 4N6FLOQSwabs™ used for crime scene evidence collection. *Forensic Science International: Genetics Supplement Series.* 4(1):336-337.

Daly, D. J., Murphy, C. & Mcdermott, S. D. 2012. The transfer of touch DNA from hands to glass, fabric and wood. *Forensic Science International: Genetics.* 6(1):41-46.

Frumkin, D., Wasserstrom, A., Budowle, B. & Davidson, A. 2011. DNA methylation-based forensic tissue identification. *Forensic Science International: Genetics.* 5(5):517-524.

Garvin, A. M. & Fritsch, A. 2013. Purifying and concentrating genomic DNA from mock forensic samples using Millipore Amicon filters. *Journal of Forensic Sciences.* 58(1):173-175.

Gill, P., Whitaker, J., Flaxman, C., Brown, N. & Buckleton, J. 2000. An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA. *Forensic Science International.* 112(1):17-40.

Gill, P. 2001. Application of low copy number DNA profiling. *Croatian Medical Journal.* 42(3):229-232.

Goodwin, J. A. & Meintjes-Van Der Walt, L. 1997. The use of DNA evidence in South Africa: Powerful tool or prone to pitfalls. *South African Law Journal.* 6(2):114-151.

Goray, M., Fowler, S., Szkuta, B. & Van Oorschot, R. 2016. Shedder status—an analysis of self and non-self DNA in multiple handprints deposited by the same individuals over time. *Forensic Science International: Genetics*. 23(1):190-196.

Gunnarsson, J., Eriksson, H. & Ansell, R. 2010. Success rate of a forensic tape-lift methodfor DNA recovery. *Problems of Forensic Science.* 83(1):243-254.

Hansson, O., Finnebraaten, M., Heitmann, I. K., Ramse, M. & Bouzga, M. 2009. Trace DNA collection—performance of minitape and three different swabs. *Forensic Science International: Genetics Supplement Series.* 2(1):189-190.

Hess, S. & Haas, C. 2017. Recovery of trace DNA on clothing: a comparison of mini-tape lifting and three other forensic evidence collection techniques. *Journal of Forensic Sciences.* 62(1):187-191.

Hudlow, W. R., Krieger, R., Meusel, M., Sehhat, J. C., Timken, M. D. & Buoncristiani, M. R. 2011. The NucleoSpin® DNA Clean-up XS kit for the concentration and purification of genomic DNA extracts: An alternative to microdialysis filtration. *Journal of Forensic Sciences.* 5(4):226-230.

Hughes-Stamm, S., Warnke, F. & Van Daal, A. 2016. An alternate method for extracting DNA from environmentally challenged teeth for improved DNA analysis. *Journal of Legal Medicine.* 18(1):31-36.

Irwin, J. A., Leney, M. D., Loreille, O., Barritt, S. M., Christensen, A. F., Holland, T. D., Smith, B. C. & Parsons, T. 2007. Application of low copy number STR typing to the identification of aged, degraded skeletal remains. *Journal of Forensic Sciences.* 52(6):322-1327.

Karlsson, S., Larsen, B. M., Eriksen, L. & Hagen, M. 2013. Four methods of nondestructive DNA sampling from freshwater pearl mussels Margaritifera margaritifera L.(Bivalvia: Unionoida). *Freshwater Science.* 32(2):525-530.

Ladd, C., Adamowicz, M. S., Bourke, M. T., Scherczinger, C. A. & Lee, H. C. 1999. A systematic analysis of secondary DNA transfer. *Journal of Forensic Science.* 44(6):1270-1272.

Lee, H. C. & Ladd, C. 2001. Preservation and collection of biological evidence. *Croatian*

Medical Journal. 42(3):225-228.

Lindstrom, J. 2012. *A More Efficient Means to Collect & Process Reference DNA Samples*. Oklahoma State Bureau of Investigation.

Locard, E. 1930. The analysis of dust traces. *American Journal of Police Sciences.* 1(3):276-280.

Lowe, A., Murray, C., Whitaker, J., Tully, G. & Gill, P. 2002. The propensity of individuals to deposit DNA and secondary transfer of low level DNA from individuals to inert surfaces. *Forensic Science International.* 129(1):25-34.

Machado, H. & Silva, S. 2014. "Would you accept having your DNA profile inserted in the National Forensic DNA database? Why?" Results of a questionnaire applied in Portugal. *Forensic Science International: Genetics*. 8(1):132-136.

Martin, B., Blackie, R., Taylor, D. & Linacre, A. 2018. DNA profiles generated from a range of touched sample types. *Forensic Science International: Genetics*. 36(1):13-19.

Menke, S., Gillingham, M. A., Wilhelm, K. & Sommer, S. 2017. Home-made cost effective preservation buffer is a better alternative to commercial preservation methods for microbiome research. *Frontiers in Microbiology.* 8(2):102-108.

Moretti, T. R., Baumstark, A. L., Defenbaugh, D. A., Keys, K. M., Smerick, J. B. & Budowle, B. 2001. Validation of short tandem repeats (STRs) for forensic usage: performance testing of fluorescent multiplex STR systems and analysis of authentic and simulated forensic samples. *Journal of Forensic Sciences.* 46(3):647-660.

Nicklas, J. A., Buel, E. 2003. Quantification of DNA in forensic samples. *Journal of Analytical and Bioanalytical Chemistry.* 376(8):1160-1167.

Nori, D. V., Mccord, B. R. 2015. The application of alkaline lysis and pressure cycling technology in the differential extraction of DNA from sperm and epithelial cells recovered from cotton swabs. *Journal of Analytical and Bioanalytical Chemistry*.

407(23):6975-6984.

Norris, J. V., Manning, K., Linke, S. J., Ferrance, J. P. & Landers, J. P. 2007. Expedited, chemically enhanced sperm cell recovery from cotton swabs for rape kit analysis. *Journal of Forensic Sciences*. 52(4):800-805.

O'brien, R., Sutherland, C. & Figarelli, D. 2012. National Forensic Science Technology Center Swab collection study. *Journal of Forensic Sciences.* 5(1):42-47.

