EVALUATING THE EFFECTIVENESS OF TOUCH DNA METHOD FOR CRIMINAL INVESTIGATIONS IN ZAMBIA

By

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A Dissertation Submitted to the University of Zambia in Partial Fulfilment of the Requirements of Degree of Master of Science in Molecular Biology.

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DECLARATION

I, **Mathews Chikabisa Zulu**, do declare that this dissertation represents my own work. It is being submitted for the Master of Science Degree in Molecular Biology at the University of Zambia, Lusaka. It has not been submitted for any degree, Diploma or other qualification at this or other University in Zambia.

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CERTIFICATE OF APPROVAL

This dissertation of **Mathews Chikabisa Zulu** was approved as fulfilling the requirements for the award of the degree of Master of Science in Molecular Biology by The University of Zambia.

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ABSTRACT

Touch DNA (trace DNA) is a forensic method for analysing DNA left at a scene of a crime such as shed skin cells and other biological material transferred from a donor to an object or a person during physical contact. This method of identification was pioneered by the British geneticist, Sir Alec John Jeffreys, who observed the similarities and distinguishing characteristics of DNA within a family in his lab in Leicester. The Zambian justice system has been making decisions to convict or exonerate crime suspects basing decisions on circumstantial evidence from either witnesses or confessions from the suspects after interrogation. This study aimed at establishing the potential application of touch DNA in criminal investigations in Zambia using established Short Tandem Repeats (STRs) from thirteen loci. DNA was extracted from twenty-one mobile phones which served to simulate crime scenes and twenty-one buccal swabs from participants which served as reference samples. DNA extraction was done using automated Qiacube Connect device, quantification was done using Rotor-Gene Q (Real-time PCR) followed by PCR-amplification while amplicons were sequenced by the Sanger dideoxy- chain-termination method in an AB 3500 xL Genetic Analyser. Of the twenty-one simulated casework samples, nineteen successful profiles were fully generated, while twenty DNA profiles from twenty-one simulated reference samples were successfully generated. A comparison of the results from simulated casework and reference samples established that touch DNA method could indeed be applied in forensic investigation in Zambia. The findings of this study support the suggestion that touch DNA could be incorporated in criminal investigations and could be used to link suspects to crime scenes in the country.

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LIST OF ABBREVIATIONS AND ACRONYMS

DNA: Deoxyribonucleic acid

PCR: Polymerase chain reaction

STR: Short Tandem Repeat

CODIS: Combine DNA Index System

SNP: Single nucleotide polymorphism

VNTR: Variable number tandem repeats

SGM: Second generation multiplex

FBI: Federal Bureau of Investigation

cRNA: Carrier ribonucleic acid

CNAs: cell-free nucleic acids

LCN: Low copy number

RFU: Relative Fluorescence Unit

NHGRI: National Human Genome Research Institute

NFSBD: National Forensic Science and Biometrics Department

SOP: Standard operation procedure

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CHAPTER ONE

INTRODUCTION

1.0 Overview

With modern technology, the simple act of picking up an object or touching a surface can lead to the identification and apprehension of a criminal. In the world of the forensic DNA analysis, the analysis of "Touch" DNA samples is no longer the exception to the rule, it is the norm (Editorial, 2007).

1.1 Background

To begin with, it's important to understand what touch DNA is and how it gets deposited on objects. Touch DNA refers to the DNA that is left behind through skin cells when an object is touched or handled. On average, humans shed around 400,000 skin cells daily (Wickenheiser, 2002). However, given that the outermost layers of skin are largely composed of dead, keratinized cells that have lost their nuclei (Kita *et al*., 2007), the source of touch DNA becomes a question (Goodwin *et al*., 2019). Research (Kita *et al*., 2007) indicated that trace amounts of DNA exist on the skin's surface, suggesting that DNA fragments may continually shed from the keratinized skin layer, with sweat possibly containing small amounts of DNA (NHGRI, 2016) as well. Quinones and Daniel, 2011 later confirmed that sweat contributes to the DNA profiles obtained from touch DNA samples. They found that cell-free nucleic acids (CNAs)—DNA fragments not enclosed within a cell nucleus—were present in the sweat of 80% of healthy individuals tested, adding to the overall DNA quantity in a sample (Kita *et al.,* 2007). Moreover, nucleated cells were also found in sweat samples. Interestingly, many DNA extraction techniques discard the aqueous portion of the extract, where CNAs are located, after centrifugation, which typically focuses on collecting cellular material. By modifying extraction methods to include DNA from CNAs, the DNA yield available for profiling could be significantly increased (Kita *et al.,* 2007).

The application of DNA as evidence in criminal case resolution has become a critical component of forensic science (Schneider, 2007; Hedman *et al*., 2010). The DNA analysis technique first pioneered by Sir Alec Jeffreys and his team at the University of Leicester, was

referred to as DNA Fingerprinting and the methods have since evolved to DNA profiling to avoid the connotation of absolute uniqueness implied by the word fingerprint (Jeffreys *et al*., 1985). The influence of DNA profiling on forensic science lies in its high discriminatory power, allowing differentiation between individuals through various genetic markers, which creates a unique DNA profile for each person (Goodwin *et al*., 2011). This unique profile can be left behind on objects and people we come into contact with (Gill *et al*., 2015). DNA evidence, alongside other types of evidence, can either include or exclude a suspect from being present at a crime scene. The discriminatory power of DNA makes it an invaluable tool in legal investigations (Walsh, 2007). DNA profiles can be generated from body fluids like blood, saliva, semen, and sweat, as well as tissues from teeth, skin, hair roots, and bones (Dissing and Søndervang, 2010).

Recent advancements in forensic DNA identification technology have significantly contributed to both the conviction of criminals and the exoneration of wrongly accused individuals (Hedman *et al*., 2010; Goodwin *et al*., 2011). DNA profiling is also crucial in identifying victims, particularly in cases where the victim is unrecognizable, such as in burn cases or decapitations. The ability to analyse and characterize an individual's DNA has transformed forensic science and impacted the criminal justice system (Schneider, 2007). DNA analysis has proven vital in solving various criminal cases, including homicides, sexual assaults, physical assaults, hit-and-run incidents, missing person investigations, and paternity determinations, among others (Castriciano *et al*., 2010; Pyrek, 2007). It has also been essential in identifying biological evidence at crime scenes, redirecting investigations, linking serial crimes, and exonerating the wrongly convicted (Dissing and Søndervang, 2010).

Considerable efforts have been made to enhance laboratory-based DNA extraction and analysis techniques (Bogas *et al*., 2011). However, regardless of the technology used, the preceding steps of sample collection, handling, and storage before laboratory receipt are crucial. Proper collection, preservation, and storage of DNA samples are fundamental to ensuring reliable forensic genetics results (Butler, 2009). Poor practices at any of these stages can compromise the sample quality and the validity of the DNA results, potentially undermining their use as evidence in criminal investigations (Bonnet *et al*., 2010). A 2013 report from the Forensics Special Interest Group to the Technology Board of the Department for Business Innovation and Skills highlighted the need for improvements in forensic biology, particularly in "total

recovery or release of biological material onto or from swabs," stressing the importance of better swabbing techniques (Hedman *et al.,* 2019).

Once collected, most biological evidence samples can be protected from contamination and degradation by keeping them dry and cold to prevent degradation by hydrolysis or DNase activity. However, if biological material degrades during sample collection and transport to the laboratory due to carelessness or ignorance, the resulting DNA analysis may be compromised. Therefore, it is vital to adhere to international standard protocols for sample collection, handling, and preservation to ensure the reliability of results as court evidence (Lee *et al*., 2012). The reliability of DNA analysis results is greatly enhanced by following well-designed protocols, making adherence to international standards when transferring evidence from the crime scene to the laboratory essential. Forensic institutes, such as the European Network of Forensic Sciences Institutes (ENFSI), have established guidelines that are regularly inspected for compliance (ENFSI, 2015).

However, there is limited published data on the development and use of protocols specifically addressing the collection, transfer, and pre-laboratory storage of biological samples destined for DNA analysis, particularly in extreme environmental conditions. The natural degradation mechanisms of DNA are well understood (Hu *et al*., 2005), with temperature and moisture content playing significant roles. In regions with extreme climatic conditions, crime scene evidence may be exposed to high or low temperatures and humidity levels before reaching the laboratory, potentially accelerating the degradation of evidence.

1.2 Statement of the problem

The Zambian justice system has been making decisions to convict crime suspects based on circumstantial evidence from either witnesses or confessions from the suspects after interrogation. Criminal convictions based on circumstantial evidence are problematic and subjective and, in some cases, could have led to innocent citizens being convicted (Goodwin *et al*., 2011) while real criminals are set free. The development and application of more objective scientific methods have led to the overturning of such convictions (Hedman *et al*., 2010). In some cases, criminals who might have gotten away with crimes have been convicted when new molecular evidence is presented before courts of law (Dissing and Søndervang,

2010). The application of touch DNA and other molecular-based evidence in the Zambian Courts is still at a very early stage with few cases using evidence from such methods (Naughton and Tan, 2011). The bulk of the cases are still being decided based on witnesses which leads to delayment of justice delivery and risk cases being thrown out of courts on insufficient evidence. It is necessary for law enforcement agencies to apply techniques such as touch DNA which would provide further collaborative evidence and link suspects to crime scene and exonerate innocent suspects (Byard *et al*., 2016).

1.3 Significance of the study

The study was significant in that it sought to enhance criminal investigations by law enforcement officers to reduce wrongful convictions with a potential for being overturned, the potential for criminals to get away with crimes and the length of time that it may take to resolve criminal cases. The method may help link crime scene site to suspects through contact trace evidence deposited or left behind at crime scenes (Van Oorschot *et al*., 2010) for example a hat and slippers left at the murder scenes of crime can be able to link suspect through touch DNA. It would also help consolidate criminal investigations through adding evidential value by connecting the perpetrators of crime at the crime scene and exonerating the innocent suspects (Goodwin *et al*., 2011).

1.4 Aim of the study

The aim of the study was to compare DNA profiles from simulations of crime scenes and reference samples respectively, to ascertain whether or not there was a match in the results between the sets of samples in all cases. The general objective was to determine the effectiveness of touch DNA as evidence to enhance criminal investigations. Simulated evidence of mobile phones was chosen due to its consistency with the methodology, aims and objectives as crime scenes and nature of criminal cases cannot be the same in the real world, and secondly an active crime scene cannot easily be accessed as it is a restricted area. Touch DNA would be expected to provide collaborative evidence in cases where there no eye witnesses.

1.5 Specific objectives

The specific objectives of the conducted research were to:

a) Profile touch DNA collected from mobile phones of volunteers for simulated criminal investigation.
b) Analyse touch DNA for its effectiveness in criminal investigations in Zambia.
c) Compare the results of the DNA profiles from mobile phones simulated as crime scene items to reference samples for forensic investigation.

1.6 Study hypothesis

Touch DNA extracted from swabbed mobile phones may yield DNA profiles identical or highly similar to those extracted from reference samples originating from the same DNA source.

1.7 Research Questions

The following research questions were framed to serve as a guide to the study:

1) How effective is touch DNA technique as a tool for collection of evidence from selected research sites?
2) Are touch DNA quantities sufficient to generate DNA sequences admissible as evidence in court?
3) What are the accuracy levels of results from collected touch DNA samples?
4) How much would touch DNA impact criminal investigations?

CHAPTER TWO

LITERATURE REVIEW

2.0 History of forensic genetics

2.1 Locard's exchange principle

In forensic science, Locard's Exchange Principle asserts that the perpetrator of a crime will both bring something to the crime scene and take something away from it, leaving behind evidence that can be used in an investigation (Raymond *et al*., 2009). This principle was articulated by Dr. Edmond Locard (1877–1966), a pioneering figure in forensic science, often referred to as the Sherlock Holmes of Lyon, France. Locard famously summarized this concept with the phrase, "Every contact leaves a trace" (Locard, 1953).

The principle is generally understood as a fundamental rule of forensic science: whenever two items come into contact, there will be an exchange of materials between them (Kirk, 1953). Kirk expanded on Locard's principle, stating: "Wherever he steps, whatever he touches, whatever he leaves, even unknowingly, will serve as a silent witness against him. Not only his fingerprints or his footprints, but his hair, the fibres from his clothes, the glass he breaks, the tool mark he leaves, the paint he scratches, the blood or semen he deposits or collects (Jeffrey, 1985). All of these and more are silent witnesses against him. This kind of evidence that does not change. It is not get disturbed by the excitement of the moment. It is always present. It is a factual evidentiary material (Van Oorschot *et al*., 2010). Physical evidence cannot be wrong, it cannot perjure on its own, and it cannot be wholly absent. Only human failure to discover it, study it, and understand it can diminish its evidential value.

Fragmentary or trace evidence refers to any material left at or taken from a crime scene, or transferred between two surfaces (Van Oorschot *et al*., 2019; Verdon *et al*., 2014; Alketbi and Goodwin, 2019)), such as fibres from clothing, soil on footwear, or hair left on a chair. When a crime is committed, collecting this fragmentary evidence is crucial. A team of specialized law enforcement officers typically arrives at the crime scene to secure and document it. They take videos and photographs of the scene, the victim(s) (if any), and any objects that could

serve as evidence. If necessary, they also conduct ballistics assessments, search for footprints, shoeprints, tire mark impressions, and hair, and examine any vehicles for fingerprints, whether whole or partial. The meticulous collection and analysis of trace evidence are vital for reconstructing the events of the crime and identifying the perpetrator (Jakovski *et al*., 2017; Santos *et al*., 2013).

2.2 Case studies illustrating Locard's exchange principle

The following case studies highlight the significance of Locard's Exchange Principle in forensic investigations (Locard, 1953). They demonstrate how the transfer of trace evidence can provide critical insights into criminal activities and emphasize the meticulous care required in collecting and analysing such evidence (Gill *et al*., 1985).

