Evaluation of capillary electrophoresis in the forensic DNA profiling of burnt teeth.

by

Adriaan Daniël Geldenhuys

GLDADR004

SUBMITTED TO THE UNIVERSITY OF CAPE TOWN

In partial fulfilment of the requirements for the degree

MPhil (Biomedical Forensic Science)

Division of Forensic Medicine and Toxicology

Faculty of Health Sciences

UNIVERSITY OF CAPE TOWN

12 July 2023

Supervisor: A/Prof. Laura Heathfield

Co-supervisors: Calvin Mole and Donna-Lee Martin

DECLARATION

I, Adriaan Daniël Geldenhuys, 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.

I have followed the referencing style according to the Forensic Science International journal and I have used the Forensic Science International referencing style as the convention for citation and referencing. Each significant contribution to, and quotation in, this dissertation from the work, or works of other people has been attributed, cited, and referenced.

This research project is funded by the National Research Foundation (NRF) Thuthuka Grant (UID: 129499) and falls under the umbrella study entitled “Optimisation and investigation of DNA recovery from different biological sample types for use in forensic human identification”.

Name: Adriaan Daniël Geldenhuys

Date: 13 February 2023

Signature: Signed by candidate

ABSTRACT

Fires are a frequent cause of death, both globally and in South Africa, and often, individuals are burnt beyond the point of visual recognition. Teeth maintain their structure and can withstand high temperatures; making them a possible source of DNA from burnt human remains. DNA profiling is the current gold standard in forensic human identification, however, limited literature pertaining to DNA profiling of burnt teeth exists. The aim of this study was thus to evaluate the success of capillary electrophoresis in the forensic DNA profiling of teeth burnt at different temperatures, using an optimised DNA extraction protocol.

Tooth samples from 25 donors (n = 100 [4 teeth per donor]) were subjected to three burning conditions, one tooth was left unburnt to act as a control and three teeth were each burnt in a muffle furnace at 100 ˚C, 200 ˚C, and 300 ˚C. The colour and weight of the teeth were recorded before and after burning. DNA was extracted using an optimised demineralisation step. Extracted DNA was quantified through real-time PCR and profiled using capillary electrophoresis with the Promega PowerPlex® ESX 16 system.

Teeth burnt at 100 ˚C resulted in the most full profiles (96 % ; n = 24/25), followed by teeth burnt at 200 ˚C (84 %; n = 21/25), with 16 % partial profiles obtained (n = 4/25). Teeth burnt at 300 ˚C resulted in a large number of failed profiles (88 % ; n = 22/25), and had a significant *decrease* in profiling success ($p = 0.001$) and concentration ($p = 0.001$), and were significantly more degraded ($p = 0.001$), compared to control samples and samples burnt at lower temperatures. These results suggest that conventional DNA profiling methods and the DNA extraction method used herein are suitable for obtaining full DNA profiles from teeth exposed to temperatures as high as 200 ˚C, however, more sensitive methods such as targeted next generation sequencing (NGS) would be recommended to obtain more insight into highly degraded and fragmented samples, such as those burnt at 300 ˚C.

ACKNOWLEDGEMENTS

I would like to thank my supervisors Assoc. Prof. Laura Heathfield and Calvin Mole for their assistance and guidance throughout the duration of this project. I especially wish to thank Donna-Lee Martin for stepping in as a co-supervisor and for the motivation, guidance and time offered to me despite her other duties.

I wish to thank the Division of Forensic Medicine and Toxicology at UCT and all individuals that have accompanied me on this journey.

Lastly, I would like to thank my family, without whom my academic journey would have been impossible.

TABLE OF CONTENTS

ABBREVIATIONS

General Abbreviations	
SAPS	South African Police Service
NGS / MPS	Next Generation Sequencing / Massive Parallel sequencing
CE	Capillary Electrophoresis
LMIC	Low- and Middle- Income Countries
PCR	Polymerase Chain Reaction
qPCR	real time PCR (quantitative)
DNA	Deoxyribonuclease Acid
STR	Short Tandem repeats
SNP	Single Nucleotide Polymorphism
CODIS	Combined DNA Index System
SNV	Single Nucleotide Variant
iSNP	identification SNP
aiSNP	ancestry-inference SNP
piSNP	Phenotypic-inference SNP
liSNP	Lineage-inference SNP
dNTP	Deoxynucleotide Triphosphate
NFDD	National Forensics DNA Database
IPC	Internal PCR Control
Ct.	Threshold value / Cut off value
DI	Degradation Index
EDTA	Ethylenediaminetetraacetic acid
DTT	Dithiothreitol
SDS	Sodium Dodecyl Sulphate
Measurement Abbreviations / Units	
bp	Base pair
µL	Microliter
mL	Milliliter
ng	Nanogram
g	Grams
˚C	Degrees Celsius
Hz	Hertz
rpm	Rotations per minute
RFU	Relative Fluorescence Unit

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1
INTRODUCTION

1.1. BACKGROUND

Unidentified human remains can be found in nearly any medico-legal mortuary, both locally and internationally [1]. The burden of such cases is particularly evident in developing countries such as South Africa, where the incidences of unnatural deaths are extremely high, and the mortuaries overburdened with high caseloads [2]. The identification of deceased individuals who are said to have died due to other than natural causes is mandated by the *Inquest Act 58 (Act No58 of 1959)*, whereby a magistrate must make a finding as to the identity of the deceased, cause of death, date of death, and whether someone should be held liable for the death. The investigation of an unnatural death is done by the Forensic Pathology Service in conjunction with the South African Police Service (SAPS).

DNA has long been proven to be the most accurate and validated method used for forensic human identification, both in the deceased and in the living. The widely accepted gold standard sample type for use in forensic DNA identification of deceased individuals is blood, but this is not always available [3]. In cases of severe decomposition, skeletonisation or burns, forensic scientists need to turn to other sources of DNA, such as bones and teeth [4–6]. Fire mortalities are a frequent occurrence in South Africa and place an immense burden on the already overburdened medico-legal mortuaries [7]. Cape Town has been identified as the city with the highest fire related fatalities in South Africa, this is mainly attributed to the high volume of fire incidences that occur in the ever-expanding informal settlements and veldfires (wildfires) of the fire prone fynbos shrublands [8–10].

In the case of fire related fatalities, many individuals are burnt beyond the point at which visual identification is possible, which results in other avenues of identification, such as DNA profiling, being used [4,11]. Many of these cases are never identified and as such the mortuaries are burdened by many individuals who remain unidentified [2]. Furthermore, conventional methods of DNA profiling do not function well with degraded DNA, often resulting in no or partial DNA profiles. Next generation sequencing (NGS) has been proven to be valuable in instances of degraded DNA, as smaller DNA segments (amplicons), which are less prone to degradation, are targeted [12]. In the instances of severely burnt remains, teeth may provide sufficient amounts of DNA for subsequent analysis as they are protected, isolated and can withstand relatively high temperatures [4].

The aim of this literature review is to assess the literature as it pertains to the use of burnt teeth in forensic DNA applications, specifically in DNA profiling through conventional capillary electrophoreses. The focus is first placed on fire related fatalities and describing these in a South African context. This is followed by examining teeth as a source of DNA and an assessment of DNA extracted from burnt bones and teeth. The focus then moves to DNA profiling, where the general principles are explained, and its application to burnt teeth. At the end of the chapter the aim and objectives of the research are formulated through a rationale.

1.2. FIRE RELATED FATALITIES

Fires are a common and frequent occurrence in South Africa that often result in deaths. Burns are frequently severe enough that visual identification of deceased individuals is not possible [8,13]. Globally, burns present as the 4th most common injury requiring urgent medical treatment, and is preceded by motor vehicle accidents, inter-personal violence, and falls [7]. Fatalities from burns have a global rate of 4.6 per 100 000, this is however not reflected by low- and middle-income countries (LMIC) such as South Africa where the fatal burn rate was estimated to be 6.1 per 100 000 in 2008 [14,15]. Risk factors identified for burn fatalities are majorly attributed to socioeconomic status and is especially high in those who live in informal settlements [14]. Informal settlements are identified, according to South Africa's National Housing Code, by their illegality and informality, vulnerability and poverty, and social stress [16].

A retrospective study (2011 – 2015) on burn fatalities in Pretoria, South Africa [17], indicated that approximately 52.2 % of burn fatalities occur indoors, 15.8 % outdoors, and in 32 % of burn fatalities the location was unknown. Of the 52.2 % of deaths that occurred indoors, 17.1 % occurred in formal dwellings whereas 69 % occurred in informal settlements and 13.9 % remained unknown. Overall, 3 % of cases admitted to the Pretoria Medicolegal Laboratories were burn fatalities, which is less than results from Cape Town [17]. South Africa stands in stark contrast when compared to the United States, where in the USA, 43 % of fire fatalities were a result of outdoor fires, and 38 % of fatalities occurred in structures [18]. Cape Town has one of the highest medico-legal caseloads in South Africa and has a burns mortality rate of 7.9 per 100 000, nearly double the global average [8,19]. Studies conducted in South Africa, although scarce, indicate that alcohol may be a common denominator in these fatalities [8]. The majority of fatal burns are accidental (69.8%), followed by homicidal (7.9 %) and suicidal (3.8 %) with 18.5 % with undetermined cause. This distribution reflects the general trend in the manner of deaths [17,19].

1.2.1 Veldfires (Bushfires)

South Africa along with other temperate regions are prone to experiencing veldfires, which result in devastating impacts on the economy and environment of the affected region, and often have human fatalities [20,21]. Factors that influence the severity of veldfires are extreme weather conditions such as droughts and strong winds, the availability of fuel, and invasion of flammable alien plant species [21]. It is important to note, veldfires are a natural phenomenon that is important for maintaining biodiversity and protecting the ecology of the region (especially fynbos) [22]. Issues and disastrous consequences arise when these fires occur too seldom or frequently, are mistimed, or too severe, which leads to ecosystem degradation [22]. If fires occur too seldom, overgrowth adds to fuel and hinder firefight capabilities which result in more severe veld fires, in contrast if fires occur too often then soil quality is reduced and invasive plant species can invade which are also prone to fire [21]. The general fire ecology of South Africa is represented in Figure 1.1 below.

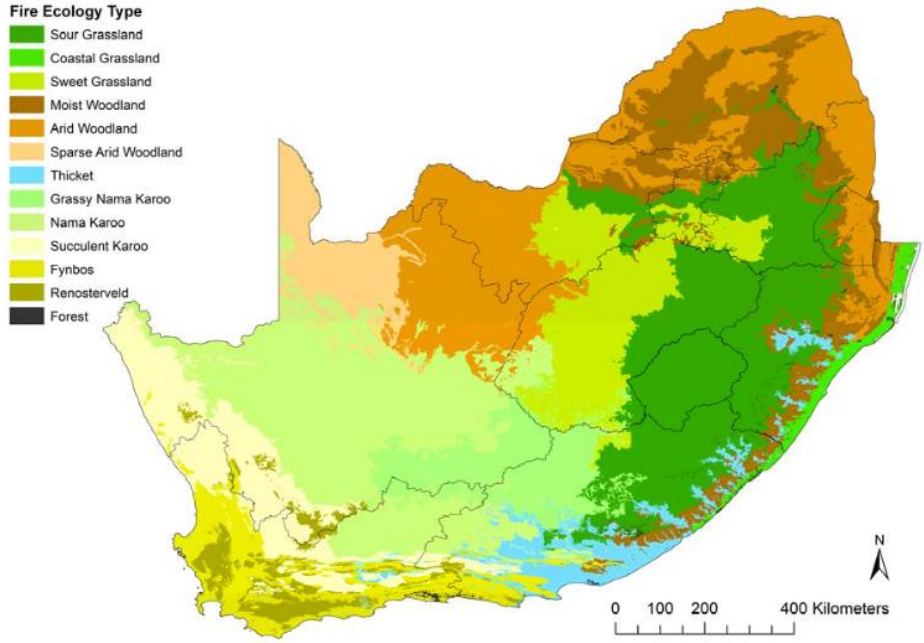

Figure 1.1: Map indicating the fire ecology of South Africa according to the CSIR report "National veldfire risk assessment: analysis of exposure of social, economic and environmental assets to veldfire hazards in South Africa".

The severity and frequency of veldfires in South Africa has become worse in the past few years [21,22]. This has been attributed to population growth, changes in land use patterns, and the expansion of the urban-rural interface, which ultimately increases the exposure risk of communities to wildfires [21,22]. Although very little information exists on fatalities and injury caused by wildfires, most of the evidence is anecdotal [22]. The California (USA) “Camp Fire” of 2018

resulted in the deaths of 85 individuals and was listed as one of the worst wildfire incidents to date where approximately 150 000 acres of land burned down [23]. It highlighted the need for rapid identification of the deceased and the importance of DNA. During the 2008 South African fire season a total of 35 000 fires were reported and approximately 380 deaths were caused by these wildfires [24]. The Knysna fire of 2017, burned over a period of four days across an area of 15 000 ha, and resulted in the deaths of seven people [21]. The most frequent causes for fire outbreaks have been attributed to anthropogenic activities or natural phenomenon such as lightning [20,25]. Other sources of ignition have been attributed to poor rural security which increase the likelihood of arson, and depopulation which results in a lack of resources such as emergency responses or fire management [22].

South Africa, as a whole, has a relatively high veldfire risk classification, with KZN having the largest area of extreme veldfire risk, as well as most of the north-eastern aspect of the country. The Western Cape has a patchwork of high and low veldfire risk, with Cape Town having a high risk along with the south-eastern coastline [22]. The north-eastern region of South Africa consists of mountainous grasslands and experience the most fires, this is followed by the Western Cape that has a high abundance of mountainous grasslands and Cape fynbos, a local plant biome that is second in terms of most frequent fauna to experience fires [24,26]. Figure 1.2 indicates the relative wildfire risk classifications in South Africa.

The Cape fynbos is the most fire prone vegetation type in the Western Cape and is associated with dry sandy nutrient-poor soil [27]. Fire is an important tool in germination and fynbos management [10,28]. Fires occur throughout the year, but the more severe and larger fires are observed in the warm and dry summer season [27,28]. The intensity of the fire is estimated to range between 500 – 20 000 kW/m, with flame hights reaching 7 meters [10,27]. Several factors exist that effect the rate of spread, intensity, and height of flames. Major contributions come from the weather and fuel moisture content, where greater wind speeds, temperature, and age of fauna aid in the rate of spread and intensity [27].

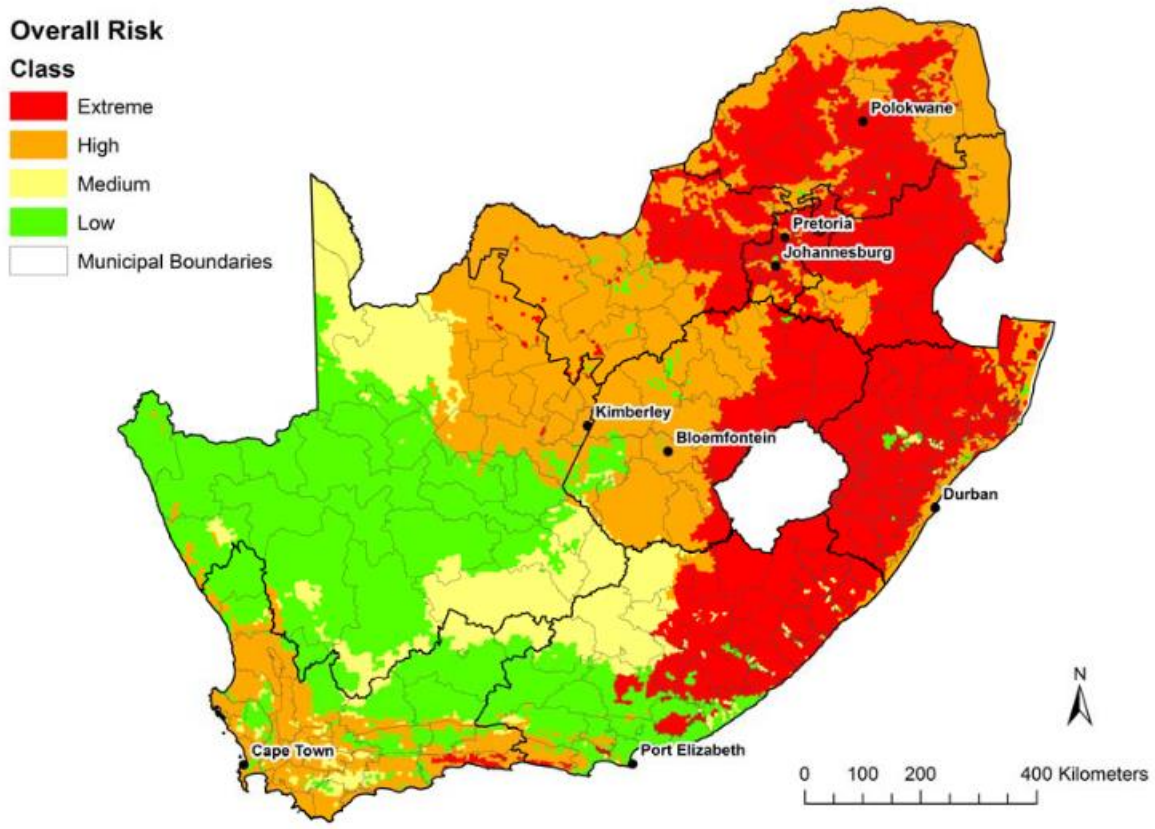

Figure 1.2: Map indicating the overall wildfire risk assessment for South Africa according to the CSIR report "National veldfire risk assessment: analysis of exposure of social, economic and environmental assets to veldfire hazards in South Africa".

1.2.2. Fires in informal settlements

As mentioned earlier, majority of fatalities due to fires occur in informal settlements. Approximately one third of the South African population live in informal dwellings. In Cape Town alone, it was estimated in 2006 that there are about 104 000 informal dwellings, and this number grows drastically yearly [29]. In 2016 it was estimated that approximately 1 in 7 households in South Africa live in informal settlements, with this number being higher in metropolitan areas [16]. It was estimated that in 2019, approximately 174 000 households live in informal settlements in the City of Cape Town [30]. In conjunction to this, the populations in informal settlements have increased significantly due to mass migration from rural areas to major cities in search of better opportunities. To this end, the rate of informal settlement expansion is estimated to be 9 % per annum [30,31]. Census data from 2011 was used in calculating these estimates. Risk factors that have been attributed to the high incidence of fires in informal settlements include; densely populated and over-crowded housing, no access to running water, illegal electricity connections, the use of kerosene stoves, and open fires used for heat and cooking [17,32].

Informal settlements are rife with flammable materials and in many instances, deaths are caused by smouldering fires in confined spaces as oxygen is removed and noxious gasses such as carbon monoxide and cyanide are released [29]. In 2017, in the informal settlement of Imizamo Yethu (Cape Town, South Africa), a fire broke out that took 14 hours to extinguish, leading to the destruction of 2194 dwellings, 9700 individuals displaced and four fatalities [31]. In December of 2021, a fire in the Masiphumelele "Masi" (Cape Town, South Africa) informal settlement destroyed approximately 1030 dwellings, leaving 4000 individuals displaced [33]. The largest factors affecting the devastation and spread of the fire was the material used in the building of the dwellings, inaccessible routes that hindered the movements of emergency personal, inadequate water supply, and the strong winds and high temperatures typical in Cape Town (Nov-April) [31]. These factors are shared with almost all informal settlements in the area. It is estimated that there are between 1200 and 1300 informal settlement fires every year in the Western Cape alone and an estimate of 10 informal settlement fires per day across South Africa [34].