Opel, K. L., Chung, D. & Mccord, B. R. 2010. A study of PCR inhibition mechanisms using real time PCR. *Journal of Forensic Sciences.* 55(1):25-33.

Ottens, R., Templeton, J., Paradiso, V., Taylor, D., Abarno, D. & Linacre, A. 2013. Application of direct PCR in forensic casework. *Forensic Science International: Genetics Supplement Series.* 4(1):47-48.

Ottoni, C., Bekaert, B., Decorte, R. 2017. DNA degradation: current knowledge and progress in DNA analysis. *Forensic Analysis of the Dead and the Depositional Environment*. 3(1):65-80.

Pang, B. & Cheung, B. 2007. Double swab technique for collecting touched evidence. *Journal of Legal Medicine.* 9(4):181-184.

Parker, L. S., London, A. J. & Aronson, J. D. 2013. Incidental findings in the use of DNA to identify human remains: an ethical assessment. *Forensic Science International: Genetics*. 7(2):221-229.

Phetpeng, S., Kitpipit, T. & Thanakiatkrai, P. 2015. Systematic study for DNA recovery and profiling from common IED substrates: from laboratory to casework. *Forensic Science Internatinal: Genetics. 17*(2):53-60.

Phipps, M. & Petricevic, S. 2007. The tendency of individuals to transfer DNA to handled items. *Forensic Science International.* 168(2):162-168.

Pineda, G. M., Montgomery, A. H., Thompson, R., Indest, B., Carroll, M. & SINHA, S. K. 2014. Development and validation of InnoQuant™, a sensitive human DNA quantitation and degradation assessment method for forensic samples using high copy number mobile elements Alu and SVA. *Forensic Science International: Genetics. 13(3):224-235.*

Plaza, D. T., Mealy, J. L., Lane, J. N., Parsons, M. N., Bathrick, A. S. & Slack, D. P. 2016. Nondestructive biological evidence collection with alternative swabs and adhesive lifters. *Journal of Forensic Sciences*. 61(2):485-488.

Prinz, M., Carracedo, A., Mayr, W., Morling, N., Parsons, T., Sajantila, A., Scheithauer, R., Schmitter, H. & Schneider, P. M. 2007. DNA Commission of the International Society for Forensic Genetics (ISFG): recommendations regarding the role of forensic genetics for disaster victim identification (DVI). *Forensic Science International: Genetics.* 1(3):3-12.

Puch-Solis, R., Rodgers, L., Mazumder, A., Pope, S., Evett, I., Curran, J. & Balding, D. J. F. S. I. G. 2013. Evaluating forensic DNA profiles using peak heights, allowing for multiple donors, allelic dropout and stutters. *Forensic Science International: Genetics.* 7(5):555-563.

Puch-Solis, R., Hopwood, A., Pope, S. & Tully, G. 2009. Low Template DNA Analysis and Interpretation. *Wiley Encyclopedia of Forensic Sciences.* 4(1):1-12.

Raymond, J. J., Van Oorschot, R. A., Gunn, P. R., Walsh, S. J. & Roux, C. 2009. Trace evidence characteristics of DNA: a preliminary investigation of the persistence of DNA at crime scenes. *Forensic Science International: Genetics.* 4(1):26-33.

Romsos, E. L. & Vallone, P. M. 2015. Rapid PCR of STR markers: Applications to human identification. Forensic Science International: Genetics. 18(1):90-99.

Rudin, N. & Inman, K. 2001. *An introduction to forensic DNA analysis*. CRC press.

Schiffner, L. A., Bajda, E. J., Prinz, M., Sebestyen, J., Shaler, R. & Caragine, T. A. 2005. Optimization of a simple, automatable extraction method to recover sufficient DNA

from low copy number DNA samples for generation of short tandem repeat profiles. *Croatian Medical Journal*. 46(4):578.

Siegel, J. A. & Saukko, P. 2012. *Encyclopedia of forensic sciences*. Academic Press.

Singh, S. C. 2011. DNA profiling and the forensic use of DNA evidence in criminal proceedings. Journal of the Indian Law Institute. 6(3):195-226.

Siriboonpiputtana, T., Rinthachai, T., Shotivaranon, J., Peonim, V. & Rerkamnuaychoke, B. 2018. Forensic genetic analysis of bone remain samples. *Forensic Science International*. 284(1):167-175.

South African Police Services, South African crime statistics 2017/2018. Available: https://africacheck.org/factsheets/factsheet-south-africas-crime-statistics-for-2017-18/ [2021, January, 17].

Stanciu, C. E., Philpott, M. K., Kwon, Y. J., Bustamante, E. E. & Ehrhardt, C. 2015. Optical characterization of epidermal cells and their relationship to DNA recovery from touch samples. *Journal of F1000 Research.* 4(1):11-20.

Stouder, S. L., Reubush, K. J., Hobson, D. L. & Smith, J. L. 2001. Trace evidence scrapings: a valuable source of DNA. *Forensic Science Communications*. 4(4):1-8.

Sweet, D., Lorente, M., Lorente, J. A., Valenzuela, A. & Villanueva, E. 1997. An improved method to recover saliva from human skin: the double swab technique. J*ournal of Forensic Sciences.* 42(2): 320-322.

Templeton, J., Ottens, R., Paradiso, V., Handt, O., Taylor, D. & Linacre, A. 2013. Genetic profiling from challenging samples: direct PCR of touch DNA. *Forensic Science International: Genetics Supplement Series. 4*(1):224-225.

Templeton, J. E., Taylor, D., Handt, O., Skuza, P. & Linacre, A. 2015. Direct PCR improves the recovery of DNA from various substrates. *Journal of Forensic Sciences.* 60(6):1558-1562.

Thomasma, S. M. & Foran, D. R. 2013. The influence of swabbing solutions on DNA recovery from touch samples. *Journal of Forensic Sciences*. 58(2):465-469.

Thompson, T. & Black, S. 2006. *Forensic human identification: an introduction*. CRC press.