2.3 The Weimar children murders

The first case involves the tragic murders of Karola and Melanie Weimar, aged 5 and 7, in Germany (Strate, 2000). The children were reported missing on August 4, 1986, and their bodies were discovered three days later. Both parents, Monika and Reinhard Weimar, became suspects. Monika initially claimed that the children had gone to a playground after breakfast but later changed her story, stating that they were already dead when she returned home the previous night. She claimed that Reinhard was sitting on the edge of Karola's bed, distraught, and that he subsequently buried the bodies. Monika was eventually convicted, partly due to trace evidence linking her to the crime scene (Pyrek, 2007), and was released after serving her sentence in 2006 (Gisela, 2006).

Investigators identified the clothing Monika wore on August 3 and 4 but were unable to determine what Reinhard had worn, leading to the identification of fibres from Monika's clothing on the children's bodies. Karola's bedding contained 14 fibres from her T-shirt, and tests simulating the friction of a lifeless child in bed suggested that Karola could have been dead when Monika claimed she had hugged her goodbye. Melanie's clothing bore 35 fibres from Monika's blouse, and additional fibres were found on the family car's rear seat covers, suggesting that Melanie's body was transported in the car after death (Strate, 2000). The discovery of 375 goose grass fruits on Melanie's clothing, including inside her trousers, further indicated that her body had been moved after death. Notably, the absence of sand on the

children's bodies or clothing contradicted Monika's initial story of a morning visit to the playground.

2.4 The Danielle van Dam case

The second case involves Danielle van Dam, a 7-year-old girl from San Diego, California, who was reported missing on February 2, 2002, and whose body was found on February 27. Neighbour David Westerfield quickly became a suspect after it was discovered that he had gone camping in his recreational vehicle (RV) around the time of Danielle's disappearance. He was ultimately convicted of her kidnapping.

Key evidence included hairs consistent with the van Dams' dogs found in Westerfield's recreational vehicle, as well as carpet fibres matching those in Danielle's bedroom. The prosecution argued that Danielle's pajamas, which she wore during her nightly ritual of wrestling with the dog, carried these hairs and fibres to Westerfield's house and RV (Kimberly, 2002). An alternative theory suggested that the evidence could have been transferred earlier in the week when the van Dams visited Westerfield's house to sell cookies (Alex, 2002). Westerfield claimed that his laundry was out during that visit, allowing for the possibility of secondary transfer of the trace evidence (Steve and Dillon, 2002).

Despite the evidence against him, several points raised doubt about Westerfield's guilt. For example, no trace of Westerfield was found in the van Dam house (Alex, 2002)., and many of the hairs found in his environment were only compared using mitochondrial DNA, meaning they could have come from another family member (Dillon, 2002). Additionally, there was no trace evidence found in his Sport Utility Vehicle (SUV), which the prosecution claimed was used to transport Danielle's body. Although some fibres found with Danielle's body were consistent with those in Westerfield's house and SUV (Kimberly, 2002), the prosecution's theory relied on the assumption that Westerfield had gone on a cleaning spree to eliminate incriminating evidence.

2.5 Advances in forensic identification

The cases of the Weimar children and Danielle van Dam highlight the evolution of forensic science, particularly in the realm of trace evidence and its role in criminal investigations. Since Sir Francis Galton's work on fingerprinting in 1880 and the discovery of the ABO blood groups by Dr. Karl Landsteiner, forensic identification methods have advanced significantly. The introduction of DNA fingerprinting by Sir Alec Jeffreys in 1985 marked a turning point in forensic genetics, enabling more precise identification in cases of crimes, filiation, and other forensic matters (Gill *et al*., 1985).

2.6 The role of DNA in forensic science

DNA analysis has become a cornerstone of forensic science, providing a powerful tool for identifying individuals involved in criminal activities (Pyrek, 2007). The amount of DNA left behind when an object is touched, known as touch DNA, can vary widely depending on numerous factors (Burrill *et al*., 2019). Studies have shown that even minute amounts of DNA (Raymond *et al*., 2009), as little as 1 nanogram, can yield a full DNA profile, with partial profiles obtainable from even smaller quantities. This capability has revolutionized forensic investigations, allowing for the detection and analysis of trace DNA evidence (Goodwin, 2019) that might have previously gone unnoticed. Ryan (2016), summarized several case studies which illustrated and underscored the importance of Locard's Exchange Principle in modern forensic science. In the summary of studies from various authors (Daly *et al*., 2011, van Oorschot and Jones, 1997), the amounts of DNA obtained from various sources including glass, fabric sources and wood ranged from 0.52 ng to 51 ng. The cited studies from which the five illustrations in Table 2.1 were obtained (Ryan, 2016) demonstrated how the careful collection and analysis of trace evidence can reveal crucial details about a crime, guiding investigators in their search for justice (Raymond *et al*., 2009; Williamson, 2012; Quinones and Daniel, 2012).

Table 2.1. Summary of quantities of DNA from various sources as cited in a review by Ryan *et al.,* 2016.

Mean DNA quantities (nanograms, ng)	Source of DNA material	Cited by	Original source
0,52	Glass material	Ryan, 2016	Daly *et al*., 2011
1.7	Assortment of items from crime scene	Ryan, 2016	Raymond *et al*., 2009
17.8	Plastic knife handle	Ryan, 2016	Van Oorschot and Jones 1997
11.68	Cotton rubbed with palm, finger, and side of hand for 15s	Ryan, 2016	Goray *et al*., 2010
4.3	Wallets	Ryan, 2016	Raymond *et al*., 2009

2.7 Amount of DNA left behind when an object is touched

In their research, van Oorschot and Jones, (1997) demonstrated that the duration an object was held (whether 5 seconds, 30 seconds, 3 minutes, or 10 minutes) does not significantly impact the amount of DNA transferred.

The variability in DNA transfer is not fully understood, but several factors have been identified. Some individuals, referred to as good shedders, tend to shed skin cells more readily than others (Lowe *et al*., 2002). However, the reliability of categorising individuals as good or bad shedders has been debated, with studies showing inconsistent shedding patterns even for the same person on different days (Phipps and Petricevic, 2006).

Other factors influencing DNA transfer include recent handwashing, which can reduce the amount of DNA on the skin, and habits such as frequently touching one's face, eyes, nose, or hair, which can load the fingers with additional DNA (Wickenheiser, 2002). Pressure and

friction also play a role; increased pressure tends to result in more DNA being transferred, and friction amplifies this effect further (Goray *et al*., 2010). Rough surfaces, like those on gun grips or concrete, are more likely to collect and retain skin cells than smooth surfaces, making them better candidates for DNA collection. Additionally, sweat can enhance DNA transfer by carrying cells to the surface of the skin and by contributing epithelial cells and CNAs to the available DNA pool (Quinones and Daniel, 2011).

2.8 Duration in which DNA stays on an object

The question of how long touch DNA can persist on an object is complex, and research in this area is limited. However, it is a critical issue, especially as defense attorneys may argue that touch DNA found at a crime scene could have been deposited during an earlier, unrelated event (Quinones and Daniel, 2012, Williamson, 2012; Martin *et al*., 2018).
For example, if a DNA profile matching a suspect is found on a brick wall outside a murder victim's home, the prosecution might argue that the suspect left the DNA while fleeing the scene. However, if the suspect previously lived at or visited the home, the defense might argue that the DNA might have been left during a prior visit, unrelated to the crime.

Raymond *et al*., (2009) conducted one of the few studies addressing the persistence of touch DNA. They applied known quantities of DNA to various surfaces and found that the likelihood of recovering DNA from outdoor crime scenes decreases significantly over time, with a major drop-off occurring around two weeks. However, control samples in indoor settings fared better, with DNA profiles still recoverable after six weeks.

Another study (Linacre *et al*., 2010) focused on the stability of touch DNA on fabrics. Volunteers rubbed their fingers on fabric samples, which were then exposed to light for up to 36 days. The researchers were able to generate nearly complete DNA profiles from the touch DNA on acrylic, nylon, and polyester fabrics even after this extended period. Determining whether a touch DNA profile was deposited during the alleged incident or earlier requires careful consideration of several factors: Rough objects are more likely to collect and retain more DNA (Sutherland *et al*., 2003). DNA is more likely to persist indoors than outdoors, where it is exposed to degrading elements like heat, humidity, UV light, and bacteria. Objects that are frequently touched by many people, such as those summarised in Table 2.2 e.g. door handles, may have a more complex DNA profile than items touched by only a few people, such

as a weapon (Alketbi, 2018; Alketbi and Goodwin, 2003). While it is impossible to precisely determine how long touch DNA evidence might last, these studies highlight the need for further research to answer the questions frequently posed by investigators and attorneys regarding the persistence of touch DNA (Raymond *et al*., 2009).

Table 2.2 Different types of items that might contain DNA when handled with bare hands.

Item of Evidence	**Type of case**	**Cited by**
Steering wheel and other vehicle swabs	Carjacking	Ryan.S. 2016
Ligatures, hand cuffs, shoestrings	Strangulation, Kidnapping, Rape etc.	Ryan. S. 2016
Fingerprints on victim	Any	Ryan .S. 2016
Hands swabs from suspects	Strangulation, rape	Ryan. S. 2016
Swabs from limbs removed from animal carcases	Poaching	Ryan. S. 2016
Cell phone swab	Robbery	Ryan.S. 2016

2.9 Biological sample collection and proceeding processes

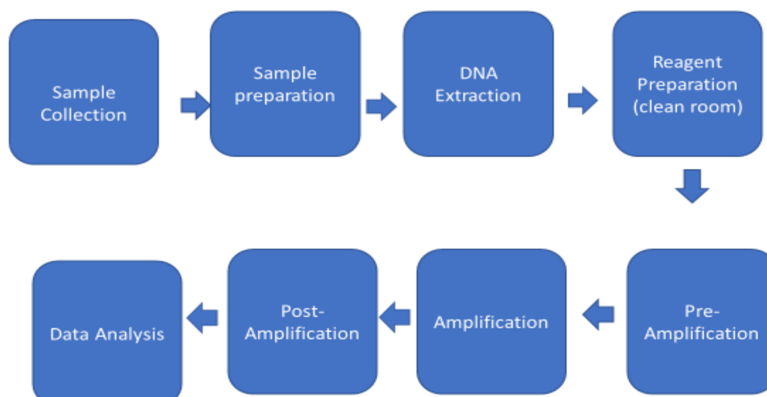

Figure 2.1 Schematic diagram showing the processes, steps and methods used in DNA analysis for crime scene investigation.

In DNA analysis the appropriate samples are collected and samples are prepared in readiness of the DNA extraction. When DNA is extracted, the regents are prepared for quantification, DNA amplification, Sequencing and bioinformatics and data analysis. The raw data is analysed so that it is understood and meaningful.

2.10 Biological samples for forensic genetic profiling

Nucleated cells in biological samples are crucial for forensic genetic profiling, as these cells contain the DNA necessary for analysis (Kupens *et al*., 2003). The types of biological samples typically used in forensic investigations include; Blood samples, semen, bone and teeth.
Bone and teeth are valuable in cases where other biological materials may not be available. The follicle attached to a hair is rich in DNA (Linacre *et al*., 2010; Quinone and Daniel, 2012) and is often targeted in forensic analysis. These contain cells that can be analysed for DNA. These cells, which slough off the body (e.g., from hands), are commonly found in touch DNA samples (Butler *et al*., 2004; Wiegand and Kleiber, 1997).

2.11 Methods used in forensic human DNA identification

2.11.1 Autosomal STR profiling

Short Tandem Repeats (STRs) are the most commonly used markers in forensic DNA typing due to their ability to distinguish between individuals. STRs, discovered in the 1980s (Reynolds *et al*., 1991), consist of repeating units of DNA sequences (mono-, di-, tri-, tetra-, penta-, and hexa-nucleotide repeats). In forensic contexts, tetra-nucleotide repeats are most frequently used (Goodwin *et al*., 2011). Individuals can be homozygous or heterozygous at a particular STR locus, depending on whether they have the same or different numbers of repeats on their two alleles.

STR profiling has become the gold standard for human identification in forensic investigations, used not only for criminal cases but also for paternity/maternity testing, disaster victim identification (DVI), and kinship testing (Yoshida *et al*., 2011). The advantage of STR markers is their ability to be tested quickly, simply, and simultaneously across multiple loci through

multiplexing (Monetti *et al*., 2001). This increases the accuracy and efficiency of identifying different biological samples (Greytak *et al.,* 2019).

In 1997, the Federal Bureau of Investigations (FBI) introduced the Combined DNA Index System (CODIS), which includes 13 autosomal loci and the amelogenin sex test (Sullivan *et al*., 1993). These loci are highly polymorphic and located in non-coding regions on different chromosomes. The efficiency of STR profiling has improved with the development of multiplex kits that amplify 16 or more loci in a single reaction (Dixon *et al*., 2005).

STR profiling has been widely accepted as an important tool in human identification and criminal justice (Greytak *et al.,* 2018). It is also used in medical genetic research, particularly in studying genetic disorders associated with trinucleotide STR loci. The most common STR kits used in forensic laboratories are manufactured by Life Technologies, Promega, and Qiagen. The forensic DNA analysis process involves multiplex PCR amplification of 10–16 STRs, followed by automated sequencing using capillary electrophoresis (CE) (Von Heeren and Thormann, 1997).

2.11.2 Analysis of the Y-Chromosome

The Y-chromosome is particularly useful in forensic investigations because it is present only in males. It consists of 60 million bp including over 400 STRs (Li, 2008; Gunn, 2006). This makes it valuable in cases involving male-female mixtures, such as rape, defilement and incest. Y-STR profiling can provide information about the male component in a sample, even when no sperm cells are present, such as in cases involving vasectomized or azoospermia rapists (Shewale *et al*., 2003). Using of Y-STRs profiling is particularly beneficial technique for samples of mixture of male and female components, allows separations of the male-specific information from the mixture (Thompson and Black, 2007; Rudin and Inman, 2002; Savino and Turvey, 2011).
Y-STR markers development provided an important breakthrough in forensic science as it is facilitating in providing the solutions for important applications than other genetic markers. It helps to determine the forensic investigations where 98% of violent crimes are done by men; it helps in biodefense particularly in male terrorist profiling and genealogical and evolutionary studies. Y-STR also established the important role in human identity testing specially in

paternal lineages, some cases where no spermatozoa and assess the number of male donors/ contributors.