A brief overview of burning time and temperatures of fires in informal settlements indicates that a typical informal house (shack) reaches its peak temperature after 10 minutes of burning, with roof gas temperatures reaching 1000 °C at this point and then steadily declines. This is all dependent on the type of fuel available. A repeat study using a timber shack replica indicated a roof temperature of 900 °C with floor temperatures reaching a maximum of 650 °C at 10 minutes [8]. Temperatures started to decrease after 15 minutes and by 20 minutes after ignition, temperatures were close to 400 °C. It is important to note that temperatures varied greatly according to ventilation as well as the location of the fire within the shack [8].

The extent of burns is highly variable and depends on factors such as exposure time, temperature of the fire, and an individual's activity at or around the time of death. These may render it near impossible for visual identification [17]. In the previously mentioned Pretoria study, visual identification was only possible in 7.8 % of burn cases with the other 92.2 % of cases being charred and requiring other forms of identification. Of the 92.2 %, only 29.3 % of the identities were confirmed through the use of DNA techniques [17]. This suggests either a low DNA yield or that severely degraded DNA was obtained in these cases due to extreme thermal stress. This motivates for further research into optimisation of methods used to obtain DNA profiles from severely degraded human remains.

1.3. TEETH AS A SOURCE OF DNA

The use of DNA for human identification is globally accepted as the most scientifically accurate and valid method of identification [35]. It is frequently used in criminal settings to identify victims and perpetrators, and in civil cases such as paternity testing. It is also frequently used in cases of unidentified human remains and in identifying the missing [35].

In cases of skeletonised, decomposed or charred remains where conventional identification methods such as forensic odontology fail due to the lack of ante mortem data, biological methods such as DNA is required for identification [4,36]. In these instances, bone and teeth are considered as potential sources of DNA as soft tissue is typically of little use [37]. Teeth have been suggested as a sample type as they are relatively isolated and protected from decomposition and the environment, and can withstand high temperatures [4,36,38–40]. Molars and premolars are the most frequently prescribed in the literature for DNA profiling, as they are multi rooted and contain large pulp chambers. In contrast, a recent study found that canines yielded the highest DNA concentrations, with the premolars having the greatest profiling success [6]. Molars on the other hand had the lowest profiling success [6]. This is attributed to molars being the most frequent tooth morphotype to undergo restorative processes which reduces the amount of DNA available and introduces polymerase chain reaction (PCR) inhibitors [6,41]. This, however, may not necessarily be the case in burnt remains, as the anterior teeth are more likely to be incinerated and are less protected than the posterior teeth due to heat contractions of the muscles that expose the anterior teeth. The molars and premolars are protected by both soft tissue and the alveolar bone, making them more resistant to heat related damage [11,42]. This suggests that the molars and premolars might be the only viable option in some cases of burnt remains, but the anterior teeth can still potentially be of use.

Various studies have been conducted on which components of teeth contain DNA and should be selected/ isolated for extraction. Initial findings suggested that dentine and tooth pulp were the only components that yielded DNA [43], later studies found that cementum also provided a source for DNA [41]. The amount of DNA obtained has been attributed to the level of degradation, PCR inhibition, and/or the DNA extraction method used. In general, a demineralisation step is widely accepted and included in the DNA extraction protocol for hard tissues as calcium and collagen act as PCR inhibitors if coextracted [44,45].

1.3.1. Tooth colour

Dental colour has been proposed as a potential tool in estimating the DNA concentration of burnt teeth, as studies have shown that a significant correlation exists between the colour of teeth and the

amount of DNA [46]. Several tools exist such as spectrophotometry and simpler visual tools like the Munsell colour chart [47,48]. The Munsell colour chart separates colour by “hue”, “value” and “chroma” and is presented as a code. For example, hue is denoted as “2.5Y”, value and chroma as “8/1” and the colour associated therewith, the full colour code would therefore be “2.5Y 8/1 White”.

(Rubio *et al.* (2018) found that at a temperature of 100 ˚C teeth turned a yellow white colour and at 200 ˚C a yellowish brown with a drastic change to dark brown at 400 ˚C after being burnt for 60 minutes [49]. DNA concentrations associated to each burning temperature indicated a strong correlation in the amount of extracted DNA. At 200 ˚C the average DNA concentration was approximately 0.1 ng/µL and at 400 ˚C DNA concentrations were below 0.001 ng/µL [49]. Beach *et al.* (2008) observed Munsell colour changes from a 5Y 8/3 (pale yellow) to 10YR 7/8 (yellow) for root structures after being burnt at 204 ˚C for 60 minutes, whereas at 30 minutes of burning no significant differences were observed [47]. They found that teeth burnt at 260 ˚C for 30 minutes had a drastic change whereby the enamel turned a pale brown (10YR 8/2) and the root a dark reddish brown (2.5YR 2.5/3). Further drastic changes were observed in the enamel of teeth burnt at 371 ˚C for 30 minutes, whereby the enamel turned a dark greyish brown (10YR 3/2) with a glossy appearance [47].

These colour changes occur due to dehydration of the dental tissue as well as the break down in collagen. Teeth also undergo a change in weight as dehydration results in a loss in mass [46–48]. Thus, colour estimations of teeth may provide useful information as to the approximate burning temperature as well as the potential amount of DNA that can be extracted.

1.4. DNA PROFILING

1.4.1. STRs and SNPs

DNA profiling makes use of variable polymorphic regions in the human genome called short tandem repeats (STRs). These STRs consist of 2-6 base pair (bp) units of DNA that repeat itself consecutively and the number of repeats differ between individuals [12]. Primers specific to each STR marker are tagged with a fluorescent dye and amplified using primers that are complementary to the 5’ and 3’ ends of the STR [50,51]. There are 13 core CODIS (Combined DNA Index System) STR loci/ genetic markers that form the essence of DNA profiling in the USA. These markers are D8S1179, CSF1PO, D3S1358, D21S11, D5S818, D7S820, D13S317, D16S539, D18S51, FGA, TH01, TPOX, and vWA. In addition to this, Amelogenin, the biological sex marker is also included [50].

Single nucleotide variants (SNVs) are sequence variations at a single nucleotide that occur approximately every few hundred bases in the human genome, similarly, single nucleotide polymorphisms (SNPs) are substitutions at a single nucleotide that occurs in approximately 1 % of a population [12]. The use of SNPs in forensics is currently being used more often in forensic investigations, and there is a plethora of research that has been conducted [12,52]. Currently SNPs are being used in four major areas: individual identification (iSNPs), ancestry-inference (aiSNPs), lineage-inference (liSNPs), and phenotypic-inference (piSNPs) [53]. SNPs have very low mutation rates, nearly 100 thousand times lower than STRs, giving them more inheritance stability [12,52]. Other benefits include the fact that there are a multitude of SNPs that can be analysed, and smaller segments are targeted making them more useful for degraded DNA samples [52,54]. The discriminatory power of SNPs is however far lower than STRs and therefore more SNPs are required (40-60 SNPs to reach the same discriminatory power of STR analysis) [12].

1.4.2. DNA amplification

STRs are amplified through a process known as the polymerase chain reaction (PCR), whereby short complementary DNA strands (primers) for each STR loci are used to amplify the targeted markers. The process starts by adding primers, buffer, dNTPs, and DNA polymerase to the extracted DNA sample [55]. The DNA is denatured at high temperatures which splits the DNA strands allowing for the primers to anneal to the targeted sequence. Once annealing of the primers has occurred, DNA polymerase extends the primers by adding dNTPs to create complementary strands. This process is repeated for several cycles (20-40 cycles), which intern increases the amount of targeted DNA exponentially [55]. This has greatly contributed to the success of DNA profiling methods as very low initial DNA concentrations are required [56].

For use in DNA profiling, several different commercial kits exist for the amplification of STR markers [56]. Typically, the primers for each STR loci are fluorescently tagged, allowing for detection of the amplified markers through capillary electrophoresis. These kits vary in the STR markers targeted as well as the type (autosomal, X and Y chromosomal markers). Commercial kits typically have a higher tolerance to inhibitors and an increased sensitivity [57].

1.4.3. Capillary electrophoresis

Capillary electrophoreses (CE) is a tool used to differentiate alleles at an STR locus by separating amplified STR markers by size and fluorescence. Genetic analysers have replaced normal gel-based techniques and have decreased the run times required when analysing multiple samples at the same

time [58]. An example of such is the Genetic Analyser 3500*xl* which has 24 capillaries with a 6-dye detection system for typing a multitude of STR markers. CE systems make use of a polymer as a matrix which is injected into the capillaries prior to sampling. The polymer acts as a sieve and thereby separating the ssDNA fragments by size, while the voltage carries the fragments by their negative charge. Smaller fragments pass through the polymer faster and therefore elute out first [59].

Fluorescently tagged amplified markers fluoresce when hit by a laser as they pass through a capillary, the fluoresced fragments are detected as peaks (RFU's) and represented on an electropherogram. STR alleles are thus generated from the fluorescent peaks and size information and are reported as the DNA profile of an individual [60,61]. The use of CE in forensic identification applications has been the gold standard for over two decades and is still currently used as such [62].

Before the samples are processed an internal size standard is added to each sample and is detected along with the STR markers. This ensures that STR alleles are sized correctly and uniformly across each capillary [59]. An allelic ladder consisting of all known STR alleles in a human population (kit specific) is run with each injection and is used to designate alleles at an STR loci [59].

1.4.4. Databases

DNA profiling results have long been used in police work and the creation of databases have further enhanced the power of forensic DNA data. They have allowed for the matching of profiles from different crime scenes and in identifying repeat offenders [63]. These have however raised public concern globally in terms of what samples are kept, what safeguards are in place for human rights, privacy, and whose profiles are stored and in what manner are they accessed/restricted [64].

In South Africa the *Criminal Law (Forensic Procedures) Amendment Act (2013),* known colloquially as the "DNA Act", has provided the provisions for DNA profiling, the collection and retention of forensic DNA samples, and further introduced the creation and maintenance of the National Forensic DNA Database (NFDD) [65]. The NFDD consists of five indices, each with its own regulation on the retention and destruction/removal of samples and DNA profiles from the database. The indices consist of an arrestee index, convicted criminals index, investigative index, elimination index, and a missing persons index [66]. In addition to this, population specific allele frequency databases exist and are required to calculate random match probabilities. Variation in allele frequencies are observed between different population groups and frequencies are generated from population studies [67,68].

1.4.5. Random match probability

Random match probability refers to the statistical weight applied to DNA evidence presented in court proceedings. It states the probability that a random individual's DNA profile (other than the profile in question), from a certain reference population group, will match the DNA profile in question [69,70]. This requires allelic frequencies from the specific population group to be known and therefore relies on available population data [71]. Such databases exist and contain the allelic frequencies for each forensic marker within a population and racial subpopulation [71,72].

Probabilities are calculated under the assumptions that STR loci are independent, in Hardy-Weinberg equilibrium, and that allele frequencies are known or in a database [73]. Similarly, the power of DNA evidence can be represented as a likelihood ratio through Bayesian statistics. This works with two hypothesis, one that the individual is the source of the DNA, and the other that the DNA originated from someone else [71,74]. If the same assumptions are met, the likelihood ratio is simply the reciprocal of the random match probability and is reported as the likelihood that the DNA originates from an individual compared to a random unrelated individual [70,73,75].

1.4.6. DNA quality

Degradation of DNA samples can severely affect the success of DNA profiling. Degradation is influenced by environmental factors such as temperature, UV radiation, humidity, inhibitors, microorganisms, and the post-mortem interval (PMI) [76]. Teeth and bone samples have a slower rate of degradation but have additional drawbacks such as the large volume of non-DNA components, the low quantity of DNA and the labour intensive and time-consuming extraction methods [76]. Often with mineral rich tissue, PCR inhibitors are also co-extracted and impact the success rates of amplification [77]. The internal PCR control threshold/cut-off value (IPC Ct.) is a useful tool in determining if inhibition is taking place. The IPC consists of an oligonucleotide that is present in equal concentrations in all samples and negative controls. It is therefore expected that IPC Ct. values will remain constant for all samples, unless PCR efficiency is affected by the presence of inhibitors [78]. IPC Ct. values greater than 30 typically indicate that PCR inhibition is taking place either due to the presence of inhibitors or an incorrect assay setup [78,79].

Degraded DNA can become chemically modified or highly fragmented, which reduces the number of intact targets. This ultimately leads to the failure of amplification and mistyping of loci [77]. Thermal degradation occurs around 130 ˚C whereby the covalent bonds between DNA molecules start to break and complete degradation occurs around 190 ˚C [80,81]. To quantify the amount or severity of DNA degradation, the degradation index (DI) is used. It is calculated as the ratio of the

small autosomal target (~80 bp) to the large autosomal target (~214 bp), with DI values less than 1.5 indicating no degradation, 1.5 - 4 indicating mild degradation, 4 – 10 indicating degradation, and greater than 10 indicating severe degradation [82]. Typical complications of degraded DNA in PCR consist of failure to amplify and preferential amplification in heterozygotes resulting in allele dropout. Smaller amplicons have a higher chance of being amplified in degraded samples, thus giving rise to newer kits with repositioned primers that are as close to the repeat motif as possible, these are referred to as mini-STRs [77,83]. The greatest genotyping success can therefore be found in the use of SNPs, as these are more likely to be intact as amplicon lengths range between 45-80 bp, far shorter than most STRs [83].

1.4.7. DNA from burnt teeth

Several studies have investigated the effects of temperature on DNA yield from hard tissues, especially teeth as they are protected and are the hardest tissue in the body [84]. One such study attempted to amplify a 317 bp fragment from the roots of teeth after incinerating the teeth at 600 ˚C, 800 ˚C, and 1000 ˚C for 10 minutes, 30 minutes and 60 minutes at each temperature. They found that regardless of temperature or length of time, the fragment could not be amplified [85]. Similarly, another study found that no amplifiable DNA could be extracted from bones burnt at temperatures higher than 250 ˚C, and at temperatures lower than 250 ˚C, DNA was severely degraded after burning for 15 minutes [86]. Tsuchimochi *et al.* (2002) attempted to amplify Y-STRs from dental pulp that had been heated. They found that DNA degradation was significant enough that they could not amplify DNA extracted after 2 minutes of burning at 400 ˚C [87].

Emery *et al.,* (2020) compared two DNA extraction methods by separating burnt bone and tooth samples into five categories based on their colouration (I: <200 ˚C, II: 200-300 ˚C, III: 300-550 ˚C, IV: 550-650 ˚C, V: >650 ˚C). They found that the average DNA concentration through qPCR for categories I-III ranged from 5.98 ng/µL – 0.62 ng/µL, categories IV and V had far lower means of 0.018 ng/µL and 0.0003 ng/µL [44]. An analysis of bones using the same burn categories found that in category I the average DNA concentration was 71.5 ng/µL, and 21.7 ng/µL in category II. In category III DNA concentrations ranged between 0.01 ng/µL and 0.84 ng/µL, and categories IV and V ranged from 0 - 0.17 ng/µL and 0 ng/µL – 0.03 ng/µL respectively [4]. Garriga *et al.* burned teeth at 100 ˚C, 200 ˚C, 300 ˚C, 400 ˚C, 500 ˚C, 600 ˚C, and 700 °C for 1 minute, 5 minutes, 10 minutes and 15 minutes durations and extracted the DNA using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). They found that DNA concentrations ranged from 31.8 ng/µL to < 0.05 ng/µL, with each burning duration the DNA concentrations declined and as the temperatures increased, specifically above (300 ˚C), DNA concentrations progressively became worse [84].

Table 1.1: Literature pertaining to DNA profiling/ amplification (PCR)/ quantification of burnt teeth/bone.

Author	Year	Sample	Species	Sample Size	Temp. Range (˚C)	Time (min)	DNA conc. (ng/ul)	Profiling/ PCR	Results
Lozano-Peral	2021	Teeth	Human	40	100-400	60	0-35.87	AmpFlSTR Identifiler Plus	Full DNA profiles < 100 ˚C
Emery	2020	Bone, Teeth	Human	123	200- >650	-	0.0003-6.27	PowerPlex ESX 17	Full DNA profiles (Categories I-III, < 350 ˚C)
Yukseloglu	2019	Bone, Teeth	Human	50	50-1000	10-60	0.02-13.48	Qubit	Quantifiable DNA < 400 ˚C
Federchook	2019	Teeth	Human	72	100-600	10-30	Not Stated	qPCR	Could not calculate DI > 200 ˚C
Mahat	2019	Teeth	Human	25	300	5-20	51.1-71.72	GlobalFiler Express	Full profiles obtained only at 5 min (did not consider DI and inhibitors)
Garriga	2016	Teeth	Human	28	100-700	1-15	>0.005-0.0318	PCR	Low levels of STR amplification at 300 ˚C
Maciejewska	2015	Teeth	Human	56	100-1000	5-10	-	AmpFlSTR SGM Plus/ MiniFiler	Full STR profiles at 100 ˚C (5 & 10min). Partial STR profiles at 300 ˚C (5 & 10 min). Unable to detect and amplify STRs at and above 500 ˚C.
Devaraju	2014	Teeth	Human	13	100-800	15-20	0-0.060	-	No genomic DNA obtained at 400 ˚C
Da Silva	2012	Teeth	Human	45	600-1000	10-60	0.045-1.188	PCR	Failure to amplify target fragment (378 bp) at all temperatures
Fredericks	2012	Bone	Bovine	>39	50-1000	120-240	-	PCR	DNA stable < 150 ˚C
Schwark	2011	Bones	Human	71	5 Burn Categories (as described par. 1.4.7)	-	0.01-114	AmpFlSTR Identifiler	Full DNA profiles in I-II, variable full/partial/failed in III.
Rees & Cox	2010	Teeth	Porcine	88	150-600	15	0.147-19.52	PCR	No amplification of 450 bp fragment > 525 ˚C (teeth still embedded in jaw when burnt)
Al Khalidi	2009	Teeth	Human	30	100-1000	10-60	3.4-26.0	PCR	Successful quantification of 312 bp fragment at 100 ˚C and 500 ˚C
Tsuchimochi	2002	Teeth	Human	46	100-500	2	-	PCR	Successful amplification < 300 ˚C

The profiling success of burnt skeletal remains, such as bone and teeth, are relatively well documented. Emery *et al.,* (2020) managed to obtain full DNA profiles (14 - 15 STRs) in 95 % of samples in burn categories I-III. A marked decline in STR detection was noted at temperatures exceeding 350 °C [44]. Similarly, Schwark *et al.,* (2011) manage to generate full profiles for category I samples and 85% full profiles for category II. Category III had variable success ranging from complete allele drop out in 34.8 % of samples to full profiles in 8.7 %. A maximum of 5 STR loci were typed successfully in categories IV and V [4]. It has been noted to be extremely difficult to use DNA from burnt hard tissues in conventional PCR and STR genotyping technologies [44]. This is typically associated with the high amounts of severely degraded DNA.

A case study conducted on partially skeletonised human remains found washed up in Cape Town found that NGS/MPS was able to generate full DNA profiles even though the DNA was degraded, compared to the partial profiles generated via capillary electrophoresis (CE) [88]. Similarly, NGS of degraded DNA obtained from old skeletal remains proved to be valuable as it can target different single nucleotide polymorphisms (SNPs) and STR markers of forensic significance [89]. One downside to MPS-STR analysis is that the input DNA amounts are greater than what is needed in conventional CE-STR methods (unless optimised) [57]. The application of NGS/MPS technology in the analysis of burnt teeth could not be found. It is however hypothesised that highly incinerated skeletal material may preserve ultra-short DNA fragments that can be analysed and genotyped through NGS and SNP analysis.