Van Hoofstat, D. E., Deforce, D. L., Hubert De Pauw, I. P. & Van Den Eeckhout, E. G. 1999. DNA typing of fingerprints using capillary electrophoresis: effect of dactyloscopic powders. *Electrophoresis: An International Journal.* 20(14):2870-2876.

Van Oorschot, R., Phelan, D., Furlong, S., Scarfo, G., Holding, N. & Cummins, M. 2003. Are you collecting all the available DNA from touched objects? *International Congress Series*. 4(2):803-807.

Van Oorschot, R. A., Ballantyne, K. N. & Mitchell, R. 2010. Forensic trace DNA: a review. *Journal of Investigative Genetics.* 1(1):14.

Van Oorschot, R. A. & Jones, M. K. 1997. DNA fingerprints from fingerprints. *Nature.* 387(6635)767.

Vance, J. M. 2005. Collection of biological samples for DNA analysis. *Genetic Analysis of Complex Diseases: Second Edition.* John Wiley & Sons, Incorporation. 4(3):153-166.

Verdon, T. J., Mitchell, R. J. & Van Oorschot, R. A. 2014. Evaluation of tapelifting as a collection method for touch DNA. *Forensic Science International: Genetic*s. 8(1):179-186.

Voorhees, J. C., Ferrance, J. P. & Landers, J. P. 2006. Enhanced elution of sperm from cotton swabs via enzymatic digestion for rape kit analysis. *Journal of Forensic Sciences.* 51(3):574-579.

Wickenheiser, R. A. 2002. Trace DNA: a review, discussion of theory, and application of the transfer of trace quantities of DNA through skin contact. *Journal of Forensic Science.* 47(3):442-450.

Williamson, A. L. 2012. Touch DNA: forensic collection and application to investigations. *Journal of the Association of Crime Scene Reconstruction*. 18(1):1-5.

Windram, K., Miller, S., Ward, D., Silenieks, T., Henry, J. 2005. Comparison of swab types for the recover of trace DNA in Forensic Investigations. *Evidence Recovery and Biology Analytical Groups, Biology Report.* 4(1):73.

Zgonjanin, D., Antov, M., Alghafri, R., Petković, S., Vuković, R., Stojiljković, G. & Toljić, D. 2017. DNA analysis from human skeletal remains in forensic casework. *Forensic Science International: Genetics Supplement Series.* 6(1):342-345.

6. Appendices

Appendix A: Human Research Ethics approval letter

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E53-46 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone (021) 406 6492
Email: s.mayah.arefdien@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

11 July 2019

HREC REF: 312/2019

Ms L Heathfield
Division of Pathology
Forensic Medicine and Toxicology
Entrance 2
Falmouth Building-FHS

Dear Ms Heathfield

PROJECT TITLE: INVESTIGATING THE EFFECT OF NUCLEOSPIN FORENSIC FILTERS IN DNA EXTRACTION YIELD FROM SWABS (MASTERS CANDIDATE: MS A HITEWA)

Thank you for your response letter dated 03 July 2019, addressing the issues raised by the Human Research Ethics Committee (HREC).

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30 July 2020.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

We acknowledge that the student: Ms A Hitewa will also be involved in this study.

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

Signature Removed

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

NHREC-registration number: REC-210208-007

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines. The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

Appendix B: Information and Informed consent form

INFORMATION AND INFORMED CONSENT FORM

Title of project: Investigating the effect of NucleoSpin® Forensic Filters in DNA extraction yield from swabs

Investigators: Dr Laura Heathfield (Principal investigator)
Dr Andrea Gibbon (Co-investigator)
Calvin Mole (Co-investigator)
Alina Hitewa (Student)

Background

Currently, there is a large number of biological samples and unclaimed human remains that are unidentified in South Africa. DNA analysis is method used in forensic science to assist with identifying who the biological remains belong to. This is done by a method called 'DNA profiling' which studies a small portion of the DNA which differs between people.

DNA is a molecule found in the cells of all living organisms, including human beings. It contains the genetic information which is inherited from both parents and contains instructions for the body to work. Most of the DNA between people is the same, but there is a small portion that varies between people. In forensics, we are interested in these variable regions because statistically, there is an extremely low chance that two people will share the same combination of variations in their DNA. Identification is achieved by a matching principle, where the 'DNA profile' from a biological sample matches that of a known individual.

Forensic DNA analysis is done by the state, but they often struggle to recover DNA from biological samples and human remains. There is a need to improve DNA recovery from these samples, as increased yield of DNA will be helpful in the overall identification process.

A new product, the NucleoSpin® Forensic Filter was recently developed for use in DNA analysis. These filters are thought to improve the recovery and purity of DNA from samples. This study wants to investigate if these filters really do improve DNA recovery from biological samples – especially those which only have a small amount of starting DNA. This project will be conducted in 2019, in Cape Town (South Africa) and it forms part of an MPhil project within the Division of Forensic Medicine and Toxicology at the University of Cape Town.

What do we need from you?

To do our project, we need to collect DNA samples from people, and we are asking if you would be willing to donate samples. We would like to collect buccal (inner cheek) cells from the inside of your mouth. This will be done by gently rubbing the inside of your cheeks up and down for about 30 seconds using two cotton and two flocked swabs. For comfort purposes, you may collect your own buccal cells under the supervision of the researcher, and this will be done in a private space. You will also be asked to wash your hands without soap, and then touch the laboratory bench surface with moderate pressure for about twenty seconds. This will transfer your skin epithelial cells onto the bench surface, which will allow for collection of touch DNA by the researcher using four swabs.

All the swabs will undergo DNA extraction, with and without NucleoSpin® Forensic Filters. The extracted DNA will then undergo a series of molecular tests in the laboratory to study the amount of DNA and the quality of the DNA that we were able to extract. This will include seeing if we are able to generate something called a 'DNA profile', which is the standard test of forensic laboratories. This DNA profile will not be able to tell anything about you, other than your sex. The swabs that we use will be discarded after DNA has been extracted. The DNA sample will be stored in an access-controlled laboratory within the Division of Forensic Medicine and Toxicology at the University of Cape Town for the duration of the project. Once the study is complete, the DNA will be discarded, unless you indicate that you would like us to keep your sample for future research that obtained ethics approval.