It can help to identify the number of males involved in sexual assault evidences. It can determine paternity of disputed child. Helps in unknown dead body identification. Patrilineal relationship testing (Dekairelle and Hoste, 2001; Goray *et al.*, 2019; Mapes *et al.*, 2019; Novroski *et al.*, 2018; Prinz *et al.*, 2001; Shewale *et al.,* 2003; Sinha *et al.*, 2003; van Oorschot *et al.*, 2019). Y-STRs helps to conduct missing person investigation (Dettlaff-Kakol and Pawlowski, 2002) and facilitate genealogical research (Jobling, 2001; Sykes and Irven, 2000). Comparison of Y chromosome can help to determine the paternity of a son in question. It is also applied to determine the genetic reason for male infertility (Krausz *et al.,* 2001; Zhou *et al.*, 2020). Moreover, there are many cases of sexual assault which has been resolved by identification of the presence of Y- peak on amelogenin locus. Almost more than 90% of violent crimes are committed by male members. In rape cases these markers give particular information because in such cases victim's female DNA and assailant male DNA is mixed with each other. The presence of Identifier STRs in the victim's vaginal swabs cast doubt on the case's validity, but the Y-Filer STR assisted in identifying the male contributor from the alleged accused. This finding emphasizes the importance of Y-STRs in forensic DNA analysis. Population genetics and genealogy: The biological variation in human beings was firstly described by the understanding of human migration particularly in Europe and Africa (Cavalli-Sforza *et al.,* 1994; Menozzi *et al.*, 1978).

2.11.3 Forensic DNA analysis

Humans share 99.9% of their genetic code, but certain sites on DNA show variation between individuals (Barbujani *et al.*, 1997). Forensic genetic analysis focuses on these variable sites, particularly in hypervariable regions, which include mini and microsatellites (Holt *et al.*, 2002). STRs, which are short repeat units, are especially suitable for human identification due to their variability (Kaiser *et al.*, 2008; Walsh *et al.*, 2010).

The sensitivity of STR profiling allows for the analysis of even degraded samples, although degradation can cause dropout of larger STR loci, leading to partial DNA profiles (Gill *et al.*, 2000). Mini-STRs were developed to address this issue, targeting smaller loci to increase the success rate in obtaining profiles from compromised samples (Aladdin *et al.*, 2010).

For standardized comparison across laboratories, a common set of STR markers is used. The United Kingdom's (UK) Forensic Science Service (FSS) developed the first widely used set of STR markers in 1994. The FBI uses a standard set of 13 specific STR regions for CODIS, a national database of DNA profiles. The likelihood of two individuals having the same 13 STR profile is about one in a billion (Piacenza and Grimme, 2004).

CHAPTER THREE

MATERIAL AND METHODS

3.0 Overview

The third chapter discusses the hypothesis, the research questions, aims and objectives. It also covers the material and methods of molecular processes, sample size and data analysis.

3.1 Study Area and Sample Size

The sampling area of the study was Evelyn Hone College where twenty-one volunteer participants were recruited after signing consent forms prior to sample collection. From the twenty-one volunteers, eleven were female and ten were male. Because of the limitation of time, subjects were recruited from Evelyn Hone College student populace which originated from the ten provinces of Zambia.

3.2 Collection of DNA samples

To investigate the effectiveness of touch DNA collected from mobile phones from volunteers for its value simulated in criminal investigation, various materials were procured and some were organised inhouse; these included personal protective equipment (PPE) such as masks, gloves, surgical head cover and disposable lab coats were used to provide protection against cross contamination of the DNA samples and preserve the sample integrity. Forensic kits used in sample DNA extraction and analysis were procured from Medysis Medical Diagnostics Limited. These materials include sterile cotton swabs with no transport media, which were used to collect reference buccal swabs and casework samples from the twenty-one participants. Filter tips of 1000µl, 200µl, 10µl, 1.5 ml and 2ml sample tubes were also used in the experimental processes in the DNA sample preparation during extraction using Qiacube Connect Instrument and for collection of pure DNAs after the purification of the lysate. QIAamp Investigator kit was procured from Qiagen through the sole distributor in the country Medysis Medical Diagnostics Limited which was used in the DNA extraction process, in both casework and reference samples respectively. The extraction kit comprised of reagents and accessories which include; QIAamp MinElute Columns, Collection tubes 2ml, Buffer ATL,

Buffer AL, Buffer AW1 and Buffer AW2 (concentrate), Buffer ATE, Carrier RNA and Proteinase K (QIAamp DNA Investigator Handbook 01/2020).

3.2.2 Touch DNA collection and storage

To investigate the use of touch DNA as admissible evidence in simulated crime scene investigation, twenty-one mobile phones owned by participants were each swabbed and touch DNA sample collected with their consent (appendix 5) in July, 2023 using wet sterile cotton swabs. The phones were handled with gloves and swabbed in zig-zag motion for twenty strikes. The collected DNA was referred to as casework or forensic samples, while buccal swabs were referred as reference sample as described in Figure 3.1. Through striking the side cheek ten times on the inside of each side buccal cavity and two complete circular swabbing movement of the gums to collect buccal swabs for reference samples. Collected DNA samples were stored in the breathable evidence bag and stored at room temperature to prevent DNA degradation through high temperatures, then the collected samples were transported to the Forensic DNA Laboratory situated at Levy Mwanawasa Medical University for analysis. The of forty-two samples were collected from twenty-one participants which was referred as reference samples and twenty-one from mobile phones which was referred to as casework samples in duplicate. Before extraction the two sets of samples were kept in the fridge at -21ºC.

Figure 3.1 Demonstration of collection of reference DNA sample by the buccal swab method. www.megacliniclabs.com/~/media/it-mmfiles/special-instructions/buccal (27/05/2025).

3.3 DNA Extraction and purification procedures

To analyse touch DNA for its application to crime scene investigation in Zambia, DNA was extracted and prepared for further analysis using a Qiagen kit according to the user instruction manual (www.qiagen.com). The extraction process involved separating and isolating the DNA from other cellular components, such as proteins and the nucleus, which is crucial for the identification of loci and Short Tandem Repeat (STR) regions critical to forensic DNA analysis (Hearn, 2010). The extraction utilised two protocols: Lysis and purification. Lysis protocol utilised the lysis buffers and proteinase K provided in the extraction kit. Cells were lysed by in a lysis buffer. During purification stage other cell components were washed off as prescribed in the SOP provided. The pure DNA was collected in the 1.5 ml sample tubes and stored at -21℃. Adherence to strict hygiene and Personal Protective Equipment (PPE) protocols was mandatory to prevent cross-contamination. The figure below was the device used for automated DNA extraction.

Figure 3.2 Qiacube Connect automated device used for DNA extraction in various forensic samples such as buccal swabs, bone tissue, blood, saliva, vaginal swabs etc. https: www.qiagen.com (21/05/2025).

3.4 Sample quantitation

Quantitation is the experimental process where the amount of startup DNA is measured in the sample after being separated from other cellular materials. This was a critical process in DNA analysis because it ensured that the DNA sample was neither over-amplified nor under-amplified during the subsequent amplification process (http//www.bmglabtech.com/uv-absorbance-dna-qualification/>.). Absorbance involved the quantification of the amount of light absorbed when it passed through a material. Essentially, absorbance was chosen in the quantification because it provided insights into the concentration of a light-absorbing compound in a sample, offering crucial data in numerous scientific fields, particularly in studying chemical concentrations. Contrastingly, fluorescence method is a phenomenon where a molecule absorbs light or photons, becomes excited, and then emits light at a longer wavelength. Therefore, fluorescence provides details about the properties of the DNA, such as its environment and concentration,. It was able to visualised and quantified biological processes.

After DNA was separated from other cell materials, its quantity was measured as summerised in Table 4.1. This step was crucial to determine how much DNA was present in the sample, which in turn ensured appropriate normalisation prior amplification.The quantitation was performed using a wavelength method, which likely refered to measuring the absorbance of DNA at specific wavelengths, such as 260 nm, which was commonly used for DNA quantification using the device in Figure 3.4 shown below (www.qiagen.com). This method was a standard practice in molecular biology to assess the purity and concentration of DNA extracted from the sample.

The quantification kit contained reagents and DNA polymerase for specific amplification of 4NS1C®, which was a 91 bp propriatary region present on several autosomes of the human genome, and for detection of the specific PCR products on the Rotor-Gene® Q System. The human quantification target region was selected to achieve high sensitivity. The human quantification target region was detected using the yellow channel on the Rotor-Gene Q.The target region for male DNA quantification was selected to achieve high sensitivity in the presence of mixed female/male DNA samples. The human quantification target region was detected using the yellow channel on the Rotor-Gene Q.

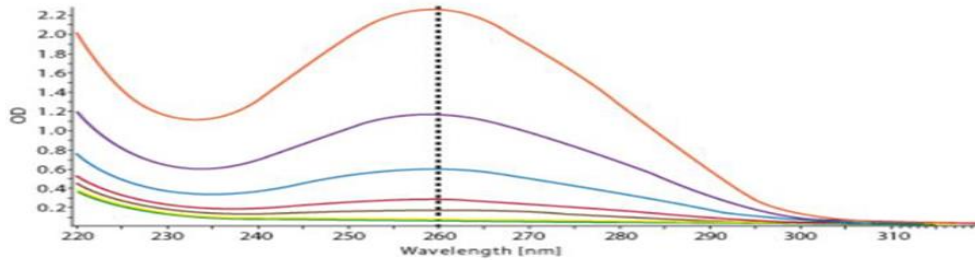

Figure 3.3 Demonstration of the wavelength method used to determine DNA quantity, purity and concentration in quantification process (http//www.bmglabtech.com/uv-absorbance-dna-qualification/>.) (21/05/2025).

Figure 3.4 Rotor Gene Q (real-time PCR) used for DNA Quantification. It was also able to determine male DNA from Female DNA in the mixed DNA sample. https://www.qiagen.com (21/05/2025).

3.5 PCR amplification

The DNA extracted from the reference and casework samples respectively was used in PCR-amplification reactions following the prescribed SOP in the instruction's manual. The materials and reagents were provided in the GlobalFiller kit and GlobalFiller Express kit. The kit was validated for use with: 1.0 ng DNA (15 µL input volume) for 29 cycles ,500 pg DNA (15 µL input volume) for 30 cycles for increased sensitivity with low-concentration samples.

The GlobalFiler™ kit also included the following primer additions and modifications DZ which referred to a specific locus (or DNA marker) that was amplified by the kit's primers. Addition of DYS391 and a novel Y indel. The TPOX reverse primers were redesigned to relocate the amplicon into the higher size range of the multiplex and optimize marker spacing. Addition of 8 new SNP specific primers for the D3S1358, vWA, D18S51, D19S433, TH01, FGA, D5S818, and SE33 loci. The second degenerated primer was added to the vWA locus to address two different SNPOs in the primer binding site. Non-nucleotide linkers are used in primer synthesis for the following loci: D19S433, vWA, CSF1PO, D2S441, TH01, FGA, and D12S391. For these primers, non-nucleotide linkers are put between the primers and the fluorescent dye during oligonucleotide synthesis (Butler 2005, Grossman *et al.,* 1994). Non-nucleotide linkers enable reproducible positioning of the alleles to facilitate interlocus spacing. The combination of a 6-dye fluorescent system and the use of non-nucleotide linkers allows simultaneous amplification and efficient separation of all 24 markers during automated DNA fragment analysis.

The Polymerase Chain Reaction (PCR) was used to make exact copies of the various STR regions isolated in the previous step using the device shown in figure 3.5 below. The process involved thermal cycles (i.e., heating and cooling) that `unzip' the DNA double helix and created an exact replica using complementary nucleic acids. It was also during this process where STR fragments were labelled with fluorescence-emitting molecules called fluorophores which allowed the STRs of varying sizes to be detected after separation.
PCR (Polymerase Chain Reaction) amplification involved three main steps denaturation (separating DNA strands), annealing (Primers binding to the template), and extension (DNA polymerase extending the primers). These steps are repeated multiple times to amplify the target DNA sequence.

1. Denaturation
   The double stranded DNA template was heated typically to 95ºC which separated the two strands into single stranded DNA. This created the template for the second time.
2. Annealing
   The temperature was lowered, typically to 50-65ºC, allowed short DNA sequences called primers to bind to specific regions on the single stranded DNA template. The primers are designed to flank the target DNA sequence which was amplified.

3. Extension

   The temperature was raised again, typically to 72ºC, allowed a thermostable DNA polymerase enzyme (like Taq polymerase) to extend the primers synthesizing new DNA strands complementary to the template.
4. Cycling and Amplification

These three steps (denaturation, annealing, and extension) were repeated multiple times (typically 20-40 cycles). With each cycle, the amount of the target DNA sequence doubled, resulting in exponential amplification. The PCR machine, also known as thermocycler, controlled the temperature changes necessary for each step.

Figure 3.5 The diagram shows Bio-Rad T100 Thermal Cycler an instrument used for DNA Amplification. https: www.bio-rad.com. (21/05/2025).

3.6 DNA sequencing and bioinformatics

The amplicons were sequenced in a 3500xL Genetic Analyser and electropherograms were generated. The basic principle relies on separation by electrophoretic mobility of DNA fragments, amplicons and alleles which were separated based on size and as each allele passed the laser, it registered as a peak in intensity of the fluorescent dye accurately. The 3500xL Genetic Analyser (shown in figure 3.6) is a 24-capillary sequencing platform which was used for a wide variety of applications, including de novo sequencing and resequencing (mutational profiling), as well as microsatellite analysis. This device used capillary electrophoresis to analyse the DNA fragments. This package included a 24-capillary instrument, 3500 Series Data Collection Software, and a Dell™ Workstation and monitor. The 3500 Series Data Collection Software supported sequencing and fragment analysis, and integrated seamlessly with several

downstream software packages for secondary analysis of genetic data these includes GeneMapper ID-X Version 1.6.