There exists a lot of variability in studies conducted on burnt teeth, this is potentially attributed to different extraction protocols and the use of different amplification/profiling kits. There is a general success in quantification and amplification of DNA obtained from teeth burnt at temperatures below 300 ˚C, above 300 ˚C amplification is rarely successful and seems to be sporadic (refer to table 1.1)

1.5. RATIONALE

Given all the available literature, it is clear that various protocols exist for the extraction of DNA and subsequent analysis of teeth within the forensic context, with total demineralisation being the preferred method [44]. An in-house method for DNA extraction from hard tissues exists and has already been optimised. As it currently stands, there is no protocol for the use of burnt teeth in the identification of burnt human remains. This is true for both the institution and Forensic Pathology Services of the Western Cape and South Africa. Furthermore, conventional DNA profiling via capillary electrophoresis is still the gold standard in South Africa and is the only technology applied in the forensic context. The reviewed literature suggests that the forensic DNA profiling of burnt teeth via CE is rife with obstacles and success may be kit specific. South Africa has a high number

of burn fatalities and a high number of bodies that remain unidentified in its medico-legal mortuaries. The optimisation of a CE method for the forensic DNA profiling of burnt teeth may assist in the identification of charred remains and of those who remain unidentified due to the failure of existing protocols when teeth are the only source of DNA available.

The above literature review suggests that the use of capillary electrophoresis in the profiling of burnt human bones and teeth is generally met with very little success at low temperatures (< 300 ˚C). Temperatures are sometimes surmised based on appearance of bone/tooth, and when burnt at specified temperatures, time ranges are quite broad. Currently a gap exists in the South African context and literature pertaining to the forensic DNA profiling of burnt teeth is scarce or outdated. It is worthwhile to investigate specific burning temperatures as the most frequent obstacle in conventional profiling methods (CE) is degraded DNA, specifically in burnt remains. As such, this study shall attempt to address this gap with the following aim and objectives.

1.5.1. Aim

The primary aim of this study is to optimise and evaluate the use of capillary electrophoresis in the forensic DNA profiling of burnt teeth.

1.5.2. Objectives

1. Apply an optimised DNA extraction method for burnt teeth.
2. Evaluate the temperatures at which forensically usable DNA can be obtained from burnt teeth.
3. Compare DNA profile generation success rates using capillary electrophoresis on DNA extracted from burnt and unburnt teeth.

CHAPTER 2
METHODS AND MATERIALS

2.1. STUDY DESIGN

This study forms part of the umbrella study "Optimisation and investigation of DNA recovery from different biological sample types for use in forensic human identification" (HREC: 222/2019). It is a prospective, quantitative, and cross-sectional study, and experimental by design.

2.2. SAMPLE COLLECTION

Tooth samples were available from wisdom tooth donors as part of the umbrella study. These samples were collected from the respective dentists who conducted the wisdom tooth extractions. Informed consent was obtained from their patients to use the extracted teeth in the research study (HREC: 222/2019). A total of 100 teeth from 25 donors (4 teeth per donor) were utilised in this study. Samples with obvious dental alterations such as fillings or implants were excluded due to the high volume of inorganic material that inhibit DNA analysis. The teeth were evenly divided between four groups. One group remained unburned while the three remaining were burned at various temperatures as per table 2.1.

2.3. BURNING

The teeth were burnt using an electric muffle furnace (Labofurn, Kiln Contracts (Pty) Ltd, Cape Town) placed inside a fume hood. The temperature of the furnace was set 40 ˚C below the intended temperature as the furnace temperatures fluctuate 40 ˚C above the input temperature when burning at 500 ˚C or lower. This was established by test burning prior to this study. The samples were placed in a crucible with a heat resistant tile on top to act as a lid to prevent potential cross contamination occurring in the furnace. The furnace was allowed to reach the set temperature first before the samples were placed inside. Samples were burned for 10 minutes and then removed from the furnace and allowed to cool naturally to room temperature.

The teeth were evenly divided between four groups. One group remained unburned, thus acting as a reference control for the donor, while the three remaining teeth from each donor were burned at various temperatures as per table 2.1. One tooth per donor (n = 25) was burned at 100 ˚C, one tooth per donor (n = 25) was burned at 200 ˚C, and one tooth per donor (n = 25) at 300 ˚C. (N=100, whereby one tooth from each donor (n=25) was subjected to each of the four conditions).

Table 2.1: Number of teeth and the burning conditions they were subjected to.

Number of teeth	Burning temperature (˚C)	Burning duration (min)
25	Unburnt	-
25	100	10
25	200	10
25	300	10
Total: N = 100	-	-

The teeth were scrubbed clean using 5 % commercial bleach, molecular biology grade water and 70 % ethanol to remove exogenous DNA and bacterial contaminants. All teeth were subjected to mechanical enamel removal, whereby the enamel of the crown of the tooth was removed by sanding down the surface using a Dremel drill with a new sanding attachment for each sample. This was only done after teeth had been burned. Following burning, all samples were pulverised into a fine powder, followed by DNA extraction, DNA quantification and DNA profiling. An assessment of the quality and success rates of the DNA profiles after the initial burning temperature was conducted to guide the temperature increase for the next batch of samples.

All burnt teeth were photographed, weighed on an electrical balance, and the colour was assessed using a Munsell colour chart. These measurements were repeated both before and after burning. Camera settings typically consisted of an aperture of f/1.88, shutter speed of 1/126, and exposure of ISO 50. All photographs were taken at the same bench with the same lighting conditions for all samples. Colour estimations were conducted using a Munsell colour chart which codes colour according to hue, value, and chroma.

2.4. DNA EXTRACTION

All teeth were pulverised into a fine powder using grinding jars in a Tissuelyser II (Qiagen, Hilden, Germany) according to an in-house optimised protocol for DNA extraction from hard tissue. Briefly, the teeth were placed in decontaminated grinding jars and chilled in liquid nitrogen for one minute, before being processed in a Tissuelyser II for one minute at a frequency of 30 Hz. The samples were scraped out using a spatula into labelled 1.5 mL microcentrifuge tubes and stored at -20 ˚C.

DNA was subsequently extracted using the QIAmp® Investigator DNA extraction kit (Qiagen, Hilden, Germany) whereby 0.05 g of sample powder was incubated in a thermomixer at 56 ˚C (450 rpm) for 20 hours in 1.5 mL demineralisation buffer. The demineralisation buffer consisted of EDTA (0.5 M, pH 8.0), buffer ATL, Proteinase K, SDS, and DTT (1 M) (Table 2.2). Samples were

processed through silica-based spin columns according to the manufacturers protocol (Qiagen, Hilden, Germany). Samples were eluted to a final volume of 30 µL and stored in a 4 ˚C fridge for the duration of the active lab work component of the study.

Table 2.2: Demineralisation buffer reagents and volumes used for one reaction.

Reagent	**x1 sample**
EDTA (0.5M, pH 8.0)	1210 µL
Buffer ATL	396 µL
Proteinase K	22 µL
SDS	0.0121 g

2.5. DNA QUANTIFICATION

Extracted DNA from the tooth samples were quantified on a 7500 real-time PCR thermal cycler using the Quantifiler® Trio kit according to the manufacturer's protocol (Applied Biosystems, CA, USA), with the exception that half-volume reactions were used (Table 2.3). The HID real-time PCR software was used for viewing and analysis of the quantification data.

Table 2.3: Half volume reactions of the Quantifiler™ Trio kit

Component	**Half-volume reaction**
Quantifiler™ Trio Primer Mix	4 µL
Quantifiler™ Trio PCR Reaction Mix	5 µL
Total volume of master mix	9 µL

The DNA quantity and quality of all 100 teeth were assessed based off the qPCR data obtained from the Quantifiler Trio kit (Applied Biosystems, CA, USA) which contains targets for a long autosomal target (214 bp), small autosomal target (80 bp), and Y chromosome target (75 bp) in human DNA. Quantity was assessed by looking at the DNA concentration, in ng/µL, of all three targets. Quality was assessed by looking at the degradation index which is calculated as the ratio of the small autosomal target to the large autosomal target. The presence of PCR inhibitors was determined by looking at the internal PCR control cut off value (IPC Ct.) where a Ct. value greater than 30 indicated that inhibition was taking place [78,79].

2.6. DNA PROFILING

DNA concentrations of the small and large targets were averaged to create dilutions to a final concentration of 0.5 ng/µL. The average was also used to inform DNA input volumes when the

concentration was below 0.5 ng/µL, and in instances where the large autosomal target failed to quantify only the small autosomal target was used.

DNA profiling was conducted using the PowerPlex® ESX 16 system (Promega, WI, USA) on the Applied Biosystems Genetic Analyser 3500*xl*. Amplification took place on a T100 thermal cycler (Bio-Rad), with the following PCR cycling conditions: 96 ˚C for 2 minutes; then 94 ˚C for 30 seconds, 59 ˚C for 2 minutes, 72 ˚C for 90 seconds, for 30 cycles; then 60 ˚C for 40 minutes, and soak at 4 ˚C (max 1 hour).

Half volumes were used for all reactions as part of the optimisation of the DNA profiling process. The following Table 2.4 shows the half volumes used for PCR amplification and HiDi Formamide master mix. To make a final volume of 12 µL for CE, 1 µL of post PCR products were added to 11 µL HiDi Master mix in a 96 well reaction plate.

Table 2.4: Half volume reactions used for PCR amplification and HiDi master mix.

PCR Amplification	**Half Volume (µl)**
5X Master Mix	2.5
10X Master Mix	1.25
DNA template	Up to 8.75
MilliQ Water	Create full volume
Total	12.5
HiDi Master Mix	
WEN ILS 500	0.5
HiDi Formamide	10.5
Total	11

The resultant electropherograms were analysed using GeneMapper IDX software and remotely on GeneMarker. Profiles were classified as either a full profile, partial profile, or a failed profile depending on the number of successfully typed STR loci. The following was used to classify each profile:

- Full Profile = 12-15 STR loci
- Partial Profile = 4 – 11.5 STR loci.
- Failed Profile = 0-3.5 STR loci

For teeth burnt at 300 ˚C, profiling was repeated using an inhouse optimisation step whereby 1 µL of AmpSolution was added to the PCR master mix and the PCR cycles increased to 32. This was done due to the very low concentrations of the extracted DNA. In addition to this the capillary injection time was increased to 34 seconds, this was done to see if the quality of the profiles could be improved as majority were failed profiles, with some obvious peaks below the analytical threshold which resulted in uncalled alleles. For all profiles, an analytical threshold of 50 RFU's was used to call alleles and a stochastic threshold of 100 RFU's for homozygote peaks.

2.7. STATISTICAL ANALYSIS

A *Kruskal-Wallis* test was conducted to determine if significant differences exist between the temperature of burning and subsequent DNA quality and quantity. Quality was assessed by looking at the DI which was calculated as the ratio of the small autosomal target to the large autosomal target. Quantity was assessed according to the large and small autosomal target concentrations of the samples, and in Y target concentrations in the male donors. An analysis of weight loss vs. temperature was also conducted. A *Chi-square* test was conducted to determine if significant differences exist in temperature vs. profiling success, tooth colour vs. temperature, and tooth colour vs. profiling success. A p-value less than 0.05 was statistically significant. All significance values were adjusted by the *Bonferroni* correction for multiple tests. Table 2.5 provides a summary of the variables and statistical tests performed.

Table 2.5: Summary of variables and statistical tests conducted.

Statistical comparisons between:		**Test**
Variable 1	**Variable 2**	
Temperature	Quantity	Kruskal-Wallis
Temperature	DI	Kruskal-Wallis
Temperature	Weight loss	Kruskal-Wallis
Temperature	Profiling success	Chi-square
Temperature	Tooth colour	Chi-square
Tooth colour	Profiling success	Chi-square

2.8. ETHICS

Ethical clearance was obtained for this research study from the University of Cape Town, Human Research Ethics Committee (HREC REF: 204/2022, Appendix A). This research study also falls

under the umbrella study entitled “Optimisation and investigation of DNA recovery from different biological sample types for use in forensic human identification” (HREC REF: 222/2019).

CHAPTER 3
RESULTS

3.1. WEIGHT

Each tooth was weighed prior to and after burning to assess the effect of burning temperature on the weight loss observed in teeth. The average difference in weight between burned and unburned teeth significantly increased with an increase in temperature for teeth burnt at 100 ˚C ($\bar{x}$ =0.0414 g), 200 ˚C ($\bar{x}$ =0.0913 g), and 300 ˚C ($\bar{x}$ =0.2105 g). These differences exist between all three burning temperatures ($p < 0.001$).

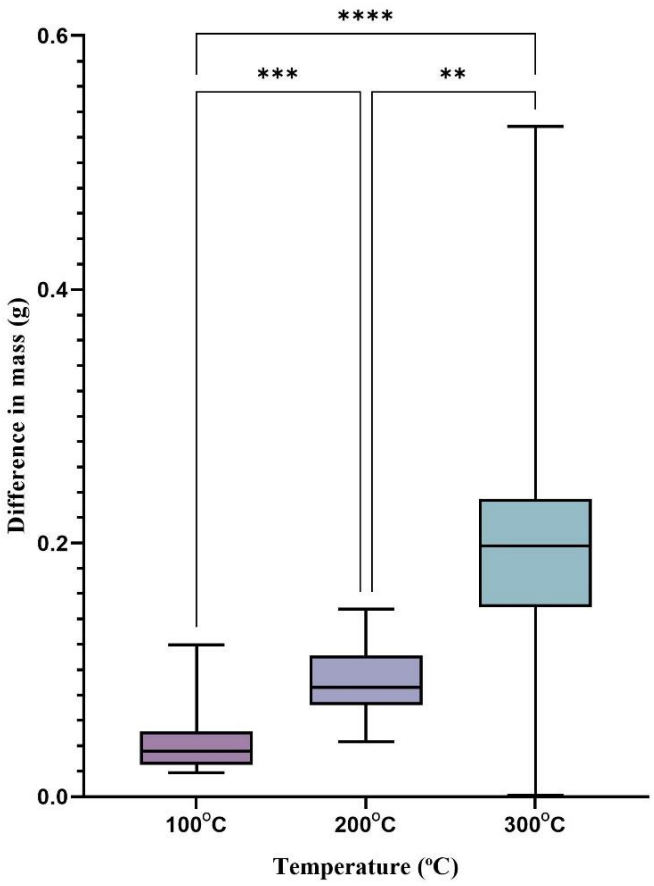

*Figure 3.1: Box and whisker plot of the difference in mass of samples burned at each temperature. "****", "***", and "**" indicate significant differences at a p-value < 0.0001, < 0.001, and < 0.01 respectively.*

3.2. COLOUR

To determine the effects of burning temperature on crown and root colour, a Munsell colour chart was used for colour estimations. Furthermore, colour was also tested against profiling success to determine if colour can be used as a tool to assess downstream profiling abilities. Colour was determined for each tooth before and after burning. The crown and root colour of the unburned

teeth remained the same hue (2.5Y), and the value and chroma ranged between 2.5Y 8/1 (white) to 2.5Y 8/4 (pale brown). Similarly, the root and crown colour of teeth burned at 100 ˚C and 200 ˚C ranged from the same white to pale brown. Teeth burned at 300 ˚C show a drastic change where the hue changes completely from 2.5Y to 10YR. Crown colour ranged from a white (10YR 8/1) to a pale brown (10YR 8/4), two teeth were black (10YR 2/1) and dark yellowish brown (10YR 4/4). The root colours were darker than the crown colours and ranged from a very pale brown (10YR 8/4) to a very dark yellow brown (10YR 2/2). Figure 3.2 below provides an example of the colour changes observed for unburned teeth, and teeth burnt at each temperature.

Significant associations between tooth colour and burning temperature ($p < 0.001$) as well as tooth colour and profiling success ($p < 0.001$) were seen. Pairwise analysis indicated these differences were only observable in the 300 ˚C group compared to other temperatures. No significant difference in colour was seen in unburned teeth or teeth burned at 100 ˚C and 200 ˚C. Similarly, pairwise analysis indicates that difference in profiling success and colour only exist in teeth burned at 300 ˚C. A table of tooth colour results as well as p-values can be found in Appendix B and C respectively.

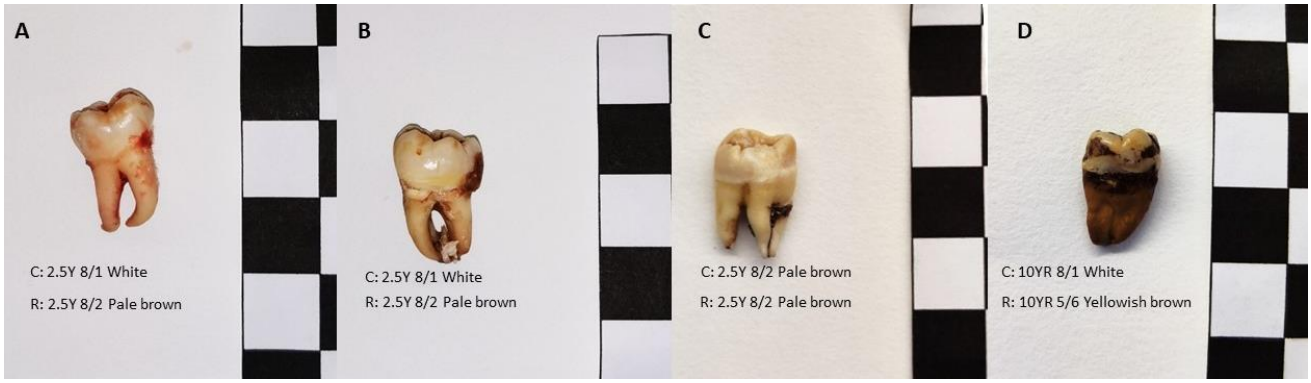

Figure 3.2: Photographs of A) unburned tooth, and teeth burned at B) 100 ˚C, C) 200 ˚C, and D) 300 ˚C. Included is the Munsell colour code for the crown "C" and root "R" of each tooth. Scale: 1 block = 1 cm.

3.4. DNA Quantity

DNA quantity was assessed by looking at the effects of temperature on subsequent DNA concentrations to determine the limits of DNA concentration on profiling success. The large autosomal target concentrations remained similar for the unburnt teeth ($\bar{x}$ =1.8914 ng/µL) and teeth burnt at 100 ˚C ($\bar{x}$ =2.1787 ng/µL), but a sharp decline was observed as the temperatures were increased to 200 ˚C ($\bar{x}$ =0.6919 ng/µL) and 300 ˚C ($\bar{x}$ =0.0009 ng/µL). Similarly, the DNA concentration of the short autosomal target was seen to be similar for the unburnt teeth ($\bar{x}$ =1.4174 ng/µL) and teeth burnt at 100 ˚C ($\bar{x}$ =1.4807 ng/µL), with a decline observed from 200 ˚C ($\bar{x}$ =0.5928 ng/µL) to 300 ˚C ($\bar{x}$ =0.0048 ng/µL). Out of the 25 donors, 11 were male, therefore the

Y chromosome target analysis was only relevant to 11 of the 25 donors. Average Y chromosome target DNA concentrations observed were $\bar{x}$ =1.6106 ng/µL, $\bar{x}$ =1.4001 ng/µL, $\bar{x}$ =0.6176 ng/µL, $\bar{x}$ =0.0071 ng/µL for the unburnt teeth, and teeth burnt at 100 ˚C, 200 ˚C, and 300 ˚C respectively. See Figure 3.3 below. A full table of results can be found in Appendix D along with the full table of p-values in Appendix C.