Benefits and Risks

The collection of inner cheek cells and touching bench surfaces has minimal risks, as they are non-invasive and will not hurt you. All the results that are generated from the samples you donate will be anonymised, and your confidentiality will be maintained. The DNA profiles will be kept in-house and will not be published or shared with anyone else. This project is also

completely separate from the South African Police Service, and there is no way that you could be linked to crime by participating in this project, whatsoever.

There is no direct benefit for you, by participating in this study and you will not be compensated. However, the results that we obtain will be helpful in possibly improving DNA extraction methods that are used in forensic science. This will mean that more samples from crime scenes or forensic mortuaries can be identified in the future.

Making your choice

Participation in this study is completely voluntary. You may also withdraw from the study at any point and you may do so without providing a reason and without any consequence to you. If you withdraw, your sample will be discarded immediately. If you choose to have your samples stored for future research, it will be stored for the next 20 years in an access-controlled freezer in the laboratory.

Contact details

If you have any questions about the research, please contact Dr Laura Heathfield at 021 406 6569 or Laura.Heathfield@uct.ac.za. Alternatively, for any questions pertaining human ethics, please contact the Chairperson of the University of Cape Town Faculty of Health Science Human Research Ethics Committee, **Professor Marc Blockman** on 021 406 6496.

Consent form

Participant number:_____

1. I confirm that I am South African and 18 years or older.
2. I confirm that I have read and understand the research information form for the above study and have had the opportunity to ask questions.
3. I agree to participate in this study and understand that my application is voluntary and that I am free to withdraw at any time, without giving any reason.
4. I consent to:

   (Tick the appropriate box)

   ☐ The use of my DNA and information to be used ONLY for this study and to be destroyed after conclusion of this project.

   ☐ The use of my DNA and information to be used for this study AND stored for the ONLY purpose of possible future continuation of this research only if approved by HREC.

   ☐ The use of my DNA and information to be used for this study AS WELL AS my samples to be stored and used for future research that is approved by the HREC.

_____	_____
Name of participant	Date
_____	_____
Signature of participant	Date
_____	_____
Name of witness	Date
_____	_____
Signature of witness	Date

Appendix C: Real-Time PCR standard curve

Figure 6.1. A standard curve generated from 5 serial dilutions of standard DNA in MBG water (dilution factor = 3), with concentrations ranging from 0.023 ng/µL to 1.850 ng/µL. The Quantifiler® Human DNA Quantification Kit (Applied Biosystems, Foster) was used to prepare the serial dilutions and quantify the samples. Red blocks represent standard DNA of know concentration used to generate the standard curve; blue blocks depict unknown samples falling within the range of the standard curve; green blocks represent unknown (flagged) samples falling out of the range of the standard curve.

Table 6.1. Quantifiler® Human DNA Quantification Kit (Applied Biosystems) qPCR quality metrics ranges.

Quality metrics	Accepted range/value
Slope	- 3.3 to - 2.9
Y-intercept	1
R^2	> 0.98
Percentage efficiency (Eff%)	90 % - 110 %