The master mix for a 96-well run was prepared by combining the following in a single microcentrifuge tube. Reagent Volume Hi-Di Formamide 988 µL. The master mix tubes were vortexed for 10-15 seconds, then spun in a microcentrifuge. In a 96-well reaction plate, 10 µL of the formamide was dispensed and size standard master mixed into each well of the run. The allelic ladder was centrifuged briefly to bring contents to the bottom, then vortexed for 15 seconds. Pulsed spun for 1-2 seconds in a microcentrifuge before was used to ensure reagent was at the bottom of the tube. Spun the amplification plate in a centrifuge for 1 minute at 2000 rpm ensured all punches and reagents were at the bottom of the wells and no bubbles were present. Loaded 1.0 µL of allelic ladder and 1.0 µL of amplified sample into the appropriate wells of the 96-well reaction plate as specified by the 3500xL plate map worksheet. Covered the reaction plate with the 96-well septa. Ensured the tray was labelled according to the labelling section of this procedure. Spun the reaction plate in a centrifuge for 1 minute at 2000 rpm ensured the contents of each well were mixed and collected at the bottom. Using a thermal cycler, I denatured the reaction plate at 95°C for 3 minutes and after that I snap froze the plate to 4°C for 3 minutes.

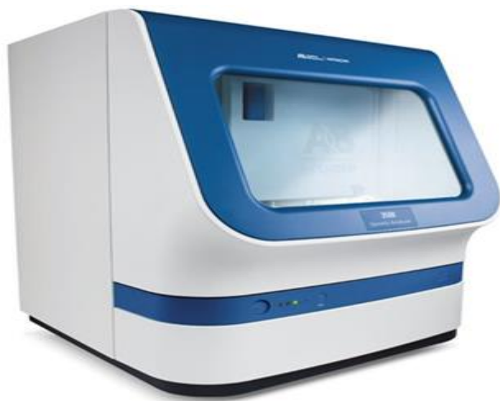

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Figure 3.6 AB 3500xL Genetic Analyser used to analyse DNA amplicons, fragments and alleles using capillary electrophoresis. https: thermofisher.com (21/05/2025).

The generated raw data (as shown in figure 4.2.2 to 4.3.2) was transferred in the data room were data analysis was done using GeneMapper software. Comparisons of casework and

reference sample results (shown in figure 4.4A to figure 4.4E) were determined and presented as in the following chapter.

CHAPTER FOUR

RESULTS

4.0 Overview

This chapter presents the findings on the characterisation of touch DNA using the STR forensic markers to determine the genetic diversification among the selected subjects. This chapter presented the sets of results from the quantification and post-amplification of the touch DNA both from reference and casework samples. The quantification process determined total human and male DNA concentrations (as summerised in table 4.1) The generated fragment data was analysed using GeneMapper™ Software 1.6.

4.1 qPCR casework results

DNA quantification was used to determine the total concentration of DNA in a sample. It was carried out before downstream experiments and it was vital in ensuring the validity of analysis results. The quantification results determined showed both human absolute and male DNA concentrations as indicated in the Tables 4.1 and 4.2 respectively.

The determined concentration was the total concentration of DNA in the sample, while PCR pre-dilution/sample volume was 15µl and PCR setup (pre-dilutions/diluent) µl were determined dilutions to achieve optimisation. When the DNA concentration of the sample was too high, such as in sample 2C, 1 µl of the sample volume was pipetted in the sample tube and 14µl of diluent (nuclease free water) was added. In this setup it was about 0.057 ng total DNA added to the reaction. The quantification kit contained reagents and a DNA polymerase for specific amplification of 4NS1C® which was a 91 bp proprietary region present on several autosomes of the human genome. The human quantification target region was detected using the yellow channel on the Rotor-Gene Q. while the target region for male DNA quantification was selected to achieve high sensitivity in the presence of mixed female/male DNA samples. The male quantification target region was detected as an 81 bp fragment using the green channel on the Rotor-Gene Q.

Note that the instrument was highly sensitive, samples which were less than 0.01ng/µl had very minute DNA such as in sample 8C and 17C in the table below, no diluent was added to the reaction mixture, this meant the whole 15µl was pipetted from the sample and approximate

amount of DNA in the sample was determined as 0.063ng and 0.014ng after quantifying respectively. The PCR sample volumes were not the same because the determined DNA concentration was not the same as these are DNA from samples collected from casework samples (mobile phones).

Table 4.1 Quantification results of absolute human and male DNA determined using Rotor Gene Q device.

Sample ID	Determined Absolute Human DNA Concentration(ng/µl)	Determined Male DNA Concentration(ng/µl)
1C	0.05	0.09
2C	1.47	0.02
3C	0.06	0.03
4C	0.06	> 0.01
5C	0.01	>0.01
6C	0.01	> 0.01
7C	0.02	0.01
8C	0.00	> 0.01
9C	0.02	0.06
10C	0.02	> 0.01
11C	0.24	> 0.01
12C	0.23	0.02
13C	0.01	0.01
14C	0.01	> 0.01
15C	0.25	0.01
16C	0.01	0.01
17C	0.00	0.01
18C	0.01	> 0.01
19C	0.02	0.03
20C	0.01	> 0.01
21C	0.18	0.01

The table consist of absolute human and male DNA results determined using Rotor-Gene Q which differentiated male DNA from total human DNA. These results can lead the forensic scientist either to proceed to upstream processes or go back to extraction in the cases of suspected sample mixture like in sexual cases, touch DNA and many others.

4.2 Post amplification results

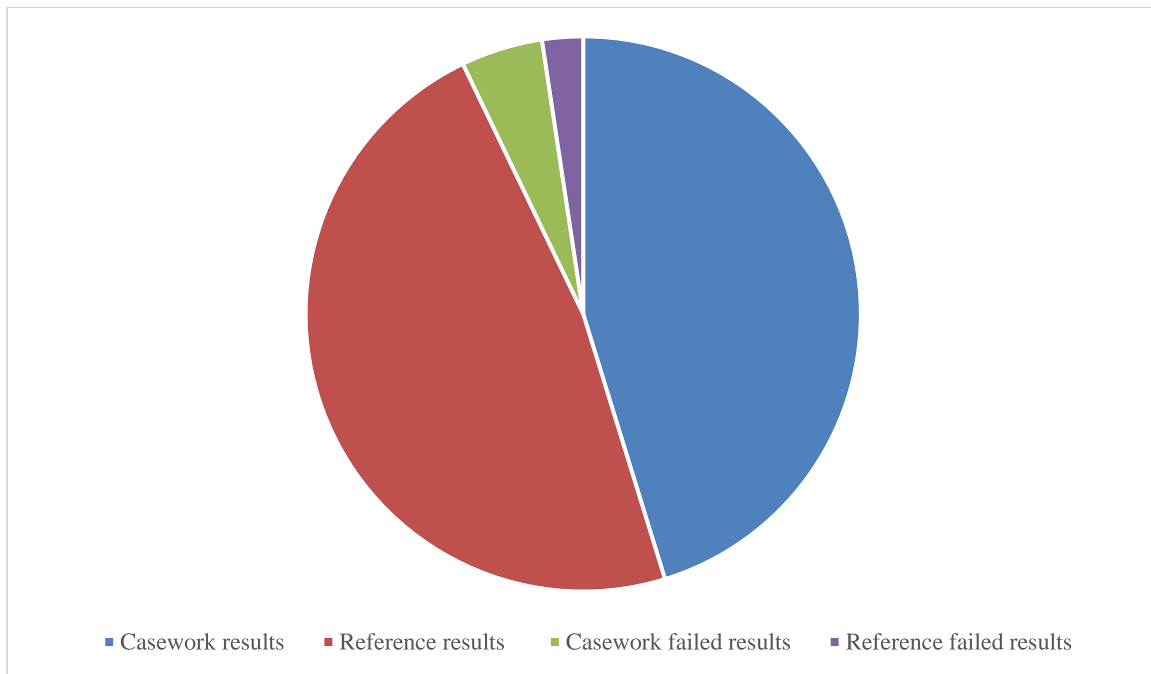


Figure 4.1 Results of post-amplification analysis of amplicon DNA.

From twenty-one simulated casework samples collected and analysed,19 were successfully extracted, amplified and full DNA profiles were generated. While 20 simulated reference samples were successfully profiled and 1 sample failed to generate meaningful DNA profile. Failure to generate meaningful DNA profile could be due to DNA degradation, allele dropout or no adequate DNA was extracted from the sample due to poor sample collection techniques.

The electropherogram figures 4.2A, 4.2B, 4.3Aand 4.3B shown below are the data generated by AB 3500xL genetic analyser (figure 3.6). Figures shown are selected as they represent the full range of observed DNA quality and DNA electropherograms peaks for both casework and reference sample results respectively. The peaks in the electropherogram represent each allele, while the numbers below the peaks represent the Short Tandem Repeats (STR). An allele can either be referred as homozygous or heterozygous. Of the 42 samples analysed 90.5% matched reference and casework sample results. the rest of the profile results are presented in appendices 1 and 2 respectively. The specific loci were identified and compared manually marker by marker during data analysis. The sample which originate from common source will have the similar DNA profiles. The graphical data presented aligns with the objectives of the study results in that sufficient DNA was extracted from the mobile phones simulated crime scene. A

comparison between simulated casework sample results and simulated reference sample results was done.

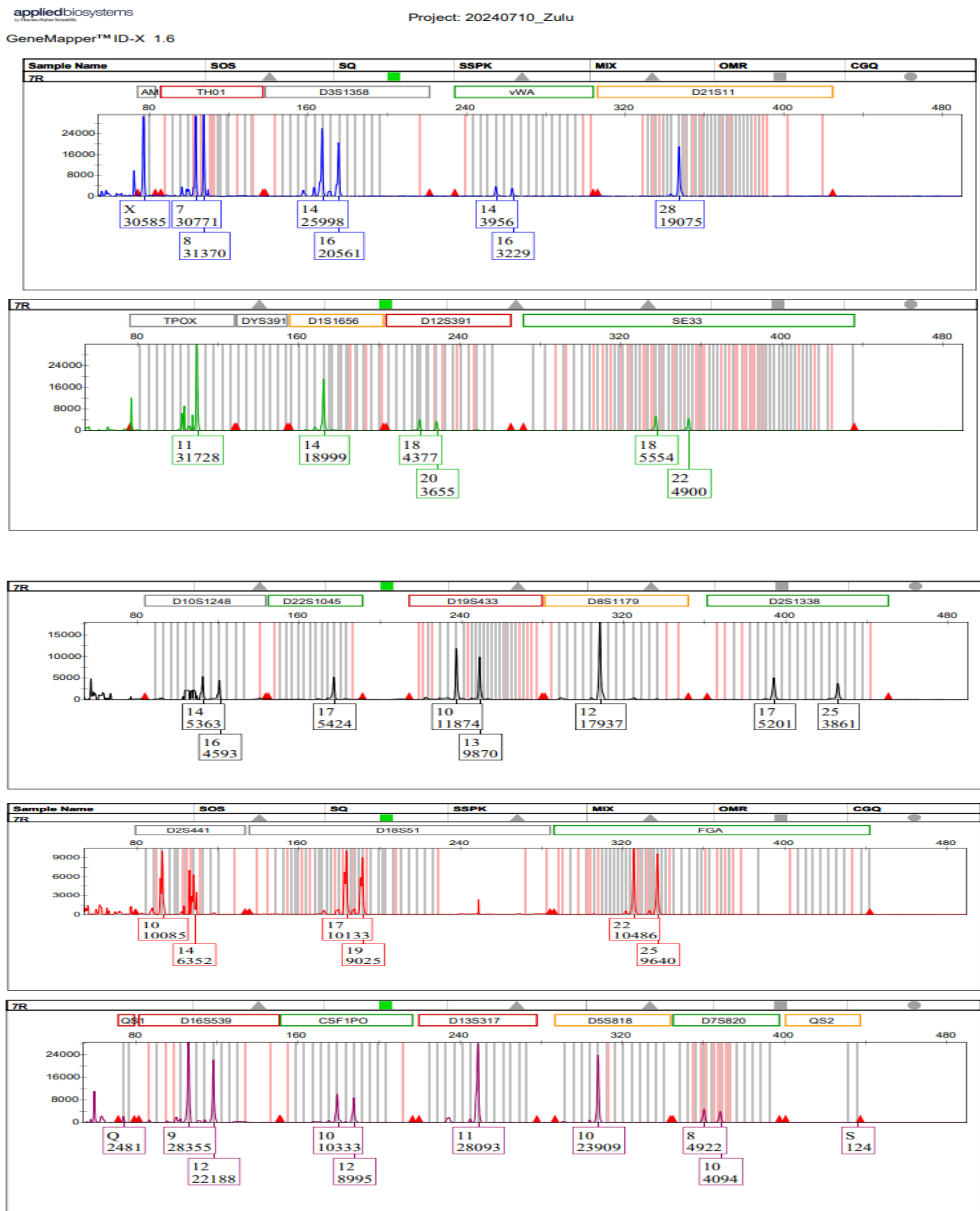


Figure 4.2A Electropherogram profile results for reference (buccal swab) sample 7R results indicating the forensic markers in all the core loci generated.

Figure 4.2B Electropherogram profile results for reference (buccal swab) sample 4R results indicating the forensic markers in all the core loci generated.