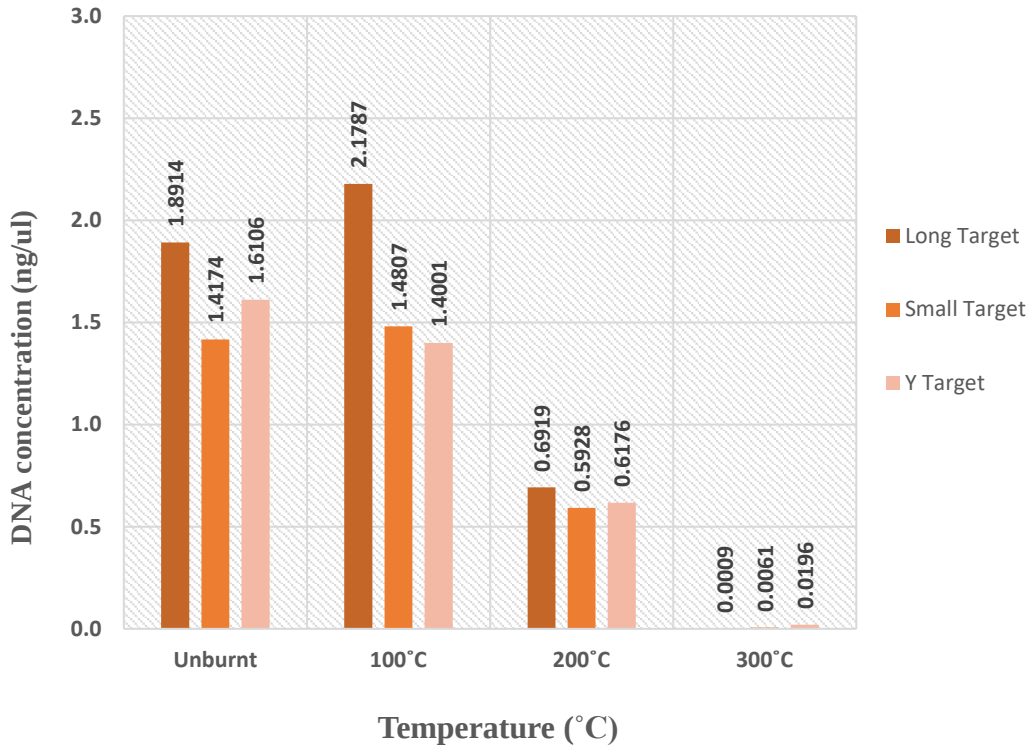

Figure 3.3: Bar graph indicating the average DNA concentration of the large autosomal target, small autosomal target, and the Y chromosome target for each burn category.

3.4.1. Large autosomal target

A significant difference ($p < 0.001$) exists between the DNA concentration of the large autosomal target and burning temperature (Figure 3.4). These differences exist specifically between 300 ˚C and the other two burn temperatures and the unburned teeth ($p < 0.001$). Following Bonferroni corrections, no significant difference was observed between the DNA concentration of the large autosomal target and the categories of unburnt, 100 ˚C, and 200 ˚C ($p > 0.05$).

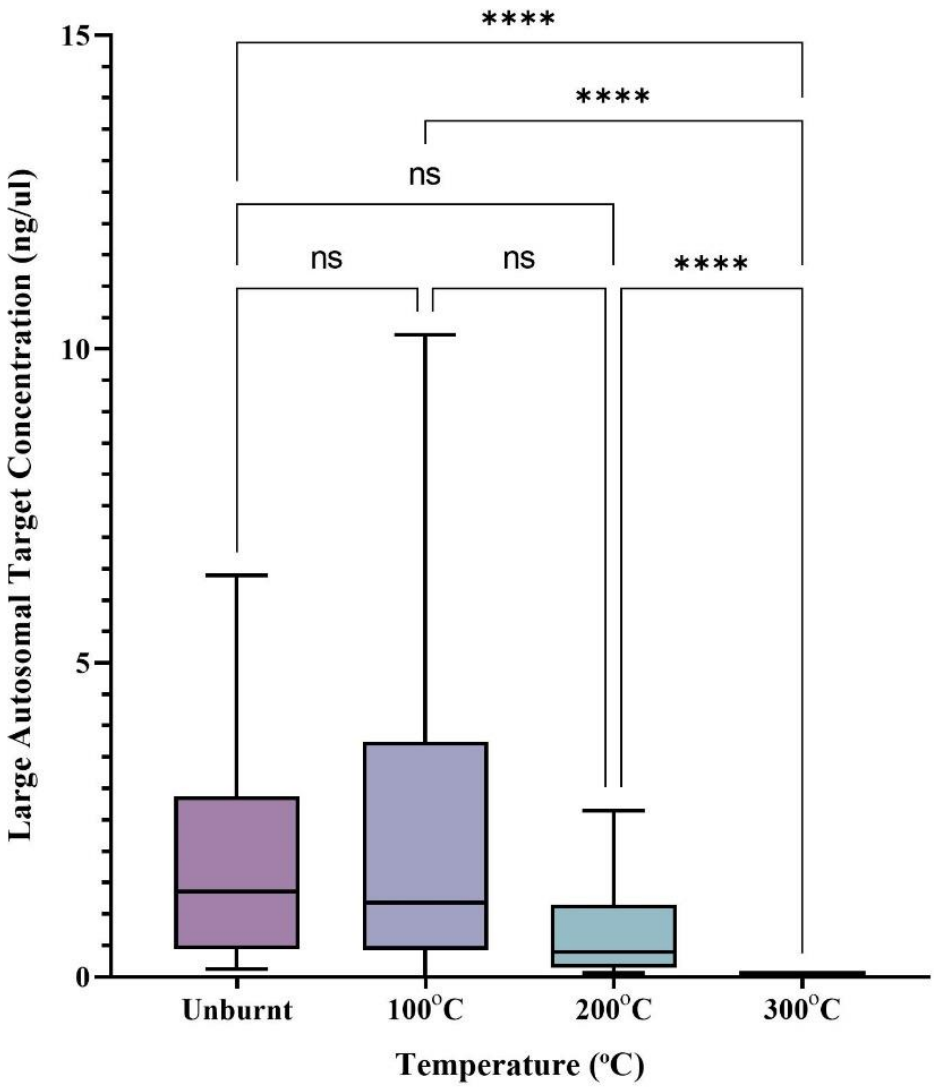

*Figure 3.4: Box and whisker plot showing the DNA concentration of the large autosomal target vs, burning temperature. "****" indicates p < 0.0001; "ns" indicates no significant difference.*

3.4.2. Small autosomal target

A significant difference ($p < 0.001$) exists between the DNA concentration of the small target and the four burn categories (Figure 3.5). These differences exist specifically between 300 ˚C and the other two burn temperatures, and the unburnt teeth ($p < 0.001$). After applying Bonferroni corrections, no significant differences exist between the DNA concentration of the small autosomal target and the categories of unburnt, 100 ˚C, and 200 ˚C ($p > 0.05$).

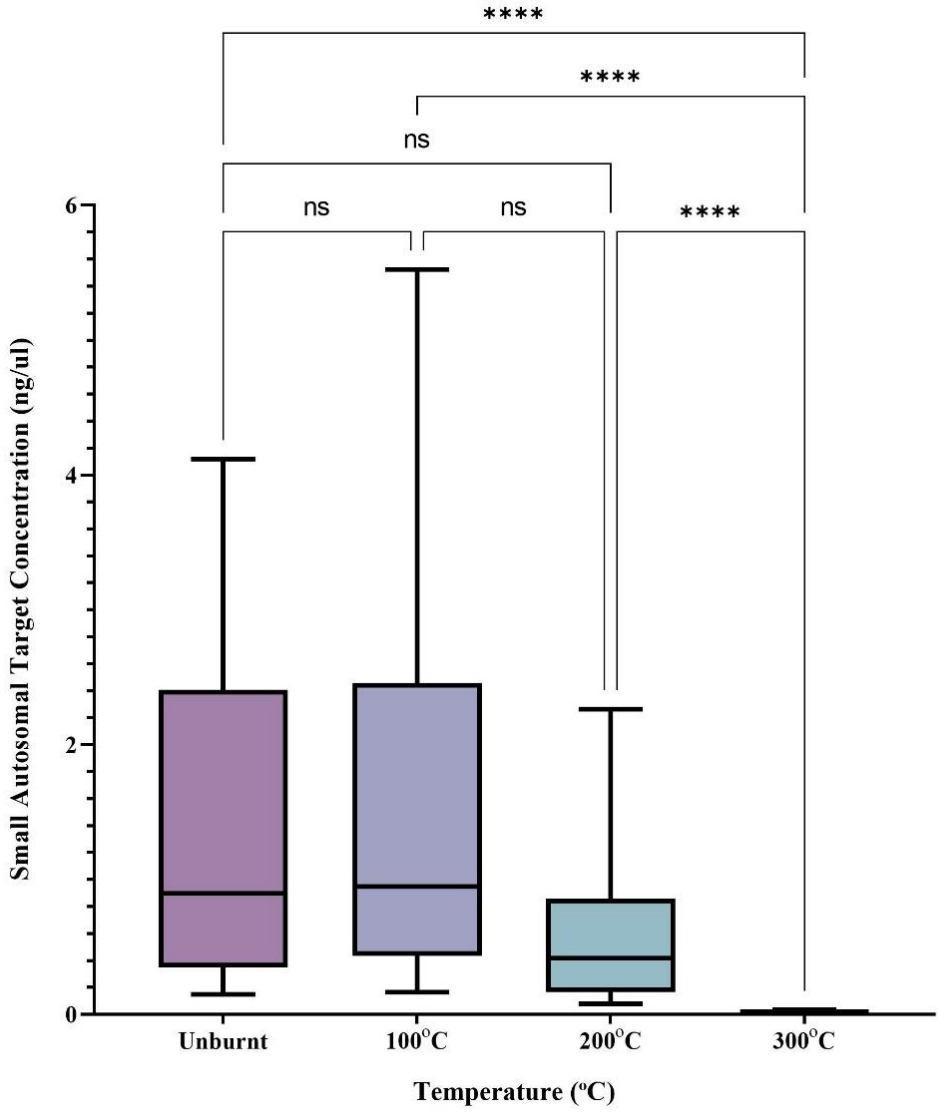

*Figure 3.5: Box and whisker plot showing the DNA concentration of the small autosomal target vs, burning temperature. "****" indicates a significant difference (p < 0.0001);"ns" indicates no significant differences.*

3.4.3. Y chromosome target

A significant difference ($p < 0.001$) exists between the DNA concentration of the Y chromosome target and the four burn categories (Figure 3.6). These differences exist specifically between 300 ˚C and the teeth burnt at 100 ˚C, 200 ˚C, and unburned teeth ($p < 0.05$). No significant differences exist in the concentration of the Y chromosome target and the categories of unburnt, 100 ˚C, and 200 ˚C ($p > 0.05$). Of the 25 donors, 11 are males and as such this statistical relationship is only applicable to the 11 male donors.

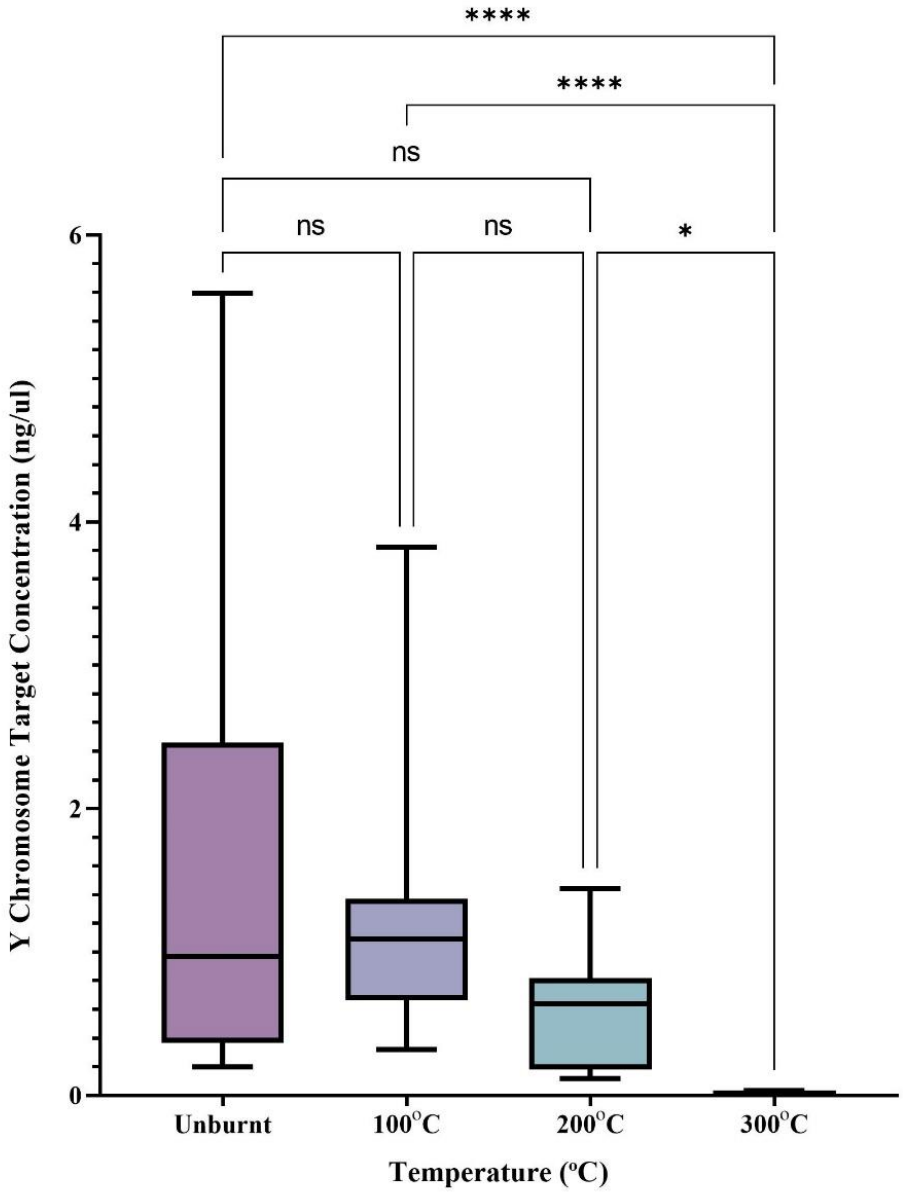

*Figure 3.6: Box and whisker plot showing the DNA concentration of the Y chromosome target vs, burning temperature. "****" and "*" indicates a significant difference p < 0.0001 and p < 0.05; "ns" indicates no significant differences.*

3.5. DNA QUALITY

DNA quality was assessed based on the degradation index and IPC data obtained from the qPCR results The DNA degradation index is less than 1.5 for both the unburned teeth ($\bar{x} = 0.8538$) and teeth burnt at 100 ˚C ($\bar{x} = 0.8041$). The degradation index can be seen to increase slightly at 200 ˚C ($\bar{x} = 1.0133$) and then increases drastically at 300 ˚C ($\bar{x} = 9.6601$). Table 3.1 below indicates degradation by category.

Table 3.1: Burning temperature and subsequent number of samples that fall within the four DNA degradation categories based on DI.

	Not degraded	Mildly degraded	Degraded	Severely degraded
Unburned	25/25	0/25	0/25	0/25
100 ˚C	24/24	0/24	0/24	0/24
200 ˚C	25/25	0/25	0/25	0/25
300 ˚C	5/15	1/15	2/15	6/15

Overall, there is a significant difference in the degradation index and burning temperature. ($p < 0.001$) Specifically the degradation index was significantly greater in the 300˚C group compared to unburned teeth and 100˚C respectively ($p < 0.05$). No significant difference in DI was seen between the 300 ˚C group and the 200 ˚C group (Figure 3.7). The significance values were adjusted by the Bonferroni correction for multiple tests.

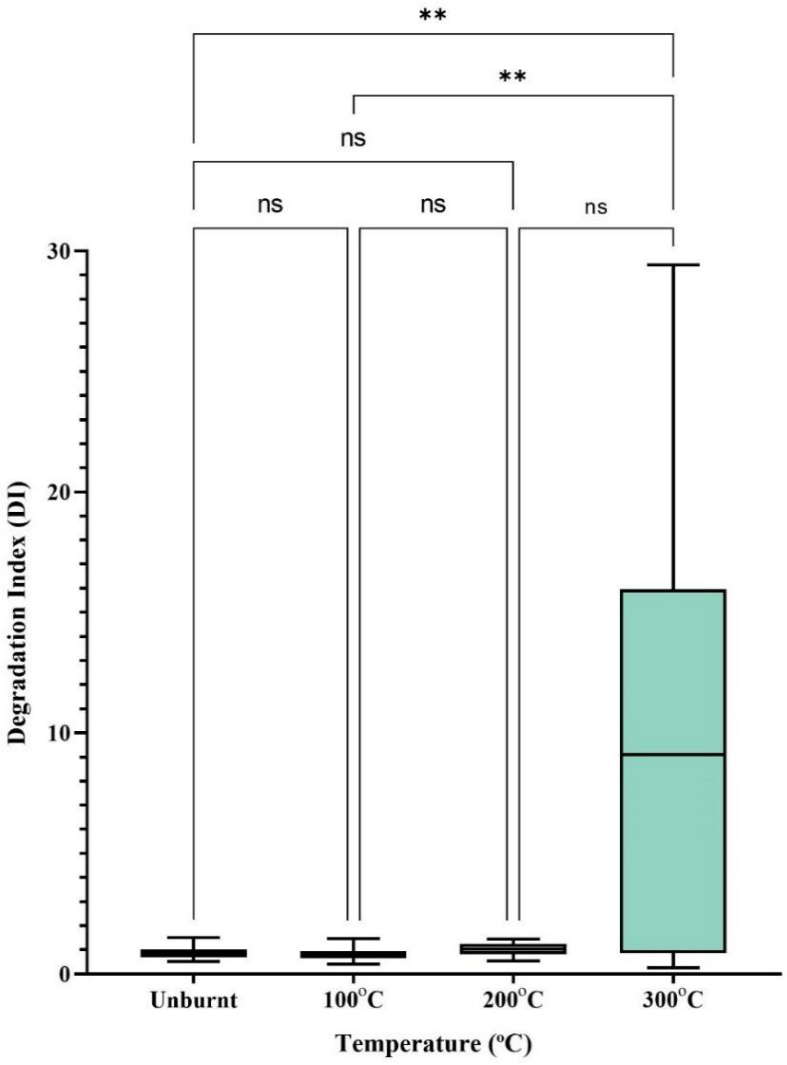

*Figure 3.7: Box and whisker plot showing the degradation index (DI) vs, burning temperature. "**" indicates a significant difference ($p < 0.01$); "ns" indicates no significant differences.*

The IPC Ct. values obtained from the qPCR data indicates that minimal to no inhibition was detected. The average IPC Ct. values for all four categories ranged between 28.3444 to 28.6760 for the unburnt teeth and those burnt at 100 ˚C, and 27.835 and 27.5303 for the teeth burnt at 200 ˚C and 300 ˚C respectively. One sample from the 100 ˚C group had a high IPC Ct. value of 37.088 but still managed to undergo PCR and a full profile was obtained.

3.6. DNA PROFILING

Profiling success was categorised as full (12 - 15 STRs), partial (4 - 11.5 STRs), and failed (0 - 3.5 STRs), where half an STR marker consists of allele dropout of a sister allele. Full profiles were obtained for 24 out of 25 (96 %) of the unburnt teeth, with one partial profile (11.5 STRs). Similarly, for teeth burned at 100 ˚C 24 out of 25 (96 %) full profiles were obtained, with one partial profile (4.5 STRs). Out of the 25 samples burnt at 200 ˚C, 21 (84 %) full profiles were obtained and four (16 %) partial profiles. A drastic decrease in profiling success can be observed in samples burnt at 300 ˚C where only two (8 %) full profiles were obtained, one (4 %) partial profile and 22 (88 %) failed profiles. Minor improvements in peak heights were noted with the optimisation steps for teeth burnt at 300 ˚C. This result was expected based on the qPCR data, see table 3.2 for a summary. The table below summarises the average DNA concentrations of the large and small autosomal targets, DI, and subsequent profiling success.

Table 3.2: Summary of the average DNA concentrations of the small and long autosomal targets, DI, and profiling success.