Appendix D: PowerPlex® ESX 16 kit positive control DNA profile

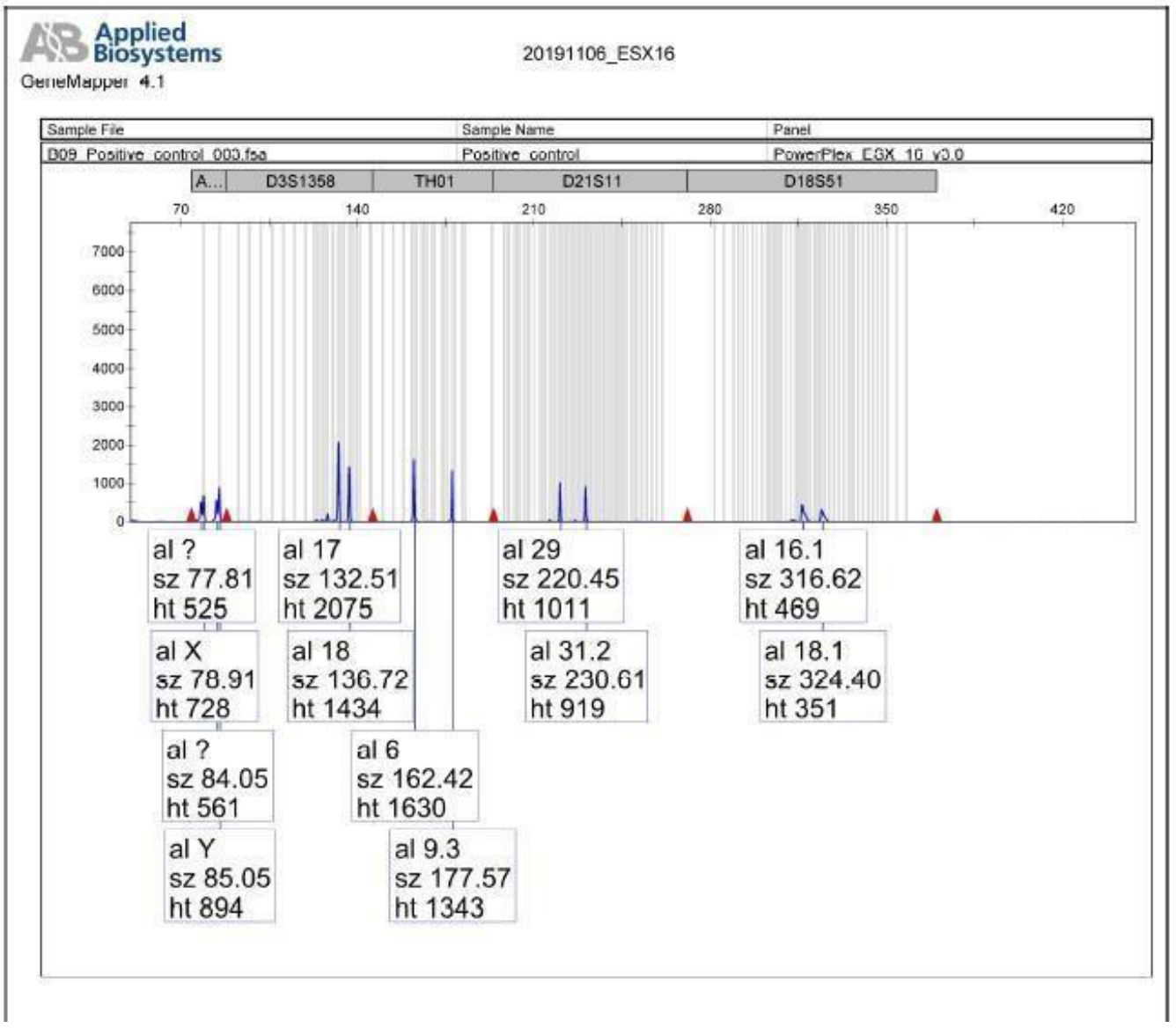

Figure 6.2. Positive control DNA profile generated from the Applied Biosystems® Gene Mapper 4.1 software using the using the PowerPlex® ESX 16 kit (Promega, Madison).

Appendix E: PowerPlex® ESX 16 kit negative control DNA profile

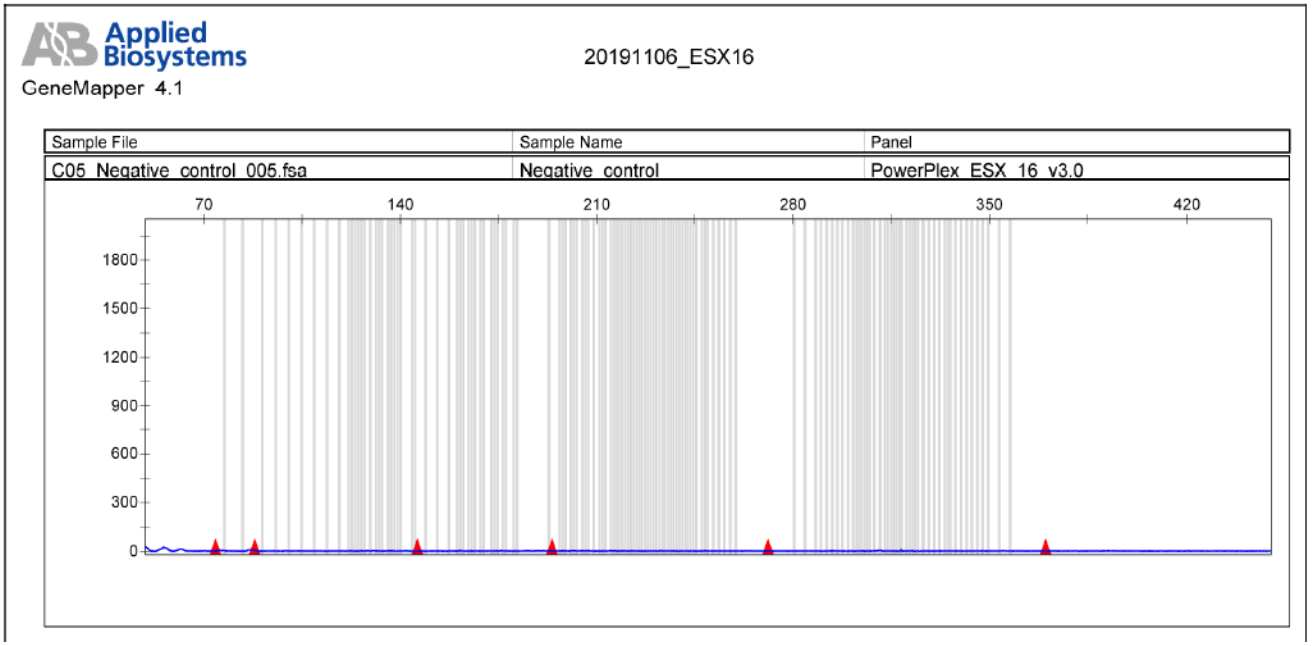

Figure 6.3. Negative control DNA profile generated from the Applied Biosystems® Gene Mapper 4.1 software using the using the PowerPlex® ESX 16 kit (Promega, Madison).

Appendix F: Table of results

Table 6.2. Raw data showing the results from all the analyses performed on touch DNA samples and buccal samples (n = 200). Presence of an asterisk (*****) indicates that the analysis was not performed for the specific sample.