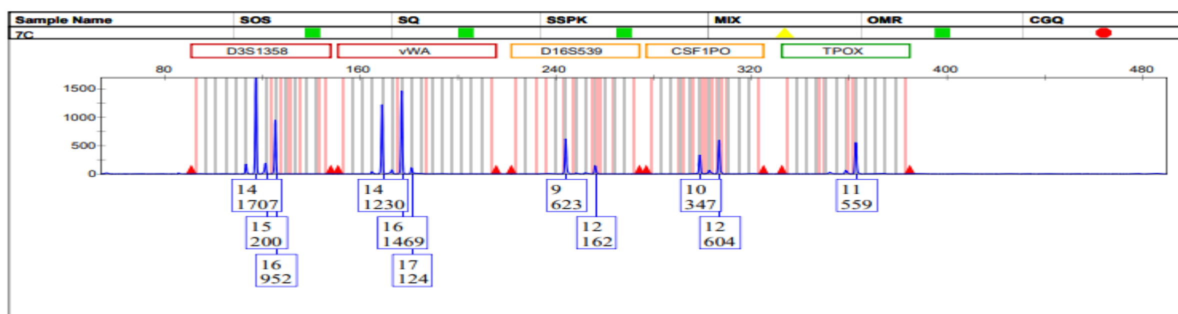
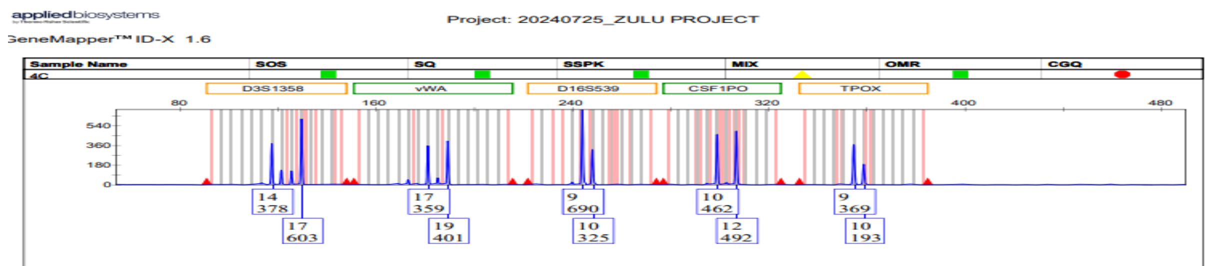


Figure 4.3A. Electropherogram profile results for reference (buccal swab) sample 7C results indicating the forensic markers in all the core loci generated.



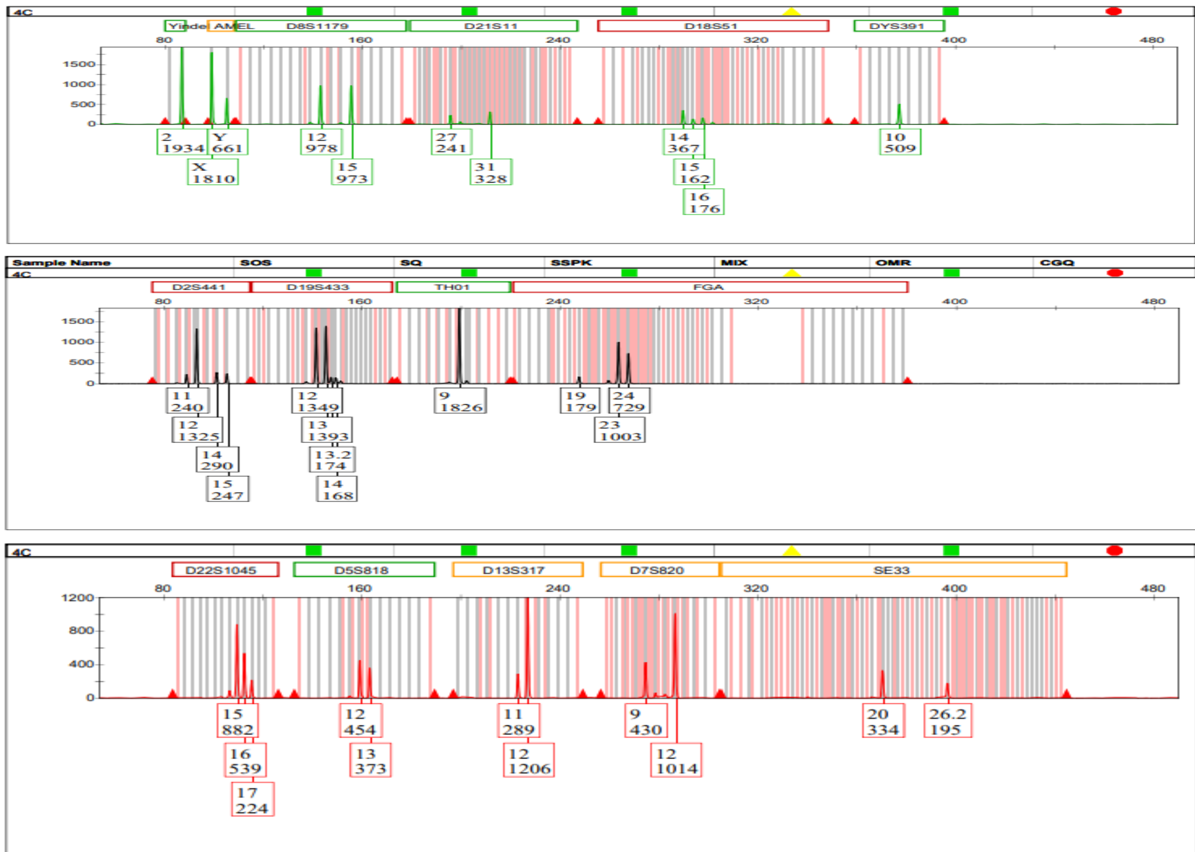


Figure 4.3B Shows the DNA profile results for casework (swab from mobile phone) sample 4C results indicating the forensic all the core loci generated.

Figures 4.4A, 4.4B, 4.4C, 4.4D and 4.4E are graphical presentation of results for both casework and reference sample results for only five samples showing possible mixtures in casework sample results, for comparisons and correlation with reference sample results. The figures show the number of STRs in the Y-axis and forensic markers in the X-axis.

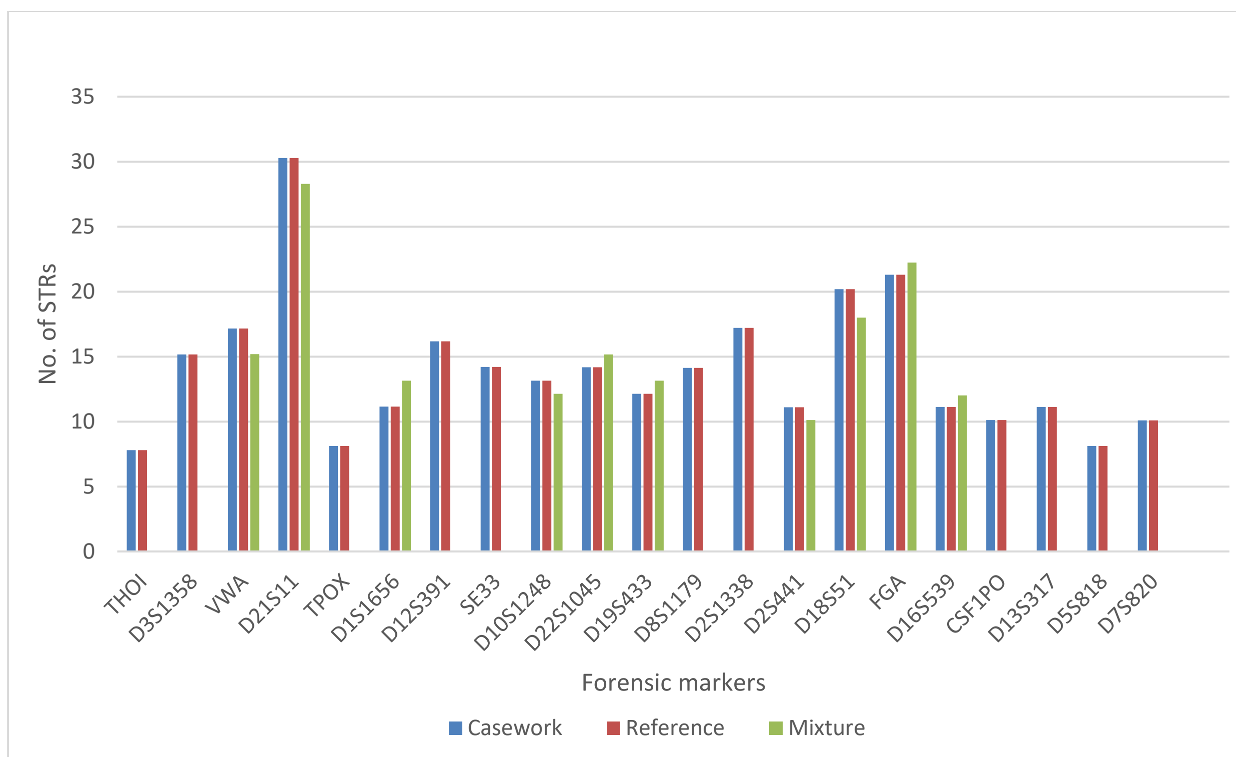


Figure 4.4A. Graphical comparison of casework and reference sample three results, showing possible mixture which is shown in green on 10 forensic markers. The Y-axis represents the number of STRs, while the X-axis represent the forensic markers. On the markers the blue bar represents casework results, while brown bars represent reference samples and these are DNA profile generated from buccal swabs. Green bars represent mixed DNA profiles that contained DNA from two or more people.

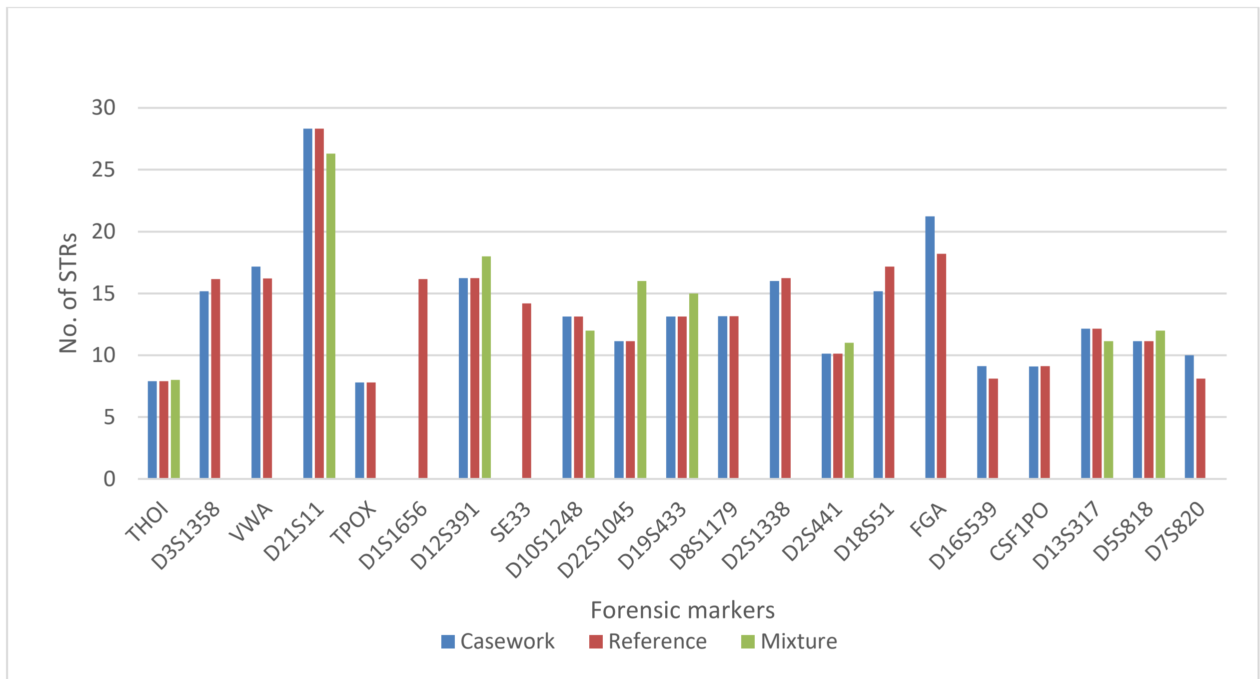


Figure 4.4B Graphical comparison of casework and reference for sample five, showing partial mixture. The Y-axis represents the number of STRs, while the X-axis represent the forensic markers. Blue bars represent casework results while brown bars represent reference samples. Green bars represent mixed DNA profiles for profiles that contained DNA from two or more than two people.

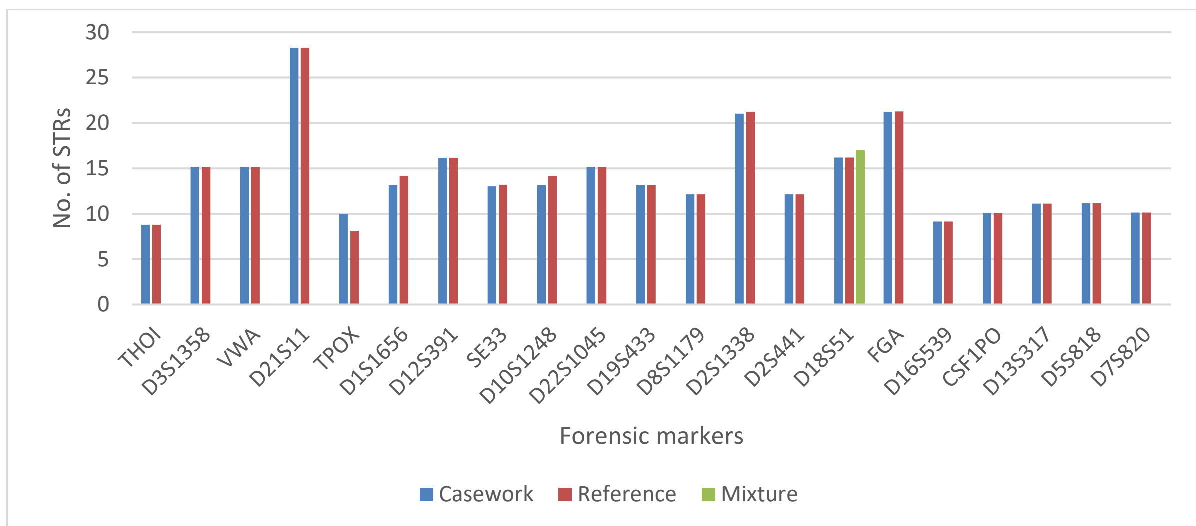


Figure 4.4C Graphical comparison of casework and reference for sample eleven, showing the possible mixture only one marker. The Y-axis represents the number of STRs, while the X-axis represent the forensic markers. Blue bars represent casework results, brown bars represent reference samples and green bars represent mixed DNA profiles that contained DNA from two or more people.