Category	L. Target ng/ul	S. Target ng/ul	DI	Profile success		
				Full	Partial	Failed
Unburnt	1.8914	1.4174	0.8538	24/25	1/25	0/25
100 ˚C	2.1787	1.4807	0.8041	24/25	1/25	0/25
200 ˚C	0.6919	0.5928	1.0133	21/25	4/25	0/25
300 ˚C	0.0009	0.0061	9.6601	2/25	1/25	22/25

Significant associations between burning temperature and profiling ($p < 0.001$) were seen. Pairwise analysis indicates that these differences only exist between teeth burnt at 300 and profiling success ($p < 0.05$).

3.7. ANOMALIES AND ARTIFACTS

Bleed-through, also known as pull-up, was observed in majority of DNA profiles. Specifically in the yellow dye channel. Figure 3.8 below indicates the channel bleed-through of the orange dye (internal lane standard) into the yellow channel of a negative control. (Note: the yellow channel is represented as black for visualisation purposes).

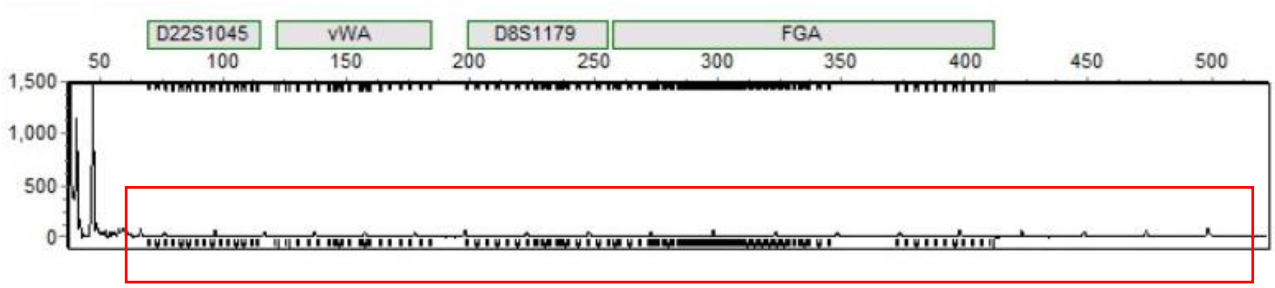

Figure 3.8: Electropherogram of the yellow channel showing bleed through of the internal lane standards.

Background noise levels appeared to increase in the yellow dye channel for majority of DNA profiles. Stutter peaks as well as bleed-through/-pull up in the yellow channel further made it challenging to interpret electropherograms, especially when peaks were below 500 RFU's as can be observed in the electropherogram segment below (Figure 3.9).

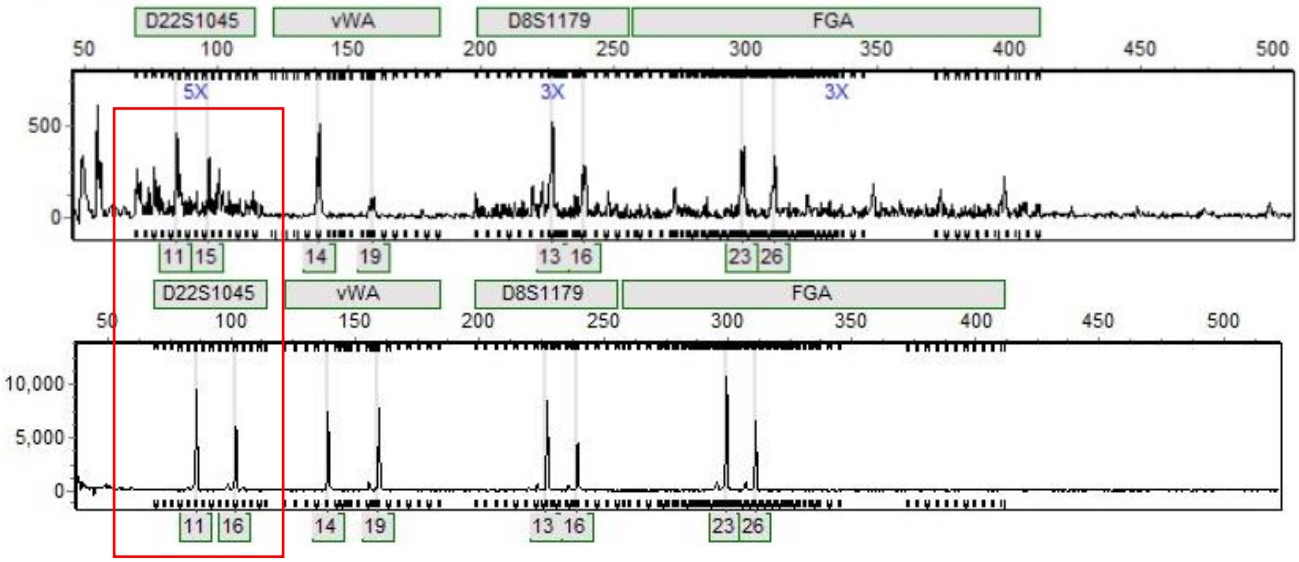

Figure 3.9: Electropherogram segment (top panel) with high background noise levels, with the addition of pull up and stutter peaks. Bottom panel (positive control) showing correctly called alleles at D22S1045 with no artifacts.

Allele dropout is associated with the loss of an allele at a locus, whereas locus dropout is the loss of a whole STR marker. Allele and locus dropout was observed in all DNA profiles of teeth burnt at 300 ˚C, in one profile from the unburnt teeth, in two profiles of teeth burnt at 100 ˚C, and in six

profiles of teeth burnt at 200 ˚C. Figure 3.10 below indicates allele dropout at the heterozygote markers D21S11 and D18S51 where allele 30 and 14 have dropped out.

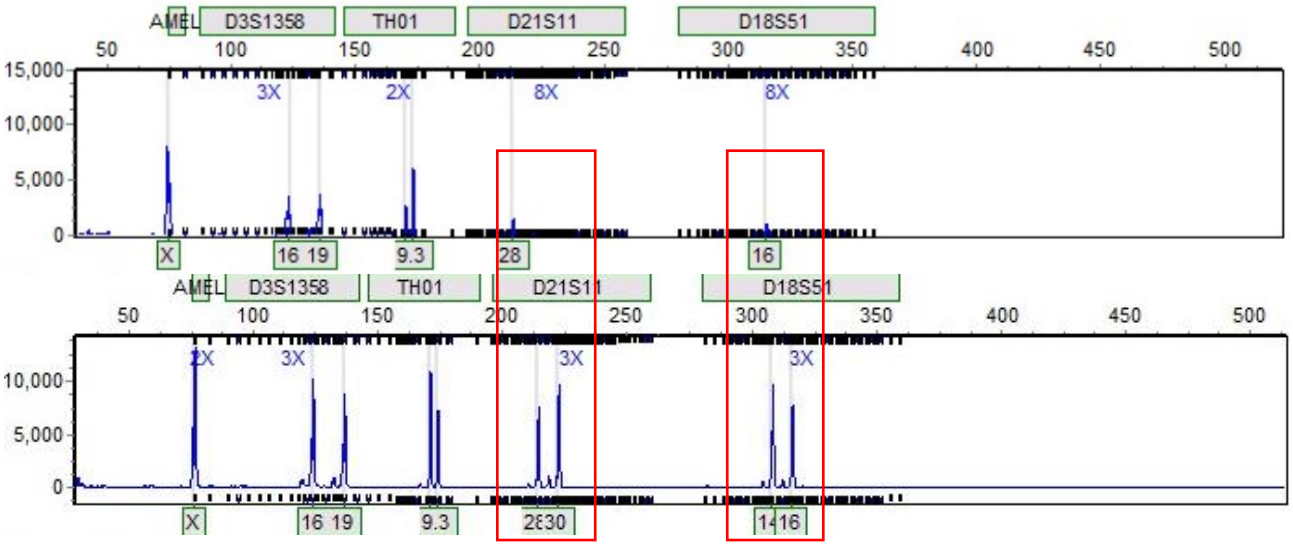

Figure 3.10: Allele dropout observed at known heterozygote STR markers. Top panel shows the allele dropout observed at 300 ˚C with the corresponding positive control (reference sample) panel below.

Allele drop-in is associated with the appearance of an allele that appears spontaneously and incorrectly in an electropherogram. Allele drop-in was observed for three DNA profiles of teeth burnt at 300 ˚C. No allele drop-in was observed at any of the other profiles. Figure 3.11 below indicates allele drop-in of allele 5.3 at the TH01 STR marker in a DNA profile of a tooth burnt at 300 ˚C where allele 5.3 was the only allele called in the entire profile.

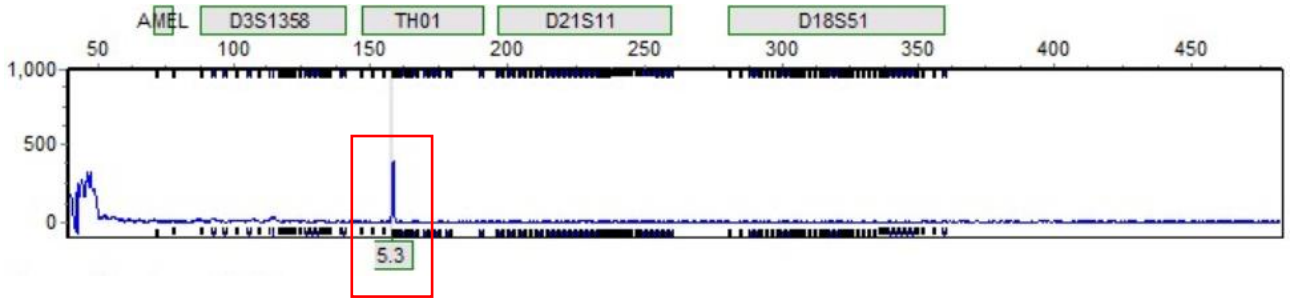

Figure 3.11: Allele drop-in observed in a failed DNA profile of a tooth burnt at 300 ˚C.

CHAPTER 4
DISCUSSION AND CONCLUSION

Fires remain one of the leading causes of injury, both globally and locally [8,9]. With fires in informal settlements being the leading cause of fire related fatalities [7]. Identification of the deceased relies heavily on orthodontic and DNA methods in the majority of burn fatalities due to visual identification being impeded [2,4]. Orthodontic methods rely on the existence of antemortem data, which is often not available, especially in impoverished households that cannot afford dental care. Therefore, DNA becomes one of the most reliable sources for identification purposes [35].

This study aimed to evaluate and optimise the use of capillary electrophoresis in the forensic DNA profiling of burnt teeth. This was achieved by applying an optimised DNA extraction method and evaluating the temperature at which forensically usable DNA can be obtained from burnt teeth. In addition to this, the DNA profiling success rates using CE on DNA obtained from unburnt and burnt teeth was compared.

The data suggests that DNA profiling is successful up to burning temperatures of 200 ˚C with a burning duration of 10 min. The quantity and quality of the DNA severely decreases at burning temperatures of 300 ˚C. Munsell colour estimations of teeth burnt at 300 ˚C show significant association with profiling success and can therefore be used as a helpful tool in selecting samples for downstream analysis.

4.1. OPTIMISATION

4.1.1. DNA extraction

The quantity of DNA extracted from hard tissue is highly variable, but teeth in general provide lower amounts of DNA compared to bone [90,91]. This may be attributed to the type of bone used and the size of the segment. Several DNA extraction methods exist for the extraction of DNA from hard tissue [92–96]. The DNA extraction method described in this study was already optimised and used for hard tissues, however its application to burnt teeth is novel.

Based on the IPC data, the demineralisation step successfully limited the effects of inhibitors. It has been shown in the literature that a demineralisation step for hard tissue DNA extraction methods improves the quality of the DNA extracted by reducing the amount of co-extracted inhibitors as it crosslinks mineral ions [44,92]. The HID software did not flag the IPC Ct. values for any of the burnt teeth samples, indicating that these samples were not affected by PCR inhibition. This was

further indicated by the actual IPC Ct. values, as IPC Ct. values for teeth burnt at all temperatures were below 30 and similar to those of the negative controls (see Appendix D) [79]. The optimised extraction protocol also allowed for the successful extraction of amplifiable DNA for all teeth burnt up to 300 ˚C for ten minutes albeit at very low amounts at 300 ˚C.

4.1.2. Capillary Electrophoresis

In an attempt to improve the quality of STR profiles for tooth samples burnt at 300 ˚C, the number of PCR cycles were increased to 32 as an increase in number of PCR cycles exponentially increases the quantity of amplified DNA every cycle. This results in an increase in the fluorescent signal of alleles (peak heights) which allows previously undetectable alleles to be called [97] The increase to 32 PCR cycles was selected as studies have shown that 32 PCR cycles is optimal for DNA quantities less than 120 pg, PCR cycles greater than 33 did not further improve alleles called [97,98]. Based on DNA concentrations of tooth samples burnt at 300 ˚C, it is expected that severe allele and locus dropout would occur as demonstrated in a study by Hedell *et al.* (2015). They found that at 32 PCR cycles and DNA concentrations of 0.008 ng/ul, allele dropout levelled off at 75% [98].

Moreover, the injection time was increased to 34 sec for the first CE injection and the injection voltage increased to 1.6 kV for the second injection in an attempt further improve STR profiles. A minor improvement was observed in the number of alleles called in the first injection with an injection time of 34 sec, but allele dropout was still present in most samples. Allele dropout occurs when one allele is preferentially amplified in a heterozygote resulting in a false homozygote allele as can be seen in Figure 3.10. No improvements were noted for the second injection with an injection voltage of 1.6 kV, the baseline noise levels were however elevated. Using the altered assay parameters, two full profiles and one partial profile were generated at 300 ˚C and had small autosomal target concentrations greater than 0.01 ng/ul. One sample burnt at 300 ˚C had a concentration of 0.0199 ng/ul but a DI greater than 15 (severely degraded), resulting in a failed profile. It should therefore be noted that although the DNA concentration was greater than 0.01 ng/ul and sufficient to result in a full profile, the added information given through qPCR such as the DI, allows for interpreting the results more comprehensively to decide whether samples should undergo further downstream processing.

4.3. EVALUATING THE EFFECTS OF BURNING TEMPERATURE

4.3.1. Tooth morphology – Weight and colour

Thermal alterations of human skeletal elements are well documented, but it was found that a lack of substantial studies exist for teeth [99]. It is well known that loss in sample weight is directly proportional to increasing temperatures due to dehydration as well as duration of thermal exposure [47,48]. Tooth colour is similarly affected by temperature [47,48,99,100]. This study found significant differences ($p < 0.05$) in weight loss and burning temperature, with the greatest weight loss observed at 300 ˚C. This indicates that teeth have undergone significant thermal stress and that DNA may be too damaged for successful STR amplification. Unfortunately, tooth weight is highly variable between people as interindividual differences play a role in tooth development, and in most instances the weight of the tooth prior to death is unknown. Therefore, the weight of the tooth may not be sufficient to use as a single method for assessing whether a sample should be subjected to downstream analysis. It may however provide high-level insight into the extent of thermal stress that was experienced.

A variety of tools exist for the colour estimation of teeth such as spectrophotometric methods and the Munsell colour chart, as used in this study due to its ease of use. An acknowledged limitation of the Munsell colour chart is that it relies on subjective interpretation of colour. However, this method is less subjective than randomly assigning a colour based on one's own knowledge. Future work should consider the use of a spectrophotometer if available [48]. In this study it was found that teeth burnt at 200 ˚C and 100 ˚C had very little change in colour after burning for 10 min, but at 300 ˚C colour changed to a darker brown colour with a change in hue from a 2.5Y to a 10YR (i.e., from a yellow hue to a red-yellow hue; Figure 3.1). The darker values were more prominent in the roots of the teeth compared to the enamel (i.e., 10YR <5). A study found similar results whereby tooth enamel colour changed to the darker red-yellow brown hues after being burnt at 260 ˚C, whereas at lower temperatures remained a paler yellow (Hue: 5Y) [47]. Another study made use of spectrophotometry and noted that teeth burnt at 200 ˚C for a duration of 30 min turned yellowish white and teeth at 400 ˚C a dark brown [46,100]. Significant differences ($p < 0.05$) were found to exist in tooth colour and profiling success especially at 300 ˚C. It is therefore suggested that teeth of a hue 10YR, especially with root values less than 5, may result in insufficient amounts of DNA that is severely degraded for current CE profiling methods.

4.3.2. DNA quantity and quality

Elevated temperatures such as those caused by fires is a common issue, along with other environmental factors, that result in the degradation of DNA and subsequent decrease in amount of usable DNA [80,81] The literature reviewed has indicated that a significant correlation exists between burning temperature and DNA quantity and quality, with DNA becoming thermally unstable at 130 ˚C [80,81]. With regards to burnt bones and teeth, usable DNA can still be obtained in most instances at burning temperatures below 250 ˚C due to them being relatively protected from the environment [40,81,101,102].

The Quantifiler® Trio kit (Applied Biosystems, CA, USA) was used for the quantification of DNA obtained from the burnt teeth as it is specific to human DNA and consists of three DNA targets of forensic interest. This study found that the most drastic change in DNA concentration is seen between teeth burnt at 200 ˚C and 300 ˚C, whereby DNA concentrations decrease significantly ($p < 0.05$) at 300 ˚C for all three DNA targets. A slight increase in DNA concentration was observed for teeth burnt at 100 ˚C compared to the unburnt teeth with no significant differences noted (Figures 3.4, 3.5, and 3.6). These findings are consistent with those in the literature where severe thermal degradation has been reported as DNA failed to amplify when temperatures exceeded 300 ˚C due to severe DNA fragmentation [87]. The short autosomal target was quantified at all temperatures indicating that shorter DNA fragments were retained at 300 ˚C and that amplification of shorter fragments remain a possibility at this temperature. An interesting observation is noted at 100 ˚C where the DNA concentrations of the large and small autosomal targets are increased compared to the unburnt teeth (Figure 3.3), a similar observation is noted by Emery *et al.* (2020). This may potentially be as a result of the breakdown of collagen resulting in an increased release of DNA trapped withing the hard tissue.

The DI was used as a measure of the quality of the DNA. The level of DNA degradation present in each tooth sample is reported as a degradation index (DI), which is calculated as the ratio of the small autosomal target to the large autosomal target [82,92]. A DNA DI value less than 1.5 indicates that DNA is intact, whereas DI values between 1.5 – 4 indicates that DNA is slightly degraded, DI values between 4 – 10 indicates degraded DNA, and greater than 10 indicates severe DNA degradation [82]. As the temperature increases the degradation index increases due to longer DNA targets fragmenting more rapidly than the smaller targets, resulting in DI's greater than 1.5 [81]. Significant differences ($p < 0.05$) in DNA degradation were observed in this study (Figure 3.7), with the highest DI values being observed in teeth burnt at 300 ˚C (Appendix D). DI values indicate that teeth burnt at 200 ˚C are not degraded whereas majority of teeth burnt at 300 ˚C ranged

between severely degraded and degraded. For 10 samples burnt at 300 ˚C the DI could not be calculated due to the long autosomal target concentrations being too low for detection. For these samples the IPC Ct. values also indicated no inhibition was taking place and it can therefore be assumed that failure to quantify is due to degradation. This poses a limitation regarding the quantification method as the quality of the DNA cannot be assessed for DNA profiling. Failure to quantify the large autosomal target is attributed to either complete degradation of the target DNA or the presence of inhibitors [82].