Sample ID	Sample type	Presence of filters	Swab type	Qubit™ (ng/µL)	qPCR (ng/µL)	DNA profiling (Number of STR markers called)
HID_004.1 G 1	Touch DNA	Filter	Cotton	0,464	0,03001	0
HID_004.1 E 1	Touch DNA	No Filter	Cotton	0,892	0,03922	15
HID_004.1 H 1	Touch DNA	Filter	Flocked	0,078	0,003367	11
HID_004.1 F 1	Touch DNA	No Filter	Flocked	0,12	0,006576	14.5
HID_005.1 G 1	Touch DNA	Filter	Cotton	0,558	0,011657	12
HID_005.1 E 1	Touch DNA	No Filter	Cotton	0,876	0,05904	14.5
HID_005.1 H 1	Touch DNA	Filter	Flocked	0,028	0,00473	10.5
HID_005.1 F 1	Touch DNA	No Filter	Flocked	0,093	0,008873	14
HID_006.1 G 1	Touch DNA	Filter	Cotton	0,0652	0	*
HID_006.1 E 1	Touch DNA	No Filter	Cotton	0,05	0	*
HID_006.1 H 1	Touch DNA	Filter	Flocked	0,0444	0	*
HID_006.1 F 1	Touch DNA	No Filter	Flocked	0,142	0,036864	15
HID_007.1 G 1	Touch DNA	Filter	Cotton	0,366	0,005568	10
HID_007.1 E 1	Touch DNA	No Filter	Cotton	0,584	0,096948	15
HID_007.1 H 1	Touch DNA	Filter	Flocked	0	0	*
HID_007.1 F 1	Touch DNA	No Filter	Flocked	0,222	0,061507	15
HID_008.1 G 1	Touch DNA	Filter	Cotton	0,367	0	*
HID_008.1 E 1	Touch DNA	No Filter	Cotton	0,871	0	*
HID_008.1 H 1	Touch DNA	Filter	Flocked	0,137	0,002453	7
HID_008.1 F 1	Touch DNA	No Filter	Flocked	0,121	0,002445	6.5
HID_009.1 G 1	Touch DNA	Filter	Cotton	0,649	0,00226	0
HID_009.1 E 1	Touch DNA	No Filter	Cotton	0,718	0,00257	0
HID_009.1 H 1	Touch DNA	Filter	Flocked	0,0292	0,002869	0
HID_009.1 F 1	Touch DNA	No Filter	Flocked	0,1	0	*
HID_010.1 G 1	Touch DNA	Filter	Cotton	0,409	0,034174	1.5
HID_010.1 E 1	Touch DNA	No Filter	Cotton	0,561	0,031991	13.5
HID_010.1 H 1	Touch DNA	Filter	Flocked	0,0596	0	*
HID_010.1 F 1	Touch DNA	No Filter	Flocked	0,12	0	*
HID_011.1 G 1	Touch DNA	Filter	Cotton	0,486	0,008634	0
HID_011.1 E 1	Touch DNA	No Filter	Cotton	0,491	0,005532	0.5
HID_011.1 H 1	Touch DNA	Filter	Flocked	0,0972	0	*
HID_011.1 F 1	Touch DNA	No Filter	Flocked	0,0568	0	*
HID_012.1 G 1	Touch DNA	Filter	Cotton	0,283	0,009438	4.5
HID_012.1 E 1	Touch DNA	No Filter	Cotton	0,363	0	*
HID_012.1 H 1	Touch DNA	Filter	Flocked	0,114	0,005971	9.5
HID_012.1 F 1	Touch DNA	No Filter	Flocked	0,113	0,002338	3

HID_013.1 G 1	Touch DNA	Filter	Cotton	0,482	0,007173	0.5
HID_013.1 E 1	Touch DNA	No Filter	Cotton	0,568	0,001805	4
HID_013.1 H 1	Touch DNA	Filter	Flocked	0,155	0,000888	1.5
HID_013.1 F 1	Touch DNA	No Filter	Flocked	0,089	0,004482	7
HID_014.1 G 1	Touch DNA	Filter	Cotton	0,407	0,001691	3.5
HID_014.1 E 1	Touch DNA	No Filter	Cotton	0,904	0,000813	0
HID_014.1 H 1	Touch DNA	Filter	Flocked	0,138	0,003403	7
HID_014.1 F 1	Touch DNA	No Filter	Flocked	0,149	0,002979	2
HID_015.1 G 1	Touch DNA	Filter	Cotton	0,435	0,003883	0
HID_015.1 E 1	Touch DNA	No Filter	Cotton	0,753	0,001093	1
HID_015.1 H 1	Touch DNA	Filter	Flocked	0,127	0,00316	12
HID_015.1 F 1	Touch DNA	No Filter	Flocked	0,103	0,002711	6
HID_016.1 G 1	Touch DNA	Filter	Cotton	0,33	0,000901	4.5
HID_016.1 E 1	Touch DNA	No Filter	Cotton	0,69	0	*
HID_016.1 H 1	Touch DNA	Filter	Flocked	0,417	0,00163	0
HID_016.1 F 1	Touch DNA	No Filter	Flocked	0,112	0	*
HID_017.1 G 1	Touch DNA	Filter	Cotton	2,06	0,486124	15
HID_017.1 E 1	Touch DNA	No Filter	Cotton	1,4	0,273868	13.5
HID_017.1 H 1	Touch DNA	Filter	Flocked	0,693	0,180842	14.5
HID_017.1 F 1	Touch DNA	No Filter	Flocked	0,503	0,123564	14.5
HID_018.1 G 1	Touch DNA	Filter	Cotton	0,381	0,009137	13.5
HID_018.1 E 1	Touch DNA	No Filter	Cotton	0,732	0,005512	8
HID_018.1 H 1	Touch DNA	Filter	Flocked	0,174	0,010868	0
HID_018.1 F 1	Touch DNA	No Filter	Flocked	0,132	0,004383	0
HID_019.1 G 1	Touch DNA	Filter	Cotton	0,575	0	*
HID_019.1 E 1	Touch DNA	No Filter	Cotton	0,446	0,001616	5.5
HID_019.1 H 1	Touch DNA	Filter	Flocked	0,085	0,000994	7
HID_019.1 F 1	Touch DNA	No Filter	Flocked	0,08	0,000904	0
HID_020.1 G 1	Touch DNA	Filter	Cotton	0,637	0,001882	3
HID_020.1 E 1	Touch DNA	No Filter	Cotton	0,638	0,002895	2.5
HID_020.1 H 1	Touch DNA	Filter	Flocked	0,136	0,00166	0
HID_020.1 F 1	Touch DNA	No Filter	Flocked	0,09	0,003971	7
HID_021.1 G 1	Touch DNA	Filter	Cotton	0,315	0	*
HID_021.1 E 1	Touch DNA	No Filter	Cotton	0,392	0	*
HID_021.1 H 1	Touch DNA	Filter	Flocked	0,072	0,001807	5
HID_021.1 F 1	Touch DNA	No Filter	Flocked	0,325	0	*
HID_022.1 G 1	Touch DNA	Filter	Cotton	0,405	0,023898	0
HID_022.1 E 1	Touch DNA	No Filter	Cotton	0,679	0,0196	0
HID_022.1 H 1	Touch DNA	Filter	Flocked	0,264	0,042571	0
HID_022.1 F 1	Touch DNA	No Filter	Flocked	0,286	0,034677	15
HID_023.1 G 1	Touch DNA	Filter	Cotton	0,572	0,00082	4.5
HID_023.1 E 1	Touch DNA	No Filter	Cotton	0,776	0,004464	6.5
HID_023.1 H 1	Touch DNA	Filter	Flocked	0,15	0,013071	10.5
HID_023.1 F 1	Touch DNA	No Filter	Flocked	0,097	0,000913	1.5