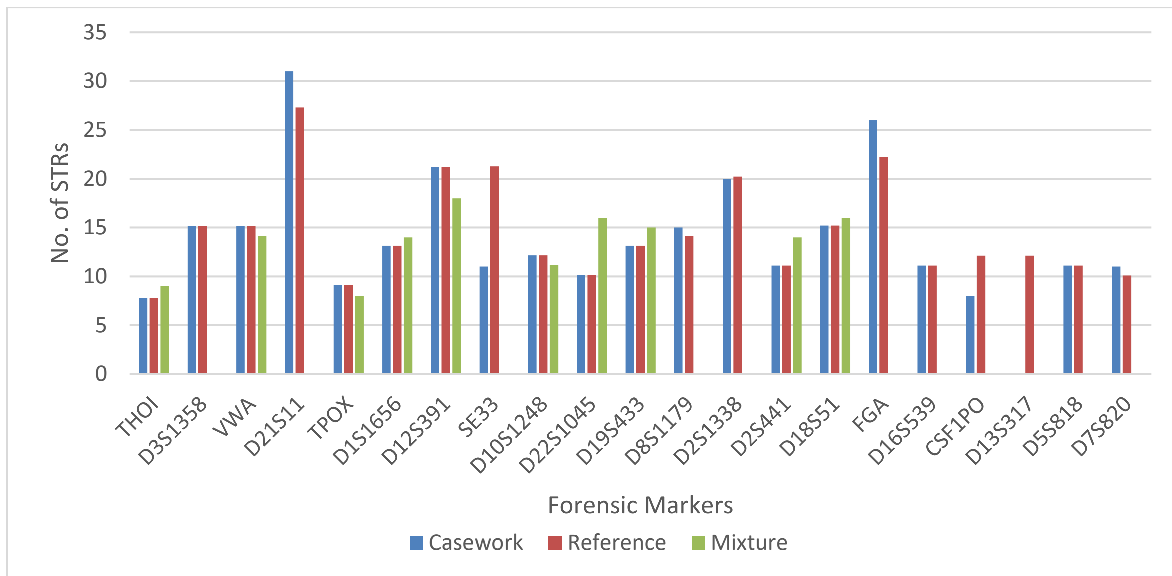


Figure 4.4D Graphical comparison of casework and reference sample eighteen, showing the possible mixture on 10 markers. The Y-axis represents the number of STRs, while the X-axis represent the forensic markers. Blue bars represent casework results, brown bars represent reference samples and green bars represent mixed DNA profile that contained DNA from two or more people.

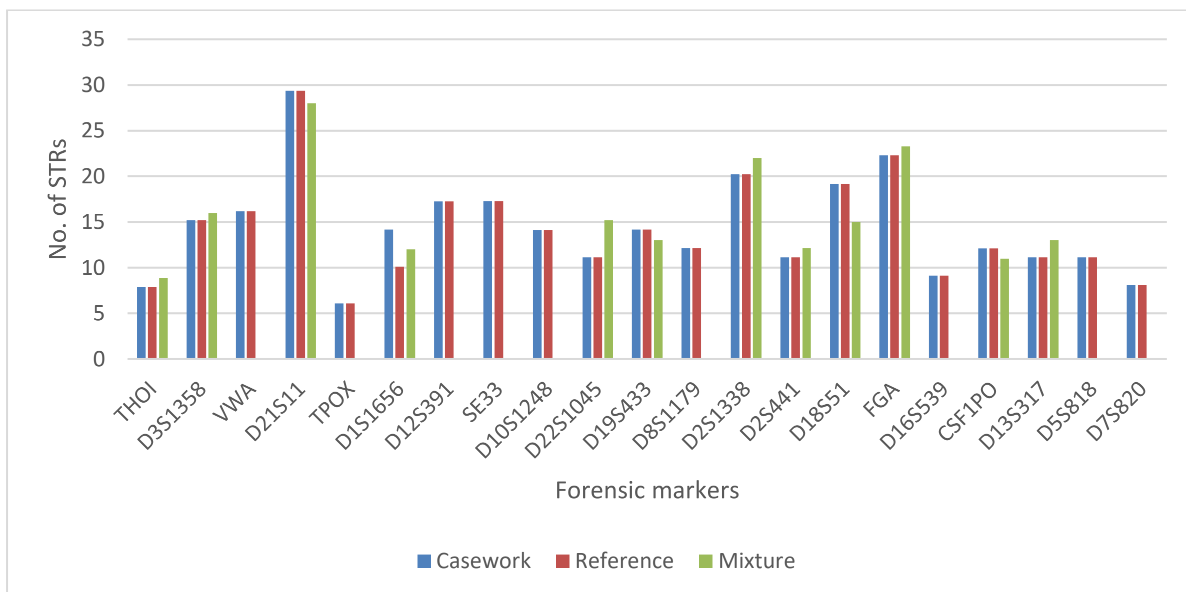


Figure 4.4E Graphical comparison of casework and reference sample twelve, showing possible mixtures on 12 markers. The Y-axis represents the number of STRs, while the X-axis represent the forensic markers. Blue bars represent casework results, brown bars represent reference samples and green bars represent mixed DNA profile that contained DNA from two or more people.

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CHAPTER FIVE

DISCUSSION

5.0 Overview

The previous chapter presented the research findings of the study in relation to the research questions. This chapter discussed the research findings on the genetic comparison of the casework and reference DNA sample profiles. The discussion was presented in accordance with the research aim and objectives of the study which was to compare DNA profiles of casework and reference samples results respectively, to establish a match or non match between the two sets of sample results.

5.1 Genetic profile assesment of the casework and reference DNA results

The current study was the first of its kind to document the assess the effectiveness of touch DNA method for criminal investigations in the country. The results from this study will augment those from recent related studies on touch DNA studies conducted in other countries such as USA (Quinones and Daniel, 2011). The results from the earlier studies concluded that touch DNA was effective as law enforcement officers encountered various crimes where trace evidence was deposited which could help resolve the crimes even when there are no eye witnesses.

The first specific objective of the study was to profile touch DNA collected from mobile phones from volunteers to determine its effectiveness in criminal investigations. To achieve this objective this study recruited twenty-one participants who owned mobile phones and were originating from each one of the ten provinces of Zambia and above 18 years of age. Both reference and casework samples were collected from the subjects and DNA extracted, quantified and amplified according to molecular techniques (Van Oorschot *et al*., 2019; Verdon *et al*., 2014; Alketbi and Goodwin, 2019). The second objective was to analyse touch DNA for its effectiveness in criminal investigations in Zambia. Forty-two DNA samples were extracted, from both set of samples. From twenty-one reference samples only one sample failed to yield DNA profile, while 19 casework samples were extracted and analysed successfully to generate full DNA profiles. The amount of DNA collected from mobile phones varied from 0.00 ng to 1.47 ng as indicated in Table 4.1. The biological source of this DNA was often unknown, but

it could be due to usage habits, saliva, or free DNA deposited through sweat glands on the hands (Wickenheiser 2002). Some of the unknown profiles were partial profiles, likely due to low amounts or degradation of DNA due to environmental factors, these highlights the challenges in forensic DNA analysis.

The third objective was to compare DNA profiles from mobile phones simulated as crime scene items to reference samples from the owners of the mobile phones for their value in forensic investigation in Zambia. The genetic comparisons were done only on samples that had successful DNA profiles generated on both sets of samples collected from mobile phones and buccal swabs of the twenty-one participants through swabbing technique. The DNA extraction for both sets of samples was done using QIAamp DNA Investigator Kit (www.qiagen.com) using Qiacube Connect Device which is an automated machine. I carefully applied the sample to the QIAamp MinElute column. Centrifugation of QIAamp MinElute columns was performed at 6000 x g (8000 rpm) to reduce centrifuge noise. Pure DNA was collected in the 1.5ml sample tubes after two washes with wash buffers. The collected pure DNA was stored at -21ºC until the next processes proceed.

The study demonstrated that sufficient DNA quantity can be collected from mobile phones through wet swab technique as shown in the quantified results in Table 4.1 and 4.2 respectively. The one reference and two casework samples which failed to generate meaningful results could be due to a number of factors such as poor swabbing technique, natural degradation mechanisms such as temperatures and moisture content playing significant role (Hu *et al*., 2005). In environments with extreme climatic conditions, crime scene evidence may be exposed to high or low temperatures and humidity levels before reaching the laboratory, potentially accelerating the degradation of evidence and can attribute to unsuccessful DNA analysis like in the three samples which failed to yield meaningful results.

Cotton swabs were used to recover trace DNA in forensic casework (Raymond *et al*., 2009; Castella and Mangin,2008). However, DNA collection methods can be inefficient or DNA retained by the cotton swabs depending on the technique (Alketbi and Goodwin, 2019) this can be evidenced by the low quantities (table 4.1 and 4.2) of touch DNA. During the purification of extracted DNA carrier RNA (cRNA) was added to enhance the recovery of DNA. The cRNA prevents the small amounts of target nucleic acid present in the sample from being irretrievably bound (www.qiagen.com. The advantage of using the cotton swab was that half or full cotton swab head can be used giving an opportunity to reuse the swab in the next run. Therefore, this

study aimed to compare the DNA profiles from casework and reference samples and a match was established in the nineteen successful DNA profiles generated. However, this study research did demonstrate that mobile phones and similar surfaces can serve as viable sources for human DNA, which can then be used in forensic investigations. Such evidence can help to collaborate and consolidate the available evidence in critical criminal cases where there are no eye witnesses.

Interestingly, some samples showed 0.00ng (table 4.1) of DNA during quantification but were able to generate full DNA profiles after amplification. This is a very interesting aspect which showed even very low quantities can generate full DNA profile with the right technology used. The efficiency of quantification and amplification methods can influence the amount of touch DNA collected as researchers have demonstrated (Ottens *et al*., 2013). However, the quantification method that used real-time PCR (qPCR) are more effective for trace samples. The study demonstrated that the quality of the human absolute DNA collected from mobile phones was sufficient to amplify the 23 core loci, enabling the identification of individuals who regularly used the device through comparison of the generated profiles.

Data analysis of the generated profiles on casework sample results, mixed DNA profiles were detected in almost all the samples. The results generally were a match between reference and casework samples results. All the mixture profiles detected were partial profiles. Mixture DNA profiles from both known and unknown users of a mobile phone were accurately detected, the findings were in tandem with the study done by (Bright *et al*., 2019 and Lodhi *et al*., 2015).

The results indicated that DNA transfer to surfaces like mobile phones varies among individuals due to factors like skin cell shedding, hand-washing habits, and frequency of touching one's face and they agrees with study done by Raymond *et al*., (2009). These factors influence the amount of DNA left behind (Burrill *et al*., 2019). The concept of "loading" fingers with DNA through touching one's face or other body parts, as described by Wickenheiser (2002), and the effects of pressure and friction on DNA transfer, as demonstrated by Goray *et al*., (2010), could be some of the factors which contributed to variations in the quantity.

5.2 Detection of Y-chromosomes

The quantification results as summerised in Table 4.2, show two categories namely absolute human DNA and male DNA. The Rotor Gene Q device has capacity to separate male DNA from female DNA and this is important in cases such as sexually related cases where the focus

is the male suspect. This study composed of ten male and eleven female participants. From the quantification results, male DNA was detected from seven (7) mobile phones from female participants. The detection of the Y chromosome shows how effective the method can be to the criminal investigations as it can give a clue to the investigation team that they are actually looking for a male suspect in the crime committed. At amplification the male DNA is detected by the presence of Y-chromosomes in the profile. Advancement in technology have developed a specific Y STR profiler kit, this kit can also be used to develop the male genetic profile which only the Y STR profile is detected. The presence of male DNA on the female mobile phones simply shows that male counterpart had access to the phones of female colleagues. The unknown male DNA detected can be probed further by analysing the reference samples from the minor contributors and the major contributor can be singled out. These study findings are in tandem with the study which was done by Lodhi *et al.*, 2015. In the case of the sexual crime committed, suspects can be brought forth and have their samples collected, extracted and analysed, and finally the profiles can be compared and perpetrators of crime can be identified and be convicted in the court of law (Thompson and Black, 2007; Rudin and Inman, 2002; Savino and Turvey, 2011).

Biological evidence can be collected from the mobile phone by simply swabbing the surface with a wet cotton swab then extracting and concentrating the DNA using molecular biology techniques. This research findings establishes several important findings. First, mobile phones can be used as a source to collect human DNA, and subsequently used for forensic investigations. Secondly, the amount (1 ng) and quality of human DNA collected from mobile phones is sufficient for amplification of the 13 loci necessary to identify the individual who regularly accesses the mobile phone. Thirdly, partial DNA profiles of unknown cell phone user(s) can be detected from the cell phone. The detection of unknown contributors (more than 2 alleles at a locus) demonstrated the usefulness of human DNA collected from a mobile phone in a forensic investigation. Most likely the partial DNA profiles are due to LCN and/or degradation of DNA collected from the cell phone. These missing markers/allele(s) were possibly due to locus and/or an allele drop out. The possibility exists that increasing the cycle number from 28 to 32 for the mobile phone DNA samples may have resulted an allele drop-in. However, these possible false alleles would not interfere with the mobile phone DNA analysis because the RFU threshold is high enough (RFU=100) to reject an incorrect interpretation of allele scoring. Therefore, the analyst should be careful when analysing results. Fourthly, in scenarios where the owner of the mobile phone is female, a male user can be detected by the

presence of the Y chromosome allele. Subsequent interviews with female mobile phone owners in which the Y chromosome allele was detected revealed that males did use their mobile phones. To establish the male contributor, a genetic profile can be developed using the Y STR profiler kit and/or by generating STR profile(s) from reference samples of the male contributor.

This study demonstrates that it is possible to obtain sufficient quantity and quality of human DNA from mobile phones for amplification to generate genetic profiles of known and unknown individual(s) from a mobile phone. The amount of collected DNA from mobile phones varied from 0.00 ng to 3.34 ng. The biological source of the DNA on the mobile phones is unknown. A possible explanation for the large amount of DNA (3.34 ng) on some cell phone may be the mobile phone usage habits of the mobile phone user(s), as well as other biological materials deposited on the mobile phone such as saliva and/or blood of mobile phone user(s). The unknown profiles in this study were all partial profiles which may be due to low amounts or degradation of DNA. My results shown 99% agreement between profiles of STR analysis between simulated reference samples and simulated casework samples, while 1 % did not correspond. This is similar to results from the study done by Lodhi *et al.*, 2015.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.0 Overview

The previous chapter looked at the discussion of the findings of this dissertation. This chapter presents the conclusion of the study and the recommendation.