4.3.3. DNA profiling

DNA profiling was successful for teeth burnt at 100 ˚C and slightly less so at 200 ˚C after being burnt for 10 minutes. Most studies previously conducted found profiling to be successful after burning at 100 ˚C, regardless of burn duration even after a duration of 60 minutes [44,81]. This is potentially due to the fact that DNA starts to degrade at 130 ˚C [80,81]. At 200 ˚C a slight decrease in profiling success was observed whereby only 21 full profiles were generated and four partial profiles. This is mainly due to potential stochastic variation in these samples prior to burning as full profiles were expected at this burning temperature and duration based on qPCR data. Allelic dropout, as observed in Figure 3.10, is commonly associated with degraded DNA and teeth exposed to thermal stress [81]. It can result in the loss of a single allele or of a complete locus. The slope of the electropherogram has the characteristic ski-slope appearance associated with degraded DNA (Figure 3.10), whereby the shorter STR markers have greater peak heights and a decrease in peak heights of the larger STR markers is observed [81]. Allelic dropout observed in the profiles of teeth burnt at temperatures lower than 300 ˚C, can be attributed to low DNA concentrations and preferential amplification of a single allele in a heterozygote, whereas the complete locus dropout observed in teeth burnt at 300 ˚C can be attributed to high levels of DNA degradation.

Allelic drop-in was observed in three DNA profiles of teeth burnt at 300 ˚C. Allelic drop-in is characterised by low level STR alleles that appear in an electropherogram but are not derived from any known donor and has been attributed to sporadic minute contamination events of a single DNA molecule [103]. All profiles where allele drop-in was observed had undergone 32 PCR cycles which in turn increases the likelihood that amplification of a single DNA molecule from outside sources can occur. These minute DNA contaminants are said to even be carried on dust particles that may have been introduced during sample preparation [103]. The increase in PCR cycles may also have elevated the background noise levels observed in Figure 3.9. Furthermore, signal bleed-through/ pull-up was observed in several DNA profiles (Figure 3.8). This occurs when the signal intensity of fluorescently tagged amplicons of one dye is strong enough to bleed into the other dye channels and

appear as allelic peaks due to spectral overlap of the dye [104]. This did not affect the analysis of the DNA profiles as it is typically easy to discern between a true peak and a peak caused due to pull-up.

Although literature has stated that DNA molecules are fully degraded when exposed to temperatures of 190 ˚C [80], teeth have proven to be resilient at 200 ˚C due to it being protected from the environment and its ability to withstand high temperatures [4,38]. It has been noted in the literature that the profile success rate at this temperature is highly dependent on the burning duration, as demonstrated by Lozano-Peral *et al.* (2021) who observed far greater DNA degradation and a severe decrease in number of STR alleles called after burning teeth for 60 minutes at 200 ˚C. A burning temperature of 10 minutes was selected based off the experimental shack fires conducted by Walls *et al.* (2017), as they show that shack fires do not tend to burn for long periods of time and are dependent on the type of fuel available [8]. At 300 ˚C teeth burnt for 10 minutes showed a severe decrease in profiling success, only two full profiles were obtained and one partial profile. This is also in line with the literature where failure to amplify DNA at temperatures greater than 250 ˚C have been reported due to DNA degradation [80,81]. This suggests that the capabilities of capillary electrophoresis are limited for hard tissues burnt at temperatures exceeding 300 ˚C, but still remains a valuable tool when teeth are the only tissue type available for DNA identification of a deceased individual.

4.4. LIMITATIONS

This study subjected individual teeth directly to each burning temperature *in vitro.* This is a limitation as teeth *in vivo* are protected by facial soft tissues and the bones of the maxilla and mandible, which creates additional resistance to fires. A study conducted using porcine teeth, still *in vivo,* managed to extract quantifiable DNA after burning the head at 625 ˚C [46]. Furthermore, this study made use of the PowerPlex® ESX 16 kit for DNA profiling but newer and more sensitive kits are currently available. There is however room for further optimisation as only samples burnt at 300 ˚C were optimised in this study. As half reactions were used, it is also possible to improve the results with full volumes as more sample can be added to the reaction, especially in the instances of very low DNA concentrations, but with the downside of an increase in resources and costs. Increasing the burning temperature and duration of the burning may give insight to the upper limits of analysis especially if future research in next generation sequencing (NGS) is applied. NGS may provide better results at higher temperatures, due to its ability to sequence smaller and highly fragmented DNA segments and thereby overcome the challenges faced by CE platforms. This is further supported by NGS being able to sequence mini-STRs (shorter STR amplicons), and a broad

range of SNPs that can be used for identification to greater statistical power as conventional tools. In turn, greater insight can be obtained into the identities of those who remain unidentified in medicolegal mortuaries.

4.5. CONCLUSION

This study sought to evaluate and optimise the use of capillary electrophoresis in the forensic DNA profiling of burnt teeth. The data indicates that DNA obtained from burnt teeth is severely degraded at temperatures of 300 ˚C with a burning duration of 10 minutes. There is a drastic decrease in DNA concentration and profiling success in teeth burnt at 300 ˚C. Obtaining sufficient amounts of good quality DNA in burnt tooth samples remains a challenge due to the thermal degradation of DNA at high temperatures. These results suggest that conventional DNA profiling methods and the DNA extraction method used herein are suitable for obtaining full DNA profiles from teeth exposed to temperatures as high as 200 ˚C. Further optimisation of the CE protocol is possible and may improve upon the current results. Colour estimation of teeth prior to analysis may give an indication as to whether sufficient DNA can be extracted and if downstream DNA profiling (CE) of teeth as a sample choice should proceed, unless if teeth are deemed the only sample type available for identification. The qPCR data of the small autosomal target indicates that small fragments of DNA (80 bp) are retained in teeth burnt at 300 ˚C for 10 min. Therefore, it is recommended that more sensitive methods such as targeted next generation sequencing (NGS) should be used to obtain more insight into highly degraded and fragmented samples, such as those burnt at 300 ˚C or higher. This in turn may assist in the identification of deceased individuals who remain unidentified due to extreme thermal conditions such as fires.

REFERENCES

[1] D. Mazzarelli, L. Milotta, L. Franceschetti, L. Maggioni, V.G. Merelli, P. Poppa, D. Porta, D. de Angelis, C. Cattaneo, Twenty-five years of unidentified bodies: an account from Milano, Italy, Int J Legal Med. 135 (2021) 1983–1991. https://doi.org/10.1007/s00414-021-02560-9.

[2] K.M. Reid, L.J. Martin, L.J. Heathfield, Bodies without names: A retrospective review of unidentified decedents at Salt River Mortuary, Cape Town, South Africa, 2010 - 2017, S Afr Med J. 110 (2020) 223–228. https://doi.org/10.7196/SAMJ.2020.v110i3.14192.

[3] O. Middleton, S. Baxter, E. Demo, C. Honeywell, J. Jentzen, F. Miller, J.K. Pinckard, R.R. Reichard, J. Rutberg, C. Stacy, H. MacLeod, National Association of Medical Examiners Position Paper: Retaining postmortem samples for genetic testing, Acad Forensic Pathol. 3 (2013) 191–194. https://doi.org/10.23907/2013.024.

[4] T. Schwark, A. Heinrich, A. Preuße-Prange, N. von Wurmb-Schwark, Reliable genetic identification of burnt human remains, Forensic Sci Int Genet. 5 (2011) 393–399. https://doi.org/10.1016/j.fsigen.2010.08.008.

[5] K.M. Reid, L.J. Martin, L.J. Heathfield, Evaluation of DNA profiles obtained from deceased individuals at Salt River Mortuary (South Africa), Aust J Forensic Sci. 51 (2019) S48–S51. https://doi.org/10.1080/00450618.2019.1569149.

[6] L.J. Heathfield, T.E. Haikney, C.G. Mole, C. Finaughty, A.M. Zachou, V.E. Gibbon, Forensic human identification: Investigation into tooth morphotype and DNA extraction methods from teeth, Sci. Justice. 61 (2021) 339–344. https://doi.org/10.1016/j.scijus.2021.05.005.

[7] L. Blom, A. van Niekerk, L. Laflamme, Epidemiology of fatal burns in rural South Africa: A mortuary register-based study from Mpumalanga Province, Burns. 37 (2011) 1394–1402. https://doi.org/10.1016/j.burns.2011.07.014.

[8] R. Walls, G. Olivier, R. Eksteen, Informal settlement fires in South Africa: Fire engineering overview and full-scale tests on “shacks,” Fire Saf J. 91 (2017) 997–1006. https://doi.org/10.1016/j.firesaf.2017.03.061.

[9] Y. Wang, L. Gibson, M. Beshir, D. Rush, Determination of critical separation distance between dwellings in informal settlements fire, Fire Technol. 57 (2021) 987–1014. https://doi.org/10.1007/s10694-020-01075-w.

[10] B.W. van Wilgen, D.C. le Maitre, F.J. Kruger, Fire behaviour in South African fynbos (Macchia) Vegetation and Predictions from Rothermel's fire model, J Appl Ecol. 22 (1985) 207–216.

[11] G.V. Reesu, J. Augustine, A.B. Urs, Forensic considerations when dealing with incinerated human dental remains, J Forensic Leg Med. 29 (2015) 13–17. https://doi.org/10.1016/j.jflm.2014.10.006.

[12] J.M. Butler, M.D. Coble, P.M. Vallone, STRs vs. SNPs: Thoughts on the future of forensic DNA testing, Forensic Sci Med Pathol. 3 (2007) 200–205. https://doi.org/10.1007/s12024-007-0018-1.

[13] L. and B.M. and R.D. Wang Yu and Gibson, Preliminary Investigation of Critical Separation Distance Between Shacks in Informal Settlements Fire, in: K.-C. and C.W.K. Wu Guan-Yuan and Tsai (Ed.), The Proceedings of 11th Asia-Oceania Symposium on Fire Science and Technology, Springer Singapore, Singapore, 2020: pp. 379–389.

[14] L. Blom, A. van Niekerk, L. Laflamme, Epidemiology of fatal burns in rural South Africa: A mortuary register-based study from Mpumalanga Province, Burns. 37 (2011) 1394–1402. https://doi.org/10.1016/j.burns.2011.07.014.

[15] M.D. Peck, Epidemiology of burns throughout the world. Part I: Distribution and risk factors, Burns. 37 (2011) 1087–1100. https://doi.org/10.1016/j.burns.2011.06.005.

[16] Socio-Economic Rights Institute of South Africa, Informal settlements and human rights in South Africa, 2018. https://www.blacksash.org.za/.

[17] K. Morobadi, R. Blumenthal, G. Saayman, Thermal fatalities in Pretoria: A 5-year retrospective review, Burns. 45 (2019) 1707–1714. https://doi.org/10.1016/j.burns.2019.05.007.

[18] A. Ost, D. Messer, D. Dirkmaat, The role of forensic anthropologists at the fatal fire recovery, J Forensic Anth. (2022). https://doi.org/10.5744/fa.2021.0015.

[19] A. van Niekerk, R. Laubscher, L. Laflamme, Demographic and circumstantial accounts of burn mortality in Cape Town, South Africa, 2001-2004: An observational register based study, BMC Public Health. 9 (2009). https://doi.org/10.1186/1471-2458-9-374.

[20] M. Kganyago, K. Govender, L. Shikwambana, V. Sivakumar, Study on blazing wildfires at the Outeniqua pass in South Africa during the october/november 2018 period, Remote Sens Appl. 21 (2021). https://doi.org/10.1016/j.rsase.2020.100464.

[21] T. Kraaij, J.A. Baard, J. Arndt, L. Vhengani, B.W. van Wilgen, An assessment of climate, weather, and fuel factors influencing a large, destructive wildfire in the Knysna region, South Africa, Fire Ecol. 14 (2018). https://doi.org/10.1186/s42408-018-0001-0.

[22] G.G. Forsyth, F.J. Kruger, D.C. le Maitre, B.W. van Wilgen, G. Forsyth, National veldfire risk assessment: analysis of exposure of social, economic and environmental assets to veldfire hazards in South Africa CSIR Natural Resources and the Environment CSIR, 2 Fred Kruger Consulting CC, 2010.

[23] K. Gin, J. Tovar, E.J. Bartelink, A. Kendell, C. Milligan, P. Willey, J. Wood, E. Tan, R.S. Turingan, R.F. Selden, The 2018 California Wildfires: Integration of rapid DNA to dramatically accelerate victim identification, J Forensic Sci. 65 (2020) 791–799. https://doi.org/10.1111/1556-4029.14284.

[24] S. Strydom, M.J. Savage, A spatio-temporal analysis of fires in South Africa, S Afr J Sci. 112 (2016). https://doi.org/10.17159/sajs.2016/20150489.

[25] M. Kganyago, L. Shikwambana, Assessment of the characteristics of recent major wildfires in the USA, Australia and Brazil in 2018-2019 using multi-source satellite products, Remote Sens (Basel). 12 (2020). https://doi.org/10.3390/rs12111803.

[26] B.W. van Wilgen, D.C. le Maitre, F.J. Kruger, Fire behaviour in South African fynbos (Macchia) vegetation and predictions from Rothermel's fire model, 1985.

[27] J.E. Keeley, W.J. Bond, R.A. Bradstock, J.G. Pausas, P.W. Rundel, Fire in the Cape Region of South Africa, in: Fire in Mediterranean Ecosystems, Cambridge University Press, 2012: pp. 168–200. https://doi.org/10.1017/cbo9781139033091.009.

[28] B.W. van Wilgen, G.G. Forsyth, H. de Klerk, S. Das, S. Khuluse, P. Schmitz, Fire management in Mediterranean-climate shrublands: A case study from the Cape fynbos, South Africa, J Appl Ecol. 47 (2010) 631–638. https://doi.org/10.1111/j.1365-2664.2010.01800.x.

[29] R. Walls, P. Zweig, Towards sustainable slums: understanding fire engineering in informal settlements, in: Lecture Notes in Networks and Systems, Springer Science and Business Media Deutschland GmbH, 2017: pp. 93–98. https://doi.org/10.1007/978-3-319-48725-0_10.

[30] Housing Development Agency, Western Cape: Informal settlements status, 2012.

[31] C. Kahanji, R.S. Walls, A. Cicione, Fire spread analysis for the 2017 Imizamo Yethu informal settlement conflagration in South Africa, IJDRR. 39 (2019). https://doi.org/10.1016/j.ijdrr.2019.101146.

[32] C.A. Keyes, K.L. Liphoko, A 5-year overview of fatal thermal and electrical burns in Johannesburg, South Africa, S Afr J Sci. 117 (2021). https://doi.org/10.17159/SAJS.2021/8288.

[33] R. Pitt, V. O'Regan, Wind-Fuelled Blaze: Four thousand Masiphumelele residents in desperate rush to rebuild homes after devastating fire, Daily Maverick. (2020). https://www.dailymaverick.co.za/article/2020-12-18-4-000-masiphumelele-residents-in-desperate-rush-to-rebuild-homes-before-christmas-after-devastating-fire/ (accessed October 1, 2022).

[34] Disaster Managements and Fire Rescue Services DMFRS, Western Cape Strategic Framework for Fire and Burn Injury Prevention, 2015.

[35] M.A. Jobling, P. Gill, Encoded evidence: DNA in forensic analysis, Nat Rev Genet. 5 (2004) 739–751. https://doi.org/10.1038/nrg1455.

[36] H. Ohira, Y. Yamamuro, Y. Kitagawa, K. Nakagawa, I. Yamamoto, Y. Yamada, Effective appropriate use of dental remains and forensic DNA testing for personal identity confirmation, Leg Med. 11 (2009). https://doi.org/10.1016/j.legalmed.2009.01.085.

[37] K.A. Rees, M.J. Cox, Comparative analysis of the effects of heat on the PCR-amplification of various sized DNA fragments extracted from Sus Scrofa molars, J Forensic Sci. 55 (2010) 410–417. https://doi.org/10.1111/j.1556-4029.2009.01286.x.

[38] B.C. Manjunath, B.R. Chandrashekar, M. Mahesh, R.M. Vatchala Rani, DNA profiling and forensic dentistry - A review of the recent concepts and trends, J Forensic Leg Med. 18 (2011) 191–197. https://doi.org/10.1016/j.jflm.2011.02.005.

[39] A.J. Hill, R. Lain, I. Hewson, Preservation of dental evidence following exposure to high temperatures, Forensic Sci Int. 205 (2011) 40–43. https://doi.org/10.1016/j.forsciint.2010.08.011.

[40] R. Amin, P. Shetty, V. Shetty, Reliability of teeth for identification after exposure to varying degrees of temperature, World J Dent. 8 (2017) 96–103. https://doi.org/10.5005/jp-journals-10015-1420.

[41] D. Higgins, J. Kaidonis, J. Austin, G. Townsend, H. James, T. Hughes, Dentine and cementum as sources of nuclear DNA for use in human identification, Aus J Forensic Sci. 43 (2011) 287–295. https://doi.org/10.1080/00450618.2011.583278.

[42] D. Higgins, J.J. Austin, Teeth as a source of DNA for forensic identification of human remains: A Review, Sci Justice. 53 (2013) 433–441. https://doi.org/10.1016/j.scijus.2013.06.001.

[43] P.C. Malaver, J.J. Yunis, Different Dental Tissues as Source of DNA for Human Identification in Forensic Cases, 44 (2003) 306–309. www.cmj.hr.

[44] M. v. Emery, K. Bolhofner, S. Winingear, R. Oldt, M. Montes, S. Kanthaswamy, J.E. Buikstra, L.C. Fulginiti, A.C. Stone, Reconstructing full and partial STR profiles from severely burned human remains using comparative ancient and forensic DNA extraction techniques, Forensic Sci Int Genet. 46 (2020). https://doi.org/10.1016/j.fsigen.2020.102272.

[45] T. Federchook, J. Pokines, K. Crowley, C. Grgicak, Recovery of DNA from Teeth Exposed to Variable Temperatures, J Forensic Anthropo. 2 (2019). https://doi.org/10.5744/fa.2019.1029.

[46] L. Rubio, J.M. Sioli, M.J. Gaitán, S. Martin-de-las-Heras, Dental color measurement to predict DNA concentration in incinerated teeth for human identification, PLoS One. 13 (2018). https://doi.org/10.1371/journal.pone.0196305.

[47] J.J. Beach, N. v Passalacqua, E.N. Chapman, Heat-related changes in tooth color: Temperature versus duration of exposure, in: The Analysis of Burned Human Remains, 2008: pp. 137–144.

[48] T.J.U. Thompson, D. Gonçalves, K. Squires, P. Ulguim, Thermal Alteration to the Body, in: Taphonomy of Human Remains: Forensic Analysis of the Dead and the Depositional Environment, 2017: pp. 318–334.

[49] L. Rubio, J.M. Sioli, J. Suarez, M.J. Gaitan, S. Martin-de-las-Heras, Spectrophotometric analysis of color changes in teeth incinerated at increasing temperatures, Forensic Sci Int. 252 (2015) 193.e1-193.e6. https://doi.org/10.1016/j.forsciint.2015.04.033.

[50] J.M. Butler, Genetics and genomics of core short tandem repeat loci used in human identity testing, J Forensic Sci. 51 (2006) 253–265. https://doi.org/10.1111/j.1556-4029.2006.00046.x.

[51] J.M. Butler, E. Buel, F. Crivellente, B.R. McCord, Forensic DNA typing by capillary electrophoresis using the ABI Prism 310 and 3100 genetic analyzers for STR analysis, Electrophoresis. 25 (2004) 1397–1412. https://doi.org/10.1002/elps.200305822.

[52] B. Mehta, R. Daniel, C. Phillips, D. McNevin, Forensically relevant SNaPshot® assays for human DNA SNP analysis: a review, Int J Legal Med. 131 (2017) 21–37. https://doi.org/10.1007/s00414-016-1490-5.

[53] K.K. Kidd, J.R. Kidd, W.C. Speed, R. Fang, M.R. Furtado, F.C.L. Hyland, A.J. Pakstis, Expanding data and resources for forensic use of SNPs in individual identification, Forensic Sci Int Genet. 6 (2012) 646–652. https://doi.org/10.1016/j.fsigen.2012.02.012.