HID_024.1 G 1	Touch DNA	Filter	Cotton	0,477	0,002838	7
HID_024.1 E 1	Touch DNA	No Filter	Cotton	0,542	0,000895	8
HID_024.1 H 1	Touch DNA	Filter	Flocked	0,063	0	*
HID_024.1 F 1	Touch DNA	No Filter	Flocked	0,078	0,003004	6
HID_025.1 G 1	Touch DNA	Filter	Cotton	0,425	0,004677	7
HID_025.1 E 1	Touch DNA	No Filter	Cotton	0,529	0,008671	11.5
HID_025.1 H 1	Touch DNA	Filter	Flocked	0,151	0,008143	3
HID_025.1 F 1	Touch DNA	No Filter	Flocked	0,129	0,000922	10.5
HID_026.1 G 1	Touch DNA	Filter	Cotton	0,566	0	*
HID_026.1 E 1	Touch DNA	No Filter	Cotton	0,705	0,000951	2
HID_026.1 H 1	Touch DNA	Filter	Flocked	0,091	0,003001	9.5
HID_026.1 F 1	Touch DNA	No Filter	Flocked	0,159	0,000894	3
HID_027.1 G 1	Touch DNA	Filter	Cotton	0,302	0	*
HID_027.1 E 1	Touch DNA	No Filter	Cotton	0,631	0,009307	0
HID_027.1 H 1	Touch DNA	Filter	Flocked	0,121	0	*
HID_027.1 F 1	Touch DNA	No Filter	Flocked	0,1	0	*
HID_028.1 G 1	Touch DNA	Filter	Cotton	0,309	0,010748	0
HID_028.1 E 1	Touch DNA	No Filter	Cotton	0,095	0,001677	0
HID_028.1 H 1	Touch DNA	Filter	Flocked	0,259	0,039366	0
HID_028.1 F 1	Touch DNA	No Filter	Flocked	0,15	0,040991	0
HID_004.1 C 1	Buccal cells	Filter	Cotton	19,7	34,63575	*
HID_004.1 A 1	Buccal cells	No Filter	Cotton	24,6	43,23519	*
HID_004.1 D 1	Buccal cells	Filter	Flocked	40,6	59,42717	*
HID_004.1 B 1	Buccal cells	No Filter	Flocked	24,1	30,75647	*
HID_005.1 C 1	Buccal cells	Filter	Cotton	25,2	26,74847	*
HID_005.1 A 1	Buccal cells	No Filter	Cotton	57	140,26536	*
HID_005.1 D 1	Buccal cells	Filter	Flocked	29,5	113,0769	*
HID_005.1 B 1	Buccal cells	No Filter	Flocked	1,04	55,63078	*
HID_006.1 C 1	Buccal cells	Filter	Cotton	10,8	9,212084	*
HID_006.1 A 1	Buccal cells	No Filter	Cotton	7	10,62137	*
HID_006.1 D 1	Buccal cells	Filter	Flocked	11,6	19,84065	*
HID_006.1 B 1	Buccal cells	No Filter	Flocked	5,97	8,156688	*
HID_007.1 C 1	Buccal cells	Filter	Cotton	6,31	10,76287	*
HID_007.1 A 1	Buccal cells	No Filter	Cotton	4,58	4,347147	*
HID_007.1 D 1	Buccal cells	Filter	Flocked	13,2	22,07169	*
HID_007.1 B 1	Buccal cells	No Filter	Flocked	45,2	46,72857	*
HID_008.1 C 1	Buccal cells	Filter	Cotton	62,8	103,73486	*
HID_008.1 A 1	Buccal cells	No Filter	Cotton	31,1	41,32247	*
HID_008.1 D 1	Buccal cells	Filter	Flocked	36,4	47,03244	*
HID_008.1 B 1	Buccal cells	No Filter	Flocked	29,2	30,75647	*
HID_009.1 C 1	Buccal cells	Filter	Cotton	52,7	67,33276	*
HID_009.1 A 1	Buccal cells	No Filter	Cotton	22,6	33,46972	*
HID_009.1 D 1	Buccal cells	Filter	Flocked	43	57,95552	*
HID_009.1 B 1	Buccal cells	No Filter	Flocked	36,9	44,14135	*