6.1 Conclusion

This study demonstrated that sufficient human DNA quantity and quality can be recovered from a mobile phone which simulate crime scene, to generate partial or full human STR profile for forensic investigations (Naughton and Tan, 2011). Through the reference samples, tracking the owner of the cell phone or source of touch DNA from among the list of suspects would not be a difficult job for the law enforcement agencies. However, exclusion or inclusion of the contributor of DNA found at the crime scene could be established through touch DNA in the criminal court. Human DNA on cell phone or surfaces of the touched objects at the crime scene could be a valuable piece of forensic evidence that would help link suspects to crime scene sites. DNA in the graphical presentations was the casework and reference sample shown same height indicating that the source of the two sets of DNAs are the same. In cases of possible mixtures, alleles other than that of the owner of the phone DNA is detected. Comparison of DNA profiles from mobile phones simulated as crime scene items to reference samples for their role in forensic investigation has been done and it has proven to be a viable technique worthy of implementing in criminal investigations by law enforcement agencies in Zambia

6.2 Recommendations

The following recommendations have been conceived based on the results of the study to assess the effectiveness of touch DNA in criminal investigations in Zambia.

Individuals have different DNA shedding abilities (Murray *et al.*, 2001; Lowe *et al.,* 2002; Phipps and Petricevic, 2007; Allen *et al.*, 2008; Quinones, 2011) which are often affected by a person's behaviour and activity.

In addition, different surfaces retain DNA deposits differently and it is important to consider the most appropriate method to implore to improve DNA collection (Alketbi and Goodwin, 2019e). It is recommended to use a cotton swab for smooth, non-porous surfaces such as fabric.

Environment conditions in combination with high temperatures can influence touch DNA deposits on surfaces (Alketbi and Goodwin, 2019b; Alketbi and Goodwin, 2019c; Alketbi and Goodwin, 2019). In addition, it is better to collect samples from surfaces as soon as possible after deposition to obtain higher DNA yields and to avoid cross contamination, particularly if items containing touch DNA are found outdoors.

Furthermore, touch DNA is usually in low quantity therefore, good swabbing techniques would be encouraged and the sample collection can be done in duplicate in case of unsuccessful DNA extraction on first attempt. During extraction cRNA was added to increase the DNA yield. At amplification and post amplification stage choice of the kit mattered, as kit may vary from one to other. Some are proving better in certain type of samples than others, hence the need to have wise choice.

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APPENDICES

Appendices 1: Represents the DNA profiles of reference

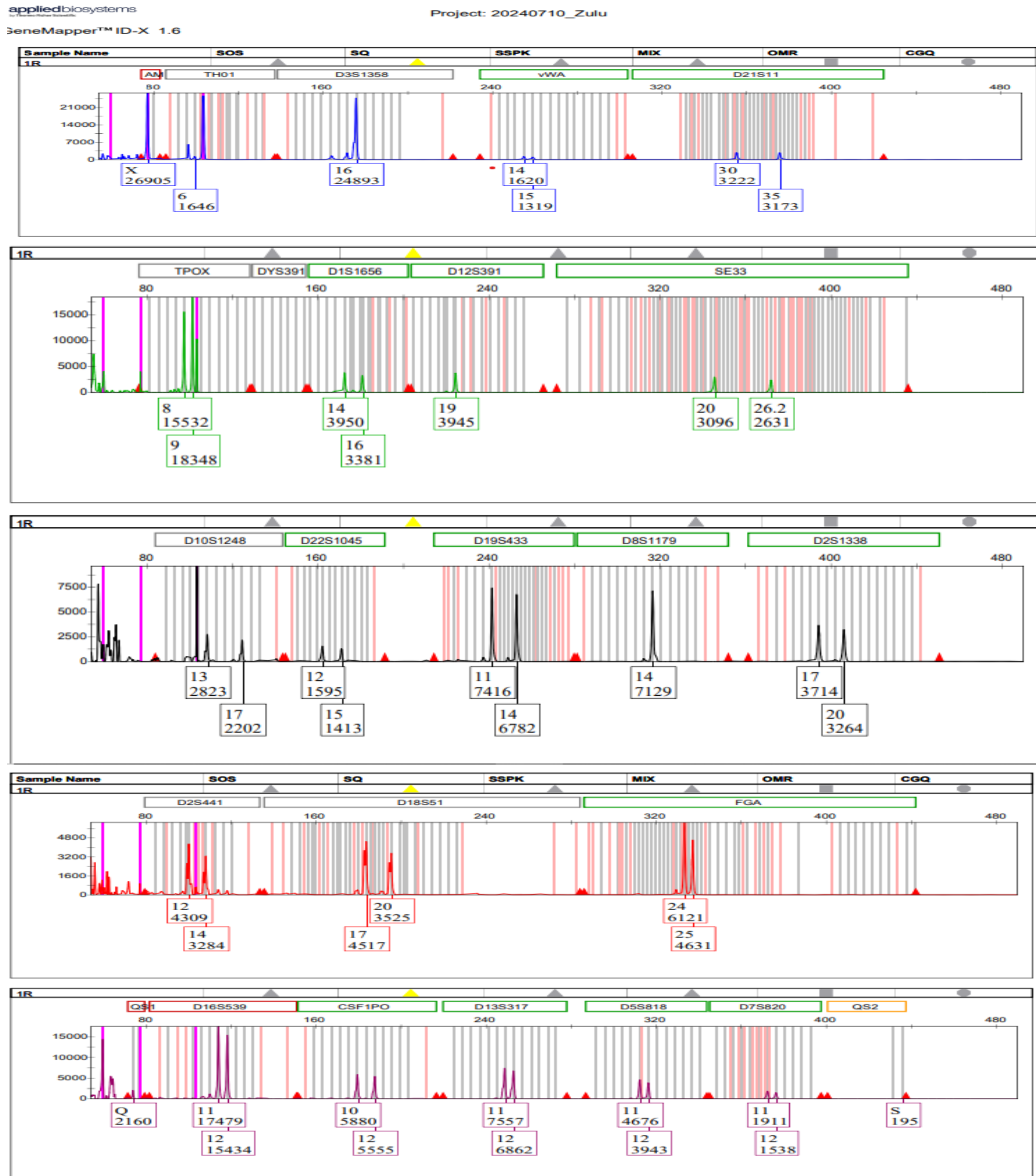

Figure1: Sample 1R DNA profile results of reference samples.

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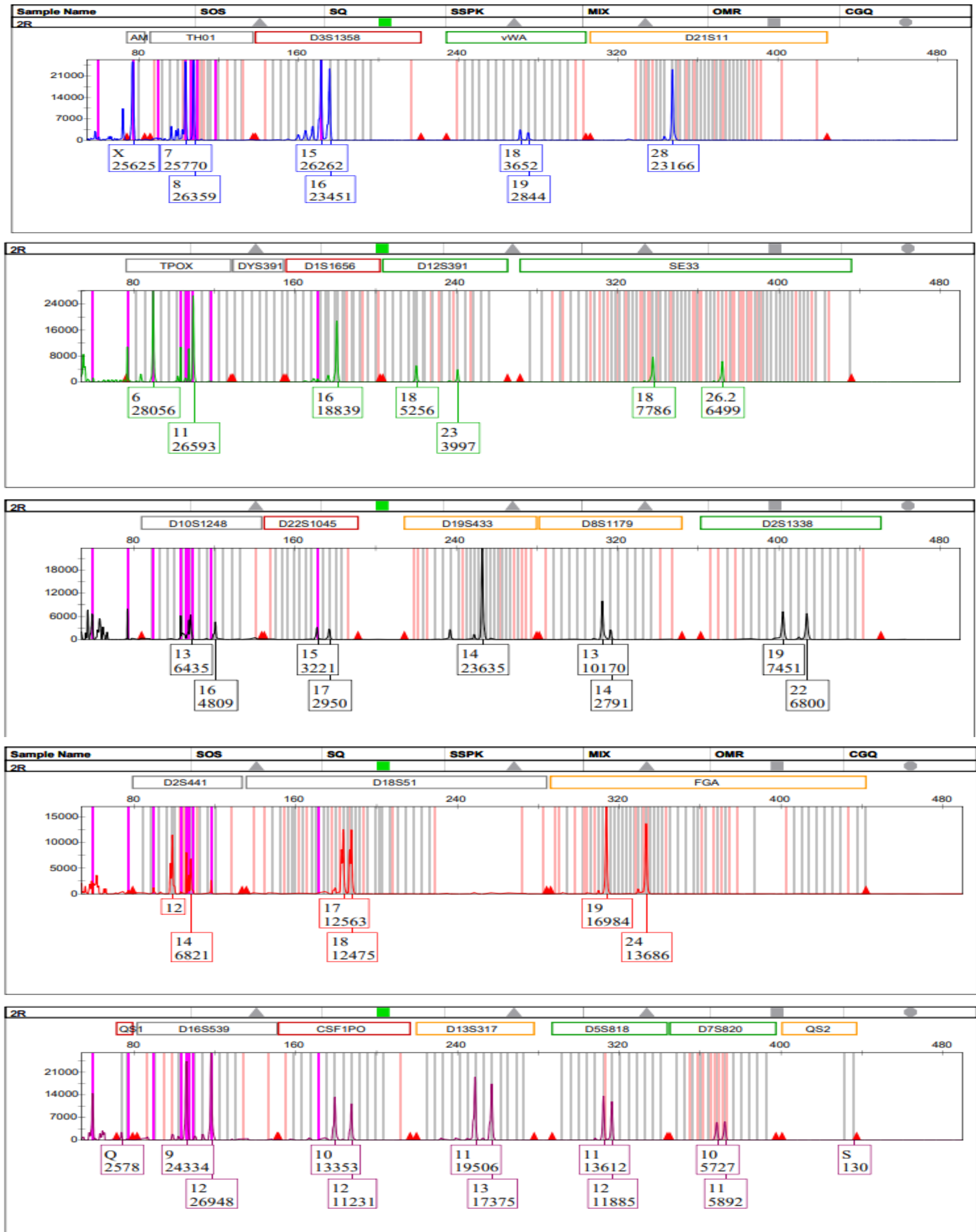


Figure 2: Sample 2R DNA profile results of reference samples

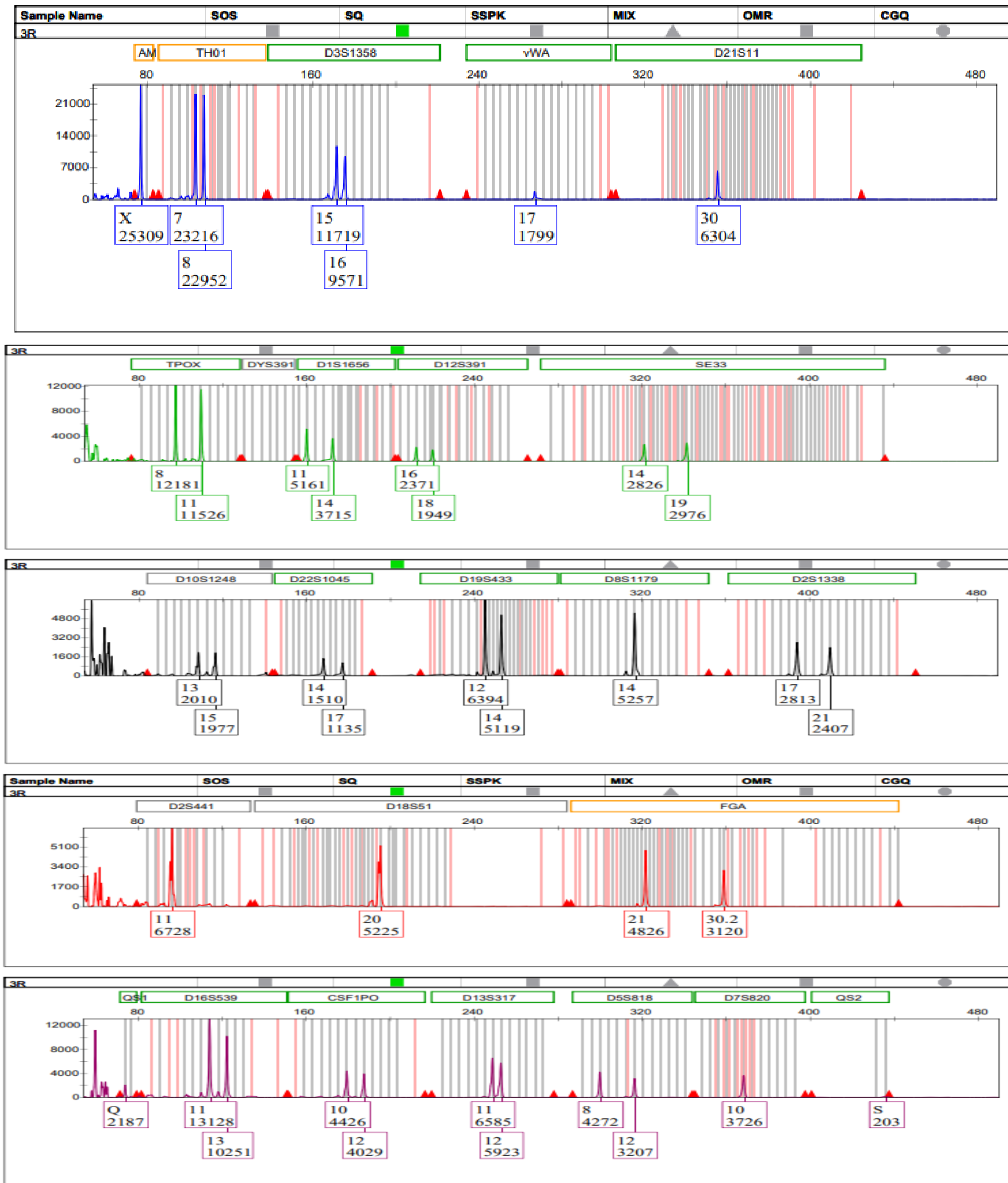


Figure 3: Sample 3R DNA profile results of reference samples.