[54] A.J. Pakstis, W.C. Speed, R. Fang, F.C.L. Hyland, M.R. Furtado, J.R. Kidd, K.K. Kidd, SNPs for a universal individual identification panel, Hum Genet. 127 (2010) 315–324. https://doi.org/10.1007/s00439-009-0771-1.

[55] G. Schochetman, C.-Y. Ou, W.K. Jones, Polymerase Chain Reaction, J Infect Dis. 158 (1988) 1154–1157. https://www.jstor.org/stable/30137034.

[56] S.E. Cavanaugh, A.S. Bathrick, Direct PCR amplification of forensic touch and other challenging DNA samples: A review, Forensic Sci Int Genet. 32 (2018) 40–49. https://doi.org/10.1016/j.fsigen.2017.10.005.

[57] K.M. Reid, L.J. Heathfield, Evaluation of direct PCR for routine DNA profiling of non-decomposed deceased individuals, Sci Justice. 60 (2020) 567–572. https://doi.org/10.1016/j.scijus.2020.08.004.

[58] R. Thompson, S. Zoppis, B. McCord, An overview of DNA typing methods for human identification: Past, present, and future, in: Methods in Molecular Biology, 2012: pp. 3–16. https://doi.org/10.1007/978-1-61779-461-2_1.

[59] H.R. Dash, P. Shrivastava, S. Das, Separation of Amplified DNA Fragments by Capillary Electrophoresis Using Genetic Analyzer 3500xL, in: Principles and Practices of DNA Analysis: A Laboratory Manual for Forensic DNA Typing, 2020: pp. 187–212. https://doi.org/10.1007/978-1-0716-0274-4_23.

[60] J.M. Butler, E. Buel, F. Crivellente, B.R. McCord, Forensic DNA typing by capillary electrophoresis using the ABI Prism 310 and 3100 genetic analyzers for STR analysis, Electrophoresis. 25 (2004) 1397–1412. https://doi.org/10.1002/elps.200305822.

[61] P. Shrivastava, H.R. Dash, J.A. Lorente, J. Imam, Forensic DNA Typing: Principles, Applications and Advancements, Springer Singapore, 2020. https://doi.org/10.1007/978-981-15-6655-4.

[62] R. Daniel, C. Santos, C. Phillips, M. Fondevila, R.A.H. van Oorschot, Carracedo, M. v. Lareu, D. McNevin, A SNaPshot of next generation sequencing for forensic SNP analysis, Forensic Sci Int Genet. 14 (2015) 50–60. https://doi.org/10.1016/j.fsigen.2014.08.013.

[63] A.O. Amankwaa, Trends in forensic DNA database: transnational exchange of DNA data, Forensic Sci Res. 5 (2020) 8–14. https://doi.org/10.1080/20961790.2019.1565651.

[64] H.M. Wallace, A.R. Jackson, J. Gruber, A.D. Thibedeau, Forensic DNA databases-Ethical and legal standards: A global review, Egypt J Forensic Sci. 4 (2014) 57–63. https://doi.org/10.1016/j.ejfs.2014.04.002.

[65] L.J. Heathfield, Policy required for entry of DNA profiles onto the National Forensic DNA Database of South Africa, S Afr J Sci. 110 (2014). https://doi.org/10.1590/sajs.2014/20130374.

[66] I. Knoetze, L. Crouse, DNA processing contemplated in the Criminal Law (Forensic Procedures) Amendment Act 37 of 2013 and the constitutional right to privacy, Obiter. 37 (2016) 36–63. http://www.fsigenetics.com/articles/S1872-4973.

[67] P.G. Ristow, K.W. Cloete, M.E. D'Amato, GlobalFiler® Express DNA amplification kit in South Africa: Extracting the past from the present, Forensic Sci Int Genet. 24 (2016) 194–201. https://doi.org/10.1016/j.fsigen.2016.07.007.

[68] C. Alves, L. Gusmão, A.M. López-Parra, M.S. Mesa, A. Amorim, E. Arroyo-Pardo, STR allelic frequencies for an African population sample (Equatorial Guinea) using AmpFlSTR

Identifiler and Powerplex 16 kits, Forensic Sci Int. 148 (2005) 239–242. https://doi.org/10.1016/j.forsciint.2004.05.007.

[69] J.J. Koehler, A. Chia, S. Lindsey, The random match probability in DNA evidence: Irrelevant and prejudicial?, Jurimetrics. 35 (1995) 201–219.

[70] J.W. Lee, H.-S. Lee, M. Park, J.-J. Hwang, Evaluation of DNA match probability in criminal case, Forensic Sci Int. 116 (2001) 139–148.

[71] W.C. Thompson, N.J. Newman, Lay Understanding of Forensic Statistics: Evaluation of Random Match Probabilities, Likelihood Ratios, and Verbal Equivalents, Law Hum Behav. 39 (2015) 332–349. https://doi.org/10.1037/lhb0000134.supp.

[72] N. Glišović, System for random match probability, in: SISY 2011 - 9th International Symposium on Intelligent Systems and Informatics, Proceedings, 2011: pp. 131–132. https://doi.org/10.1109/SISY.2011.6034307.

[73] J. Brookfield, More to DNA than meets the eye: Interpreting DNA Evidence: Statistical Genetics for Forensic Scientists, Trends in Genetics. 15 (1999) 83. https://doi.org/10.1016/S0168-9525(98)01647-3.

[74] A.P. Dawid, J. Mortera, P. Vicard, Object-oriented Bayesian networks for complex forensic DNA profiling problems, Forensic Sci Int. 169 (2007) 195–205. https://doi.org/10.1016/j.forsciint.2006.08.028.

[75] L.A. Foreman, A.F.M. Smith, I.W. Evett, Bayesian analysis of DNA profiling data in forensic identification applications, J. R. Stat. Soc. (1997) 429–469.

[76] H. Mansour, O. Krebs, H.O. Pinnschmidt, N. Griem, I. Hammann-Ehrt, K. Püschel, Factors affecting dental DNA in various real post-mortem conditions, Int J Legal Med. 133 (2019) 1751–1759. https://doi.org/10.1007/s00414-019-02151-9.

[77] R. Alaeddini, S.J. Walsh, A. Abbas, Forensic implications of genetic analyses from degraded DNA-A review, Forensic Sci Int Genet. 4 (2010) 148–157. https://doi.org/10.1016/j.fsigen.2009.09.007.

[78] W.R. Hudlow, M.D. Chong, K.L. Swango, M.D. Timken, M.R. Buoncristiani, A quadruplex real-time qPCR assay for the simultaneous assessment of total human DNA, human male DNA, DNA degradation and the presence of PCR inhibitors in forensic samples: A

diagnostic tool for STR typing, Forensic Sci Int Genet. 2 (2008) 108–125. https://doi.org/10.1016/j.fsigen.2007.09.001.

[79] S.B. Seo, A. Zhang, H.Y. Kim, J.A. Yi, H.Y. Lee, D.H. Shin, S.D. Lee, Technical note: Efficiency of total demineralization and ion-exchange column for DNA extraction from bone, Am J Phys Anthropol. 141 (2010) 158–162. https://doi.org/10.1002/ajpa.21193.

[80] M. Karni, D. Zidon, P. Polak, Z. Zalevsky, O. Shefi, Thermal degradation of DNA, DNA Cell Biol. 32 (2013) 298–301. https://doi.org/10.1089/dna.2013.2056.

[81] D. Lozano-Peral, L. Rubio, I. Santos, M.J. Gaitán, E. Viguera, S. Martín-de-las-Heras, DNA degradation in human teeth exposed to thermal stress, Sci Rep. 11 (2021). https://doi.org/10.1038/s41598-021-91505-8.

[82] S. Vernarecci, E. Ottaviani, A. Agostino, E. Mei, L. Calandro, P. Montagna, Quantifiler® Trio kit and forensic samples management: a matter of degradation, Forensic Sci Int Genet. 16 (2015) 77–85. https://doi.org/10.1016/j.fsigen.2014.12.005.

[83] S.R. Hughes-Stamm, K.J. Ashton, A. van Daal, Assessment of DNA degradation and the genotyping success of highly degraded samples, Int J Legal Med. 125 (2011) 341–348. https://doi.org/10.1007/s00414-010-0455-3.

[84] J.A. Garriga, D.H. Ubelaker, S. C. Zapico, Evaluation of macroscopic changes and the efficiency of DNA profiling from burnt teeth, Sci Justice. 56 (2016) 437–442. https://doi.org/10.1016/j.scijus.2016.06.006.

[85] R.H. Alves Da Silva, R. Quiezi, C. Danielli, P. Bertolacini, S. Papile, M. Carvalho, K. Cristina, S. Gasque, C. Thais De Almeida-E-Silva, L. Arilho, R. Bicudo, Human identification analysis using PCR from the root portion of dental elements under different conditions of temperature and exposure time, (n.d.).

[86] K. Imaizumi, K. Taniguchi, Y. Ogawa, DNA survival and physical and histological properties of heat-induced alterations in burnt bones, Int J Legal Med. 128 (2014) 439–446. https://doi.org/10.1007/s00414-014-0988-y.

[87] T. Tsuchimochi, M. Iwasa, Y. Maeno, H. Koyama, H. Inoue, I. Isobe, R. Matoba, M. Yokoi, M. Nagao, Chelating resin-based extraction of DNA from dental pulp and sex determination from incinerated teeth with Y-chromosomal alphoid repeat and short tandem repeats., Am J

Forensic Med Pathol. 23 (2002) 268–71. https://doi.org/10.1097/00000433-200209000-00013.

[88] C. Finaughty, K.M. Reid, I.H. Alli, L.J. Heathfield, A first for forensic genetics in Africa: successful identification of skeletal remains from the marine environment using massively parallel sequencing, Forensic Sci Int Genet. 49 (2020). https://doi.org/10.1016/j.fsigen.2020.102370.

[89] S. Cho, K.J. Shin, S.J. Bae, Y.L. Kwon, S.D. Lee, Improved STR analysis of degraded DNA from human skeletal remains through in-house MPS-STR panel, Electrophoresis. 41 (2020) 1600–1605. https://doi.org/10.1002/elps.202000070.

[90] J. Samsuwan, T. Somboonchokepisal, T. Akaraputtiporn, T. Srimuang, P. Phuengsukdaeng, A. Suwannarat, A. Mutirangura, N. Kitkumthorn, A method for extracting DNA from hard tissues for use in forensic identification, Biomed Rep. 9 (2018) 433–438. https://doi.org/10.3892/br.2018.1148.

[91] C.J. Adler, W. Haak, D. Donlon, A. Cooper, Survival and recovery of DNA from ancient teeth and bones, J Archaeol Sci. 38 (2011) 956–964. https://doi.org/10.1016/j.jas.2010.11.010.

[92] Q. Liu, L. Liu, M. Zhang, Q. Zhang, Q. Wang, X. Ding, L. Shao, Z. Zhou, S. Wang, A Simple and efficient method of extracting DNA from aged bones and teeth, J Forensic Sci. 63 (2018) 824–828. https://doi.org/10.1111/1556-4029.13603.

[93] I.Z. Pajnič, Extraction of DNA from human skeletal material, Methods in Molecular Biology. 1420 (2016) 89–108. https://doi.org/10.1007/978-1-4939-3597-0_7.

[94] S.M. Edson, Getting Ahead: Extraction of DNA from skeletonized cranial material and teeth, J Forensic Sci. 64 (2019) 1646–1657. https://doi.org/10.1111/1556-4029.14123.

[95] J. Jakubowska, A. Maciejewska, R. Pawłowski, Comparison of three methods of DNA extraction from human bones with different degrees of degradation, Int J Legal Med. 126 (2012) 173–178. https://doi.org/10.1007/s00414-011-0590-5.

[96] E. Yukseloglu, K. Dastan, F. Yonar, G. Rayimoglu, O. Karatas, D. Islek, M. Dogan, The comparison of DNA extraction techniques in human bone and tooth samples exposed to high heat, Med Sci Int Medical J. (2019) 489. https://doi.org/10.5455/medscience.2019.08.9051.

[97] M. Harrel, D. Gangitano, S. Hughes-Stamm, The effects of extra PCR cycles when amplifying skeletal samples with the GlobalFiler ® PCR Amplification Kit, Int J Legal Med. 133 (2019) 745–750. https://doi.org/10.1007/s00414-018-1860-2.

[98] R. Hedell, C. Dufva, R. Ansell, P. Mostad, J. Hedman, Enhanced low-template DNA analysis conditions and investigation of allele dropout patterns, Forensic Sci Int Genet. 14 (2015) 61–75. https://doi.org/10.1016/j.fsigen.2014.09.008.

[99] R.A. Rahmat, M.A. Humphries, J.J. Austin, A.M.T. Linacre, P. Self, The development of a tool to predict temperature-exposure of incinerated teeth using colourimetric and hydroxyapatite crystal size data, Int J Legal Med. 135 (2021) 2045–2053. https://doi.org/10.1007/s00414-021-02538-7.

[100] R. Kiran, J. Chapman, M. Tennant, A. Forrest, L.J. Walsh, Effect of heat on the fluorescence properties of tooth-colored restorative materials and their forensic implications, J Forensic Sci. 64 (2019) 1698–1706. https://doi.org/10.1111/1556-4029.14122.

[101] C.J. Griffiths, G.D. Bellamy, N.S.W. Forensic, Protection and radiography of heat affected teeth, Forensic Sci Int. 60 (1993) 57–60.

[102] S. Karkhanis, J. Ball, D. Franklin, Macroscopic and microscopic changes in incinerated deciduous teeth, Homo. 61 (2010) 212–212. https://doi.org/10.1016/j.jchb.2010.01.022.

[103] D. Moore, T. Clayton, J. Thomson, A comprehensive study of allele drop-in over an extended period of time, Forensic Sci Int Genet. 48 (2020). https://doi.org/10.1016/j.fsigen.2020.102332.

[104] Z.-F. Wang, S.-P. Dai, J.-Y. Lian, H.-F. Chen, W.-H. Ye, H.-L. Cao, Allele Size Miscalling due to the Pull-Up Effect Influencing Size Standard Calibration in Capillary Electrophoresis: A Case Study Using HEX Fluorescent Dye in Microsatellites, in: I. Abdurakhmonov (Ed.), Genotyping, IntechOpen, Rijeka, 2017: p. Ch. 3. https://doi.org/10.5772/intechopen.73028.

APPENDICES

Appendix A – Ethics

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

Room 45 E-52-E-Floor- Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: hrec-submissions@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

31 March 2022

HREC REF: 204/2022

Dr L Heathfield
Division of Forensic Medicine & Toxicology
FHS
Email: Laura.heathfield@uct.ac.za
Student: gldadr004@myuct.ac.za

Dear Dr Heathfield

PROJECT TITLE: EVALUATION OF NEXT GENERATION SEQUENCING IN THE FORENSIC DNA PROFILING OF BURNT TEETH- SUB-STUDY LINKED TO 222/2019 (MPHIL CANDIDATE-MR ADRIAAN GELDENHUYS-)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

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

This approval is subject to strict adherence to the HREC recommendations regarding research involving human participants during COVID -19, our letter dated 02 February 2022 provides guidance found on our website:
http://www.health.uct.ac.za/fhs/research/humanethics/forms

Approval is granted for one year until the 30 April 2023.

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)

The HREC acknowledge that the student: Mr Adriaan Geldenhuys will also be involved in this study.

Please quote the HREC REF 204/2022 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

PROFESSOR M BLOCKMAN
CHAIRPERSON, FACULTY OF HEALTH SCIENCES 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 2020), 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 – Table of results for tooth colour

Table B.1: Crown and root colour prior to and after burning at 100 ˚C, 200 ˚C and 300 ˚C.

Sample ID	Temp (˚C)	Crown Colour		Root Colour	
		Before	After	Before	After
HID_116	100	2.5Y 8/1 White	2.5Y 8/1 White	2.5Y 8/1 White	2.5Y 8/1 White
HID_117	100	2.5Y 8/1 White	2.5Y 8/2 Pale Brown	2.5Y 8/1 White	2.5Y 8/2 Pale Brown
HID_118	100	2.5Y 8/1 White	2.5Y 8/3 Pale Brown	2.5Y 8/1 White	2.5Y 8/2 Pale Brown
HID_119	100	2.5Y 8/1 White	2.5Y 8/4 Pale Brown	2.5Y 8/1 White	2.5Y 8/4 Pale Brown
HID_120	100	2.5Y 8/1 White	2.5Y 8/2 Pale Brown	2.5Y 8/1 White	2.5Y 8/2 Pale Brown
HID_121	100	2.5Y 7/2 Light Grey	2.5Y 8/2 Pale Brown	2.5Y 7/2 Light Grey	2.5Y 8/2 Pale Brown
HID_122	100	2.5Y 8/1 White	2.5Y 8/2 Pale Brown	2.5Y 8/1 White	2.5Y 8/2 Pale Brown
HID_123	100	2.5Y 8/1 White	2.5Y 8/3 Pale Brown	2.5Y 8/1 White	2.5Y 8/2 Pale Brown
HID_124	100	2.5Y 8/1 White	2.5Y 8/3 Pale Brown	2.5Y 8/1 White	2.5Y 8/2 Pale Brown
HID_125	100	2.5Y 8/2 Pale Brown	2.5Y 8/4 Pale Brown	2.5Y 8/1 White	2.5Y 8/3 Pale Brown
HID_126	100	2.5Y 8/2 Pale Brown	2.5Y 8/3 Pale Brown	2.5Y 8/2 Pale Brown	2.5Y 8/3 Pale Brown
HID_127	100	2.5Y 8/1 White	2.5Y 8/2 Pale Brown	2.5Y 8/1 White	2.5Y 8/2 Pale Brown
HID_128	100	2.5Y 8/2 Pale Brown	2.5Y 8/6 Yellow	2.5Y 8/1 White	2.5Y 8/4 Pale Brown
HID_129	100	2.5Y 8/1 White	2.5Y 8/3 Pale Brown	2.5Y 8/1 White	2.5Y 8/2 Pale Brown
HID_130	100	2.5Y 8/1 White	2.5Y 8/3 Pale Brown	2.5Y 8/1 White	2.5Y 8/3 Pale Brown
HID_131	100	2.5Y 8/1 White	2.5Y 7/3 Pale Brown	2.5Y 8/1 White	2.5Y 8/2 Pale Brown
HID_132	100	2.5Y 8/1 White	2.5Y 8/3 Pale Brown	2.5Y 8/1 White	2.5Y 8/2 Pale Brown
HID_133	100	2.5Y 8/2 Pale Brown	2.5Y 8/6 Yellow	2.5Y 8/1 White	2.5Y 8/4 Pale Brown
HID_134	100	2.5Y 8/2 Pale Brown	2.5Y 8/2 Pale Brown	2.5Y 8/1 White	2.5Y 8/2 Pale Brown
HID_135	100	2.5Y 8/1 White	2.5Y 8/2 Pale Brown	2.5Y 8/1 White	2.5Y 8/2 Pale Brown
HID_136	100	2.5Y 8/1 White	2.5Y 8/3 Pale Brown	2.5Y 8/1 White	2.5Y 8/2 Pale Brown
HID_137	100	2.5Y 7/2 Light Grey	2.5Y 8/3 Pale Brown	2.5Y 8/1 White	2.5Y 8/2 Pale Brown
HID_138	100	2.5Y 7/2 Light Grey	2.5Y 8/3 Pale Brown	2.5Y 8/2 Pale Brown	2.5Y 8/2 Pale Brown
HID_139	100	2.5Y 8/2 Pale Brown	2.5Y 8/2 Pale Brown	2.5Y 7/2 Light Grey	2.5Y 8/2 Pale Brown
HID_140	100	2.5Y 8/1 White	2.5Y 8/3 Pale Brown	2.5Y 7/2 Light Grey	2.5Y 8/3 Pale Brown
HID_116	200	2.5Y 8/2 Pale Brown	2.5Y 8/2 Pale Brown	2.5Y 8/1 White	2.5Y 8/2 Pale Brown
HID_117	200	2.5Y 8/3 Pale Brown	2.5Y 8/2 Pale Brown	2.5Y 8/2 Pale Brown	2.5Y 8/2 Pale Brown
HID_118	200	2.5Y 8/3 Pale Brown	2.5Y 8/4 Pale Brown	2.5Y 8/1 White	2.5Y 8/2 Pale Brown
HID_119	200	2.5Y 8/2 Pale Brown	2.5Y 8/3 Pale Brown	2.5Y 8/1 White	2.5Y 8/2 Pale Brown
HID_120	200	2.5Y 8/2 Pale Brown	2.5Y 8/3 Pale Brown	2.5Y 8/1 White	2.5Y 8/2 Pale Brown
HID_121	200	2.5Y 8/2 Pale Brown	2.5Y 8/3 Pale Brown	2.5Y 8/2 Pale Brown	2.5Y 8/2 Pale Brown