OPT_046.1	Buccal cells	No Filter	Cotton	9,34	1,319291	*
OPT_046.2	Buccal cells	No Filter	Flocked	42,1	8,947326	*
OPT_046.3	Buccal cells	Filter	Cotton	5,19	9,359045	*
OPT_046.4	Buccal cells	Filter	Flocked	5,48	23,26988	*
OPT_047.1	Buccal cells	No Filter	Cotton	37,7	15,53289	*
OPT_047.2	Buccal cells	No Filter	Flocked	4,97	14,05569	*
OPT_047.3	Buccal cells	Filter	Cotton	2,41	12,87523	*
OPT_047.4	Buccal cells	Filter	Flocked	25,8	83,32227	*
OPT_048.1	Buccal cells	No Filter	Cotton	1,16	6,868811	*
OPT_048.2	Buccal cells	No Filter	Flocked	7,69	24,71611	*
OPT_048.3	Buccal cells	Filter	Cotton	17	7,389314	*
OPT_048.4	Buccal cells	Filter	Flocked	9,34	33,54068	*
OPT_049.1	Buccal cells	No Filter	Cotton	5,64	22,68587	*
OPT_049.2	Buccal cells	No Filter	Flocked	13	46,90216	*
OPT_049.3	Buccal cells	Filter	Cotton	6,27	29,03755	*
OPT_049.4	Buccal cells	Filter	Flocked	15	49,46185	*
OPT_050.1	Buccal cells	No Filter	Cotton	8,6	28,55254	*
OPT_050.2	Buccal cells	No Filter	Flocked	12,2	44,1716	*
OPT_050.3	Buccal cells	Filter	Cotton	2,19	10,41765	*
OPT_050.4	Buccal cells	Filter	Flocked	15,4	50,5566	*
OPT_051.1	Buccal cells	No Filter	Cotton	13	0	*
OPT_051.2	Buccal cells	No Filter	Flocked	40	0	*
OPT_051.3	Buccal cells	Filter	Cotton	4,05	2,182515	*
OPT_051.4	Buccal cells	Filter	Flocked	6,87	27,76701	*
OPT_052.1	Buccal cells	No Filter	Cotton	11,4	42,37275	*
OPT_052.2	Buccal cells	No Filter	Flocked	11,2	44,65322	*
OPT_052.3	Buccal cells	Filter	Cotton	4,32	21,15126	*
OPT_052.4	Buccal cells	Filter	Flocked	9,15	33,84863	*
OPT_053.1	Buccal cells	No Filter	Cotton	18,7	8,07635	*
OPT_053.2	Buccal cells	No Filter	Flocked	12,4	37,84806	*
OPT_053.3	Buccal cells	Filter	Cotton	1,93	13,16233	*
OPT_053.4	Buccal cells	Filter	Flocked	10,6	37,59569	*
OPT_054.1	Buccal cells	No Filter	Cotton	3,98	32,75222	*
OPT_054.2	Buccal cells	No Filter	Flocked	8,1	30,70256	*
OPT_054.3	Buccal cells	Filter	Cotton	12,5	46,57648	*
OPT_054.4	Buccal cells	Filter	Flocked	37,2	123,0588	*
OPT_055.1	Buccal cells	No Filter	Cotton	3,31	15,2516	*
OPT_055.2	Buccal cells	No Filter	Flocked	17,2	64,80853	*
OPT_055.3	Buccal cells	Filter	Cotton	17,1	60,72326	*
OPT_055.4	Buccal cells	Filter	Flocked	22,6	76,81903	*
OPT_056.1	Buccal cells	No Filter	Cotton	4,3	15,17614	*
OPT_056.2	Buccal cells	No Filter	Flocked	10,1	11,7121	*
OPT_056.3	Buccal cells	Filter	Cotton	17,7	72,2508	*
OPT_056.4	Buccal cells	Filter	Flocked	48,4	182,2616	*

OPT_057.1	Buccal cells	No Filter	Cotton	11,4	48,6866	*
OPT_057.2	Buccal cells	No Filter	Flocked	25	86,42796	*
OPT_057.3	Buccal cells	Filter	Cotton	35,7	116,5793	*
OPT_057.4	Buccal cells	Filter	Flocked	64	186,70804	*
OPT_058.1	Buccal cells	No Filter	Cotton	1,19	9,288075	*
OPT_058.2	Buccal cells	No Filter	Flocked	4,94	22,20477	*
OPT_058.3	Buccal cells	Filter	Cotton	1,17	9,726451	*
OPT_058.4	Buccal cells	Filter	Flocked	26,7	75,68258	*
OPT_059.1	Buccal cells	No Filter	Cotton	7,47	24,43144	*
OPT_059.2	Buccal cells	No Filter	Flocked	10,9	39,01499	*
OPT_059.3	Buccal cells	Filter	Cotton	1,19	10,65385	*
OPT_059.4	Buccal cells	Filter	Flocked	16,3	57,5778	*
OPT_044.1A	Buccal cells	Filter	Flocked	59	16,989628	*
OPT_044.1B	Buccal cells	No Filter	Flocked	1,73	12,20719	*
OPT_044.1C	Buccal cells	Filter	Cotton	2,03	9,687248	*
OPT_044.1D	Buccal cells	No Filter	Cotton	16,6	6,121189	*
OPT_05.1C	Buccal cells	Filter	Flocked	1,79	10,64985	*
OPT_05.1D	Buccal cells	No Filter	Flocked	1,13	9,546407	*
OPT_05.1E	Buccal cells	Filter	Cotton	1,04	9,650854	*
OPT_05.1F	Buccal cells	No Filter	Cotton	13	5,016959	*
OPT_043.1A	Buccal cells	Filter	Flocked	13,9	5,954733	*
OPT_043.1B	Buccal cells	No Filter	Flocked	18,8	7,318074	*
OPT_043.1C	Buccal cells	Filter	Cotton	13,5	5,322404	*
OPT_043.1D	Buccal cells	No Filter	Cotton	5,92	2,381949	*
OPT_01.1D	Buccal cells	Filter	Flocked	6,67	2,791808	*
OPT_01.1E	Buccal cells	No Filter	Flocked	3,43	1,622508	*
OPT_01.1F	Buccal cells	No Filter	Cotton	5,13	2,26797	*
OPT_01.1G	Buccal cells	Filter	Cotton	5,52	2,210382	*
OPT_09.1C	Buccal cells	Filter	Flocked	9,12	3,210719	*
OPT_09.1D	Buccal cells	No Filter	Flocked	3,89	1,770019	*
OPT_09.1E	Buccal cells	Filter	Cotton	13,6	4,642054	*
OPT_09.1F	Buccal cells	No Filter	Cotton	1,18	3,991612	*

Appendix G: Table of *p*-values

Table 6.3. Table showing the different groups analysed and the *p*-values recovered for each of the 16 combinations.

DNA quantification method	**Sample type**	**Swab type**	**Presence of filters**	***p*-value**
Real-time PCR	Buccal cells	*Cotton swab*	*No filter vs filter*	0.801
		Flocked swab	*No filter vs filter*	0.035
		Cotton vs Flocked	*No filter*	0.470
		Cotton vs Flocked	*Filter*	0.021
	Touch DNA	*Cotton swab*	*No filter vs filter*	0.930
		Flocked swab	*No filter vs filter*	0.914
		Cotton vs Flocked	*No filter*	0.652
		Cotton vs Flocked	*Filter*	0.743
DNA profiling	Touch DNA	*Cotton swab*	*No filter vs filter*	0.429
		Flocked swab	*No filter vs filter*	0.408
		Cotton vs Flocked	*No filter*	0.726
		Cotton vs Flocked	*Filter*	0.665
Spearman's rank correlation coefficient				0.00003