appliedbiosystems
GeneMapper™ ID-X 1.6
Project: 20240710_Zulu

Sample Name | SOS | SQ | SSPK | MIX | OMR | CGQ
4R
AM | TH01 | D3S1358 | vWA | D21S11
X 25526 | Y 20849 | 9 31428 | 14 19691 | 17 14682 | 17 2041 | 19 2062 | 27 8476 | 31 6386

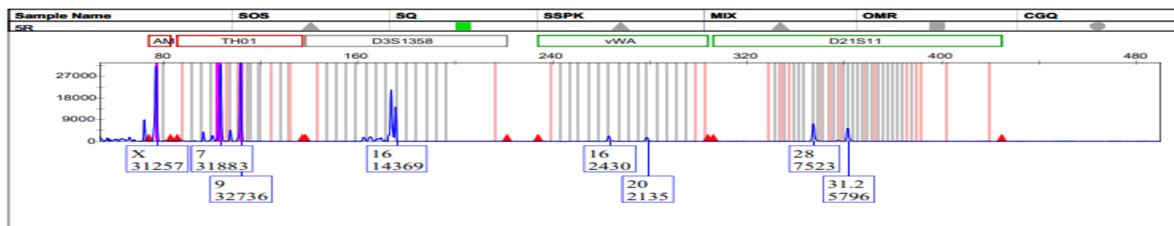
4R
TPOX | DYS391 | D1S1656 | D12S391 | SE33
9 31720 | 10 4837 | 11 9610 | 14 7445 | 15 4653 | 16 4765 | 20 6061 | 26.2 4774

4R
D10S1248 | D22S1045 | D19S433 | D8S1179 | D2S1338
13 5027 | 15 4542 | 15 2774 | 16 2902 | 12 12331 | 13 9605 | 12 7683 | 15 6067 | 17 5744 | 22 5643

Sample Name | SOS | SQ | SSPK | MIX | OMR | CGQ
4R
D2S441 | D18S51 | FGA
12 6603 | 15 5517 | 14 9821 | 16 8498 | 23 11023 | 24 11307

4R
QS1 | D16S539 | CSF1PO | D13S317 | D5S818 | D7S820 | QS2
Q 4424 | 9 30878 | 10 22891 | 10 8446 | 12 6645 | 12 26471 | 12 9485 | 13 8056 | 9 4224 | 12 3582 | S 313

Figure 4: Sample 4R DNA profile results of reference samples.



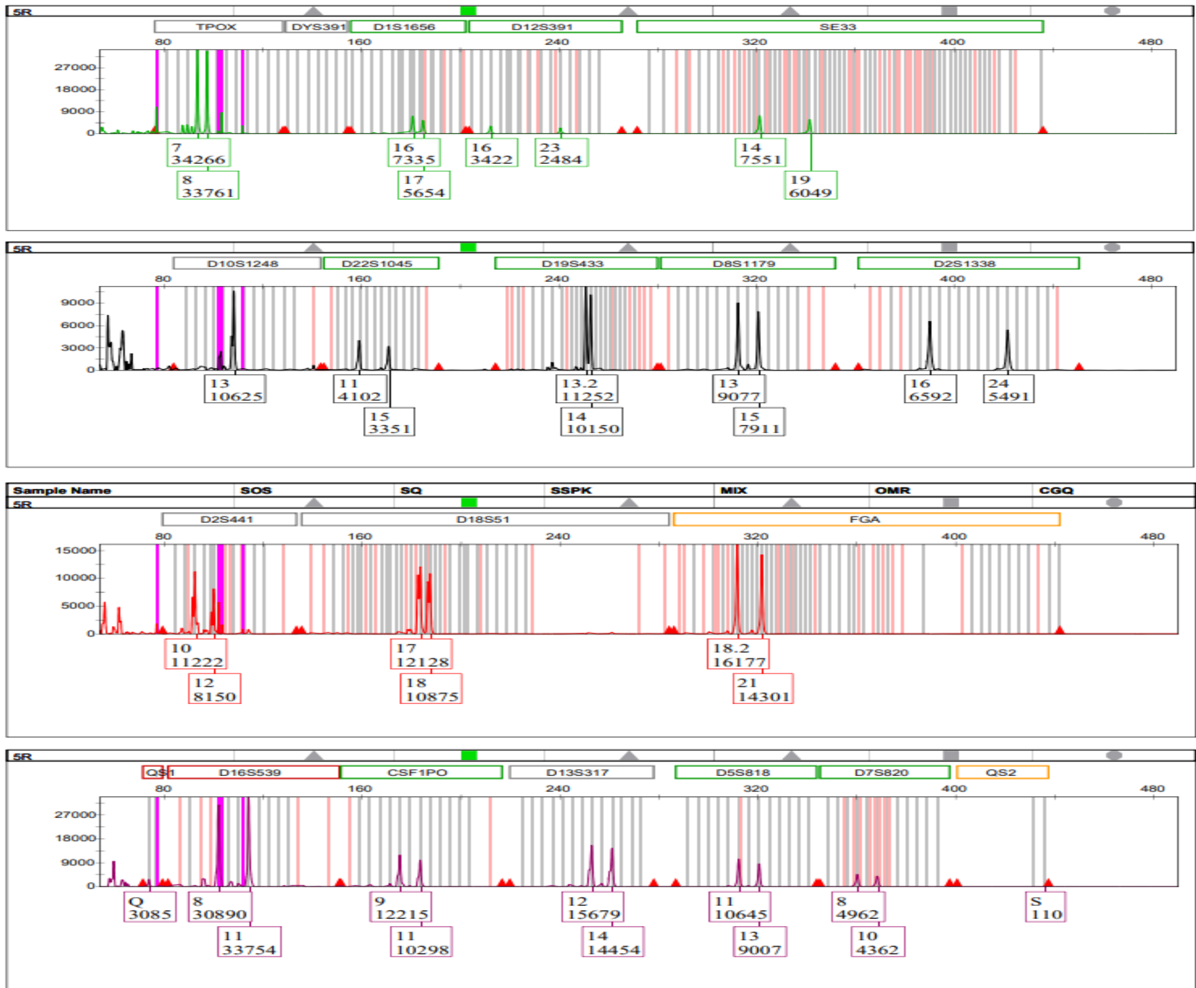


Figure 5: Sample 5R DNA profile results of reference samples.

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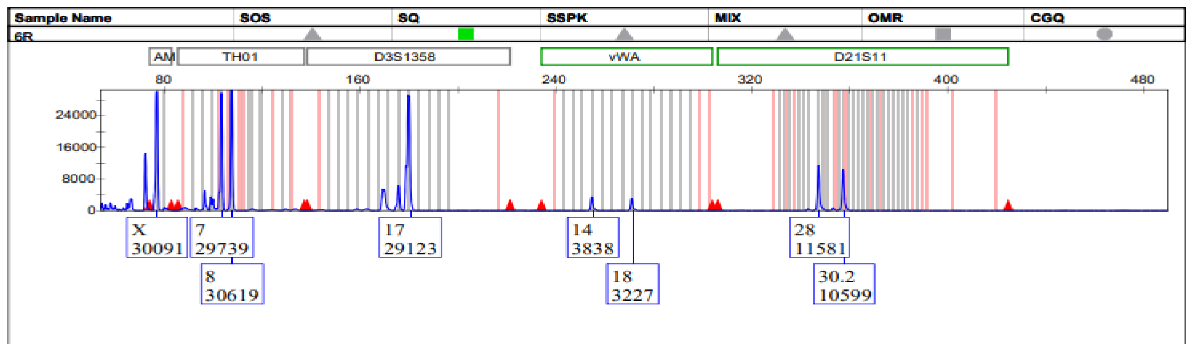
Project: 20240710_Zulu

GeneMapper™ ID-X 1.6



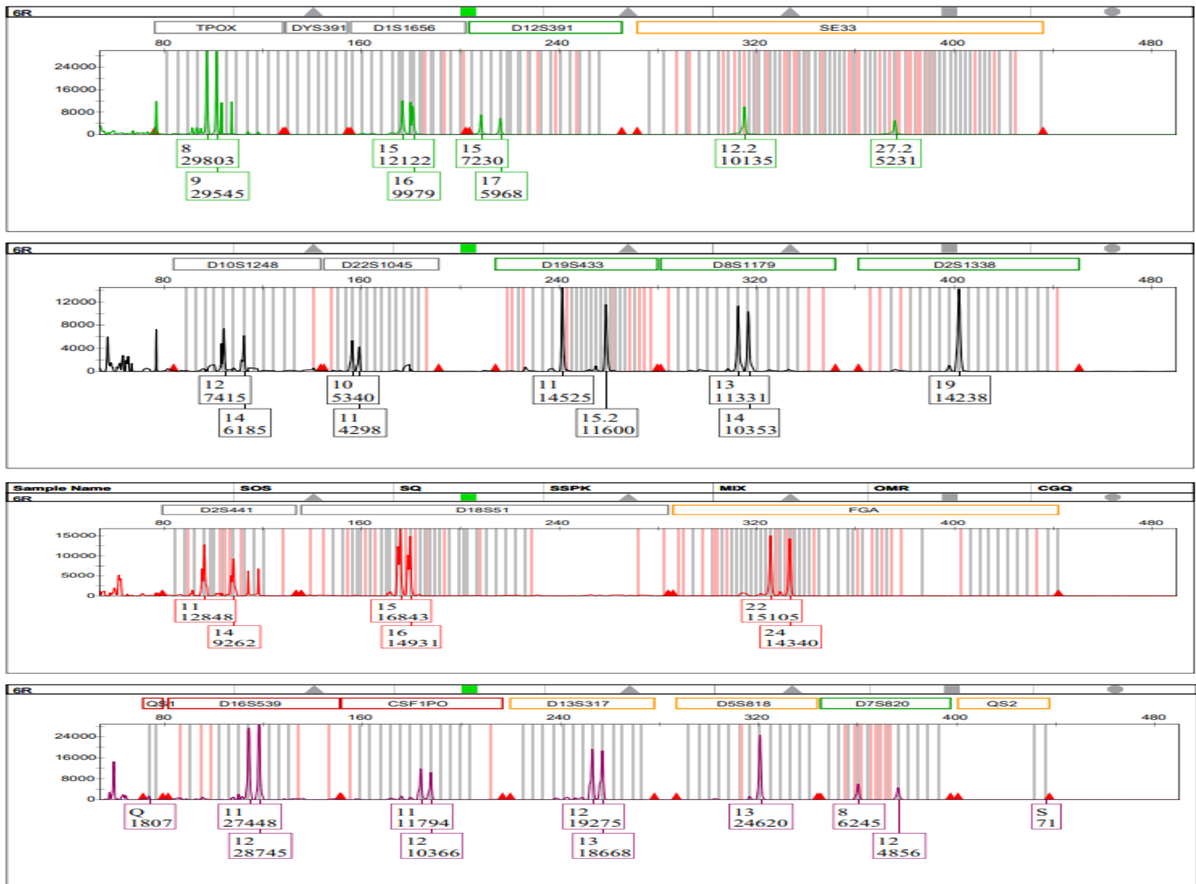


Figure6: Sample 6R DNA profile results of reference samples.

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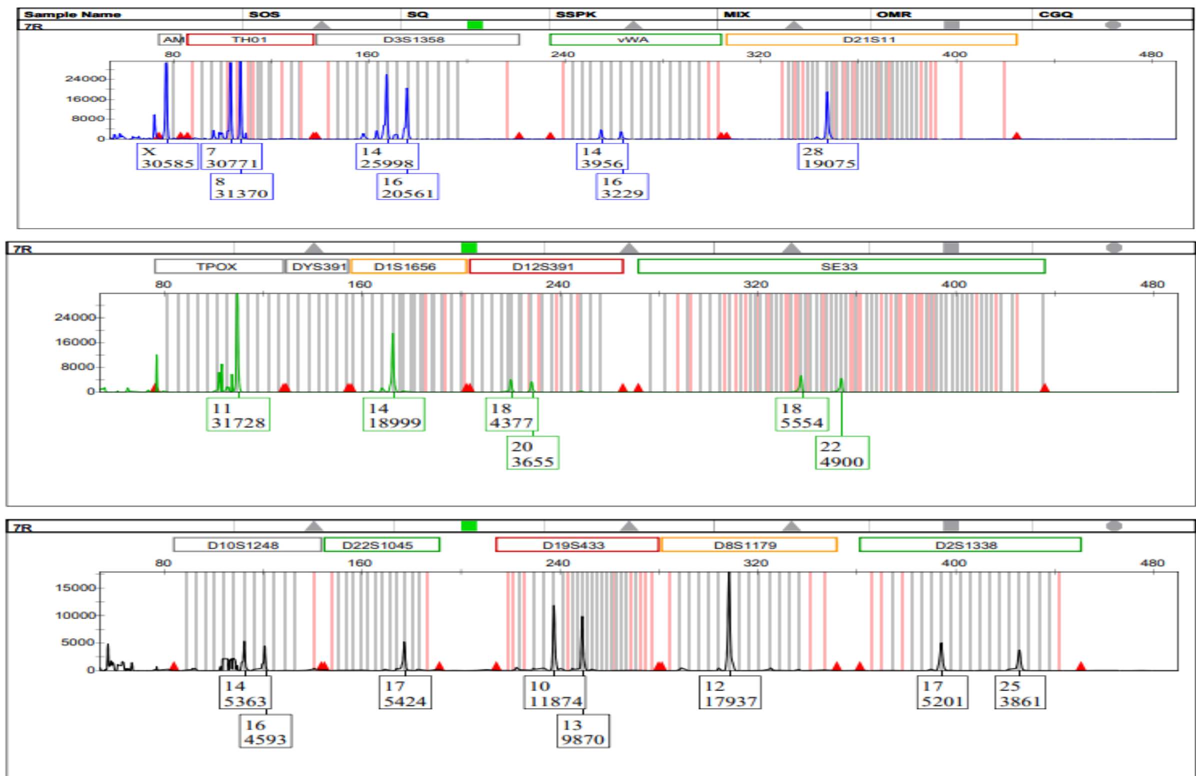
appliedbiosystems

GeneMapper™ ID-X 1.6

Project: 20240710_Zulu