HID_122	200	2.5Y 8/1 White	2.5Y 8/3 Pale Brown	2.5Y 8/1 White	2.5Y 8/2 Pale Brown
HID_123	200	2.5Y 8/1 White	2.5Y 8/3 Pale Brown	2.5Y 8/1 White	2.5Y 8/2 Pale Brown
HID_124	200	2.5Y 8/2 Pale Brown	2.5Y 8/3 Pale Brown	2.5Y 8/1 White	2.5Y 8/2 Pale Brown
HID_125	200	2.5Y 8/2 Pale Brown	2.5Y 8/3 Pale Brown	2.5Y 8/1 White	2.5Y 8/2 Pale Brown
HID_126	200	2.5Y 8/2 Pale Brown	2.5Y 8/3 Pale Brown	2.5Y 8/2 Pale Brown	2.5Y 8/3 Pale Brown
HID_127	200	2.5Y 8/1 White	2.5Y 8/2 Pale Brown	2.5Y 8/1 White	2.5Y 8/2 Pale Brown
HID_128	200	2.5Y 8/2 Pale Brown	2.5Y 8/4 Pale Brown	2.5Y 8/1 White	2.5Y 8/2 Pale Brown
HID_129	200	2.5Y 8/1 White	2.5Y 8/3 Pale Brown	2.5Y 8/1 White	2.5Y 8/2 Pale Brown
HID_130	200	2.5Y 8/1 White	2.5Y 8/2 Pale Brown	2.5Y 8/1 White	2.5Y 8/2 Pale Brown
HID_131	200	2.5Y 8/2 Pale Brown	2.5Y 8/3 Pale Brown	2.5Y 8/1 White	2.5Y 8/2 Pale Brown
HID_132	200	2.5Y 8/2 Pale Brown	2.5Y 8/3 Pale Brown	2.5Y 8/1 White	2.5Y 8/2 Pale Brown
HID_133	200	2.5Y 8/1 White	2.5Y 8/3 Pale Brown	2.5Y 8/1 White	2.5Y 8/3 Pale Brown
HID_134	200	2.5Y 8/1 White	2.5Y 8/2 Pale Brown	2.5Y 8/1 White	2.5Y 8/2 Pale Brown
HID_135	200	2.5Y 8/1 White	2.5Y 8/2 Pale Brown	2.5Y 8/1 White	2.5Y 8/2 Pale Brown
HID_136	200	2.5Y 8/1 White	2.5Y 8/2 Pale Brown	2.5Y 8/1 White	2.5Y 8/2 Pale Brown
HID_137	200	2.5Y 8/2 Pale Brown	2.5Y 8/2 Pale Brown	2.5Y 8/2 Pale Brown	2.5Y 8/2 Pale Brown
HID_138	200	2.5Y 8/1 White	2.5Y 8/3 Pale Brown	2.5Y 8/2 Pale Brown	2.5Y 8/2 Pale Brown
HID_139	200	2.5Y 8/2 Pale Brown	2.5Y 8/3 Pale Brown	2.5Y 8/2 Pale Brown	2.5Y 8/2 Pale Brown
HID_140	200	2.5Y 8/2 Pale Brown	2.5Y 8/3 Pale Brown	2.5Y 8/1 White	2.5Y 8/2 Pale Brown
HID_116	300	2.5Y 8/4 Pale Brown	10YR 8/1 White	5Y 8/1 White	10YR 5/6 Yellowish Brown
HID_117	300	2.5Y 8/2 Pale Brown	10YR 7/1 Light Grey	2.5Y 8/3 Pale Brown	10YR 8/4 Very Pale Brown
HID_118	300	2.5Y 7/4 Pale Brown	10YR 6/1 Grey	2.5Y 8/3 Pale Brown	10YR 4/4 Dark Yellowish Brown
HID_119	300	2.5Y 7/2 Light Grey	10YR 7/1 Light Grey	2.5Y 8/2 Pale Brown	10YR 3/4 Dark Yellowish Brown
HID_120	300	2.5Y 8/3 Pale Brown	10YR 8/2 Very Pale Brown	2.5Y 8/3 Pale Brown	10YR 4/6 Dark Yellowish Brown
HID_121	300	2.5Y 8/4 Pale Brown	10YR 8/2 Very Pale Brown	2.5Y 8/1 White	10YR 6/6 Brownish Yellow
HID_122	300	2.5Y 8/2 Pale Brown	10YR 2/1 Black	2.5Y 8/3 Pale Brown	10YR 4/6 Dark Yellowish Brown
HID_123	300	2.5Y 8/2 Pale Brown	10YR 7/2 Light Grey	2.5Y 8/1 White	10YR 7/6 Yellow
HID_124	300	2.5Y 8/2 Pale Brown	10YR 8/2 Very Pale Brown	2.5Y 8/4 Pale Brown	10YR 3/3 Dark Brown
HID_125	300	2.5Y 8/2 Pale Brown	10YR 8/1 White	2.5Y 8/4 Pale Brown	10YR 5/6 Yellowish Brown
HID_126	300	2.5Y 8/2 Pale Brown	10YR 8/2 Very Pale Brown	2.5Y 8/2 Pale Brown	10YR 5/6 Yellowish Brown
HID_127	300	2.5Y 8/1 White	10YR 8/1 White	2.5Y 8/2 Pale	10YR 6/6 Brownish Yellow

				Brown	
HID_128	300	2.5Y 8/2 Pale Brown	10YR 8/1 White	2.5Y 8/3 Pale Brown	10YR 8/4 Very Pale Brown
HID_129	300	2.5Y 8/2 Pale Brown	10YR 8/1 White	2.5Y 8/2 Pale Brown	10YR 8/4 Very Pale Brown
HID_130	300	2.5Y 8/2 Pale Brown	10YR 8/2 Very Pale Brown	2.5Y 8/1 White	10YR 8/4 Very Pale Brown
HID_131	300	2.5Y 8/3 Pale Brown	10YR 8/2Very Pale Brown	2.5Y 8/2 Pale Brown	10YR 8/4 Very Pale Brown
HID_132	300	2.5Y 8/2 Pale Brown	10YR 8/2 Very Pale Brown	2.5Y 8/3 Pale Brown	10YR 8/4 Very Pale Brown
HID_133	300	2.5Y 8/2 Pale Brown	10YR 8/2 Very Pale Brown	2.5Y 7/8 Yellow	10YR 8/4 Very Pale Brown
HID_134	300	2.5Y 8/3 Pale Brown	10YR 2/1 Black	2.5Y 8/2 Pale Brown	10YR 3/6 Dark Yellowish Brown
HID_135	300	2.5Y 8/2 Pale Brown	10YR 7/2 Light Grey	2.5Y 8/4 Pale Brown	10YR 2/2 Dark Yellowish Brown
HID_136	300	2.5Y 8/1 White	10YR 7/2 Light Grey	2.5Y 8/2 Pale Brown	10YR 2/2 Dark Yellowish Brown
HID_137	300	2.5Y 8/3 Pale Brown	10YR 7/2 Light Grey	2.5Y 8/2 Pale Brown	10YR 5/6 Yellowish Brown
HID_138	300	2.5Y 8/3 Pale Brown	10YR 4/4 Dark Yellowish Brown	2.5Y 8/3 Pale Brown	10YR 3/4 Dark Yellowish Brown
HID_139	300	2.5Y 8/2 Pale Brown	10YR 7/2 Light Grey	2.5Y 8/3 Pale Brown	10YR 8/4 Very Pale Brown
HID_140	300	2.5Y 8/2 Pale Brown	10YR 8/2 Very Pale Brown	2.5Y 8/4 Pale Brown	10YR 7/6 Yellow

Appendix C – Table of p-values

Table C.1: Statistical results and p-values.

Test	Variable 1	Variable 2	p-value (Adj. Sig)	
Kruskal Wallis	Difference in mass	Temperature	100 vs 200	$p < 0.001$
			100 vs 300	$p < 0.001$
			200 vs 300	$p < 0.001$
Chi-Square	Tooth colour	Temperature	*Pearson*	$p < 0.001$, $p < 0.05$
Chi-Square	Tooth colour	Profile success	*Pearson*	$p < 0.001$, $p < 0.05$
Kruskal Wallis	Large Target	Temperature	Unburnt – 100	$p = 1.000$
			Unburnt – 200	$p = 0.229$
			Unburnt – 300	$p = 0.000$
			100 – 200	$p = 0.233$
			100 – 300	$p = 0.000$
			200 – 300	$p = 0.000$
Kruskal Wallis	Small Target	Temperature	Unburnt – 100	$p = 1.000$
			Unburnt – 200	$p = 0.277$
			Unburnt – 300	$p = 0.000$
			100 – 200	$p = 0.222$
			100 – 300	$p = 0.000$
			200 – 300	$p = 0.000$
Kruskal Wallis	Y Target	Temperature	Unburnt – 100	$p = 1.000$
			Unburnt – 200	$p = 1.000$
			Unburnt – 300	$p = 0.000$
			100 – 200	$p = 0.781$
			100 – 300	$p = 0.000$
			200 – 300	$p = 0.013$
Kruskal Wallis	Degradation Index	Temperature	Unburnt – 100	$p = 1.000$
			Unburnt – 200	$p = 0.332$
			Unburnt – 300	$p = 0.007$
			100 – 200	$p = 0.078$
			100 – 300	$p = 0.001$
			200 – 300	$p = 0.691$
Chi-Square	Profile success	Temperature	*Pearson*	$p < 0.001$, $p < 0.05$

Appendix D – Table of qPCR results and DNA profiling

Table D.1: qPCR results and profiling success.

Sample ID	Temperature (˚C)	IPC Ct.	Long Target (ng/ul)	Small Target (ng/ul)	Y Target (ng/ul)	DI	Profile Success
HID_116	Unburnt	28.176	6.3960	4.1192	5.5938	0.6440	Full
HID_117	Unburnt	28.411	0.4772	0.4742	0.8618	0.9937	Full
HID_118	Unburnt	27.669	0.5003	0.3584	0.4288	0.7164	Full
HID_119	Unburnt	29.317	5.1762	3.1738	N/A	0.6131	Full
HID_120	Unburnt	28.402	1.4075	0.7828	N/A	0.5561	Full
HID_121	Unburnt	28.913	1.7439	0.8993	N/A	0.5157	Full
HID_122	Unburnt	28.254	1.1842	0.7701	1.3336	0.6503	Full
HID_123	Unburnt	28.477	4.0619	2.4746	2.7696	0.6092	Full
HID_124	Unburnt	27.693	2.7692	1.9908	2.3658	0.7189	Full
HID_125	Unburnt	27.614	0.1286	0.1488	N/A	1.1566	Full
HID_126	Unburnt	27.777	1.3597	1.4984	N/A	1.1020	Full
HID_127	Unburnt	27.636	0.2430	0.2948	N/A	1.2132	Full
HID_128	Unburnt	28.692	1.2619	1.0608	0.9683	0.8406	Full
HID_129	Unburnt	28.184	0.4381	0.3315	0.3645	0.7567	Full
HID_130	Unburnt	28.691	2.9917	2.4472	2.4617	0.8180	Partial
HID_131	Unburnt	27.930	0.2261	0.3408	N/A	1.5070	Full
HID_132	Unburnt	27.803	0.2175	0.2327	N/A	1.0699	Full
HID_133	Unburnt	28.785	3.9544	3.3152	N/A	0.8383	Full
HID_134	Unburnt	29.206	2.3551	1.6877	N/A	0.7166	Full
HID_135	Unburnt	27.619	0.4621	0.4838	N/A	0.8856	Full
HID_136	Unburnt	27.861	0.4443	0.4587	0.3667	1.0325	Full
HID_137	Unburnt	29.185	2.6477	2.3448	N/A	0.8856	Full
HID_138	Unburnt	29.282	2.6695	2.3629	N/A	0.8852	Full
HID_139	Unburnt	29.210	3.9654	3.2189	N/A	0.8118	Full
HID_140	Unburnt	27.822	0.2041	0.1652	0.2016	0.8091	Full
HID_116	100˚C	27.129	0.9297	1.1023	1.3709	1.1857	Full
HID_117	100˚C	37.088		0.5237	0.8013		Full
HID_118	100˚C	27.474	0.9059	0.8432	1.0737	0.9307	Full
HID_119	100˚C	27.741	1.2509	1.1484	N/A	0.9181	Full
HID_120	100˚C	28.503	1.3703	1.1525	N/A	0.8411	Full
HID_121	100˚C	29.797	5.3952	3.7796	N/A	0.7005	Full
HID_122	100˚C	27.984	1.4230	0.9478	1.0927	0.6661	Full

HID_123	100˚C	29.310	4.4239	2.9323	3.8229	0.6628	Full
HID_124	100˚C	27.577	0.4906	0.7146	1.0991	1.4565	Full
HID_125	100˚C	27.592	0.2437	0.2314	N/A	0.9498	Full
HID_126	100˚C	29.067	4.3352	3.9841	N/A	0.9190	Full
HID_127	100˚C	27.703	0.2061	0.1657	N/A	0.8039	Full
HID_128	100˚C	27.614	0.2850	0.2692	0.3675	0.9446	Full
HID_129	100˚C	27.968	0.3185	0.2165	0.3188	0.6798	Full
HID_130	100˚C	27.760	0.3654	0.4807	0.6657	1.3155	Full
HID_131	100˚C	28.639	0.7196	0.3919	N/A	0.5446	Full
HID_132	100˚C	28.423	0.5480	0.2506	N/A	0.4573	Partial
HID_133	100˚C	27.810	1.1870	0.4871	N/A	0.4104	Full
HID_134	100˚C	28.477	1.5489	1.2344	N/A	0.7970	Full
HID_135	100˚C	28.565	3.2555	1.9392	N/A	0.5957	Full
HID_136	100˚C	27.779	0.8294	0.8444	1.3457	1.0181	Full
HID_137	100˚C	28.970	3.4007	2.2752	N/A	0.6690	Full
HID_138	100˚C	29.325	4.0881	2.6427	N/A	0.6464	Full
HID_139	100˚C	29.630	10.2252	5.5246	N/A	0.5403	Full
HID_140	100˚C	28.976	4.5436	2.9346	3.4425	0.6459	Full
HID_116	200˚C	26.734	1.1110	1.1600	1.4406	1.0441	Full
HID_117	200˚C	27.822	0.0973	0.1414	0.2429	1.4525	Full
HID_118	200˚C	27.346	0.3941	0.4185	0.5005	1.0619	Full
HID_119	200˚C	27.404	0.1515	0.2076	N/A	1.3700	Full
HID_120	200˚C	27.650	0.1452	0.1354	N/A	0.9330	Full
HID_121	200˚C	27.554	0.1926	0.1956	N/A	1.0156	Full
HID_122	200˚C	27.330	0.5527	0.6944	0.6406	1.2564	Partial
HID_123	200˚C	27.857	0.8011	0.9236	0.7763	1.1529	Full
HID_124	200˚C	27.706	0.0648	0.0798	0.1194	1.2320	Full
HID_125	200˚C	27.566	0.1894	0.2701	N/A	1.4256	Full
HID_126	200˚C	27.915	0.2623	0.2899	N/A	1.1050	Full
HID_127	200˚C	28.606	1.2085	1.1042	N/A	0.9137	Full
HID_128	200˚C	27.780	0.1376	0.1259	0.1818	0.9148	Full
HID_129	200˚C	27.499	0.1246	0.1296	0.1775	1.0398	Full
HID_130	200˚C	27.640	0.5585	0.6251	0.7551	1.1191	Partial
HID_131	200˚C	27.729	0.3152	0.4065	N/A	1.2899	Partial
HID_132	200˚C	27.763	0.0674	0.0853	N/A	1.2642	Full
HID_133	200˚C	27.917	0.7164	0.4962	N/A	0.6926	Full
HID_134	200˚C	27.934	0.2652	0.2209	N/A	0.8330	Partial

HID_135	200˚C	28.750	1.2253	0.6492	N/A	0.5298	Full
HID_136	200˚C	28.146	1.2000	0.7946	0.8185	0.6622	Full
HID_137	200˚C	28.219	2.4125	1.6024	N/A	0.6642	Full
HID_138	200˚C	27.794	0.8926	0.7357	N/A	0.8242	Full
HID_139	200˚C	28.508	2.6495	2.2646	N/A	0.8547	Full
HID_140	200˚C	28.716	1.5627	1.0643	1.1400	0.6811	Full
HID_116	300˚C	27.695	0.0003	0.0003	0.0000	0.8554	Fail
HID_117	300˚C	27.851	0.0001	0.0000	0.0000		Fail
HID_118	300˚C	27.744		0.0002	0.0000		Fail
HID_119	300˚C	27.583		0.0003	N/A		Fail
HID_120	300˚C	27.697	0.0002	0.0005	N/A	3.1407	Fail
HID_121	300˚C	27.602		0.0001	N/A		Fail
HID_122	300˚C	27.613	0.0007	0.0002	0.0000	0.2659	Fail
HID_123	300˚C	27.462	0.0002	0.0000	0.0000		Fail
HID_124	300˚C	27.534		0.0002	0.0000		Fail
HID_125	300˚C	27.593	0.0001	0.0000	N/A		Fail
HID_126	300˚C	27.519		0.0000	N/A		Fail
HID_127	300˚C	27.633	0.0003	0.0070	N/A	20.8761	Fail
HID_128	300˚C	27.472	0.0013	0.0121	0.0222	9.4313	Partial
HID_129	300˚C	27.366	0.0003	0.0046	0.0089	14.1547	Fail
HID_130	300˚C	27.569	0.0012	0.0199	0.0357	15.9688	Fail
HID_131	300˚C	27.440	0.0003	0.0091	N/A	29.4216	Fail
HID_132	300˚C	27.278	0.0045	0.0210	N/A	4.6855	Full
HID_133	300˚C	27.374	0.0038	0.0343	N/A	9.1143	Full
HID_134	300˚C	27.340		0.0011	N/A		Fail
HID_135	300˚C	27.376	0.0002	0.0001	N/A	0.7174	Fail
HID_136	300˚C	27.315		0.0000	0.0000		Fail
HID_137	300˚C	27.607	0.0001	0.0001	N/A	1.0175	Fail
HID_138	300˚C	27.537	0.0011	0.0004	N/A	0.3407	Fail
HID_139	300˚C	27.493	0.0003	0.0040	N/A	15.9523	Fail
HID_140	300˚C	27.565	0.0003	0.0056	0.0116	18.9593	Fail
NTC	Unburned	27.169					
NTC	Unburned	27.673					
NTC	100˚C	27.558					
NTC	100˚C	27.278					
NTC	200˚C	28.096					
NTC	200˚C	27.502					

NTC	300˚C	27.554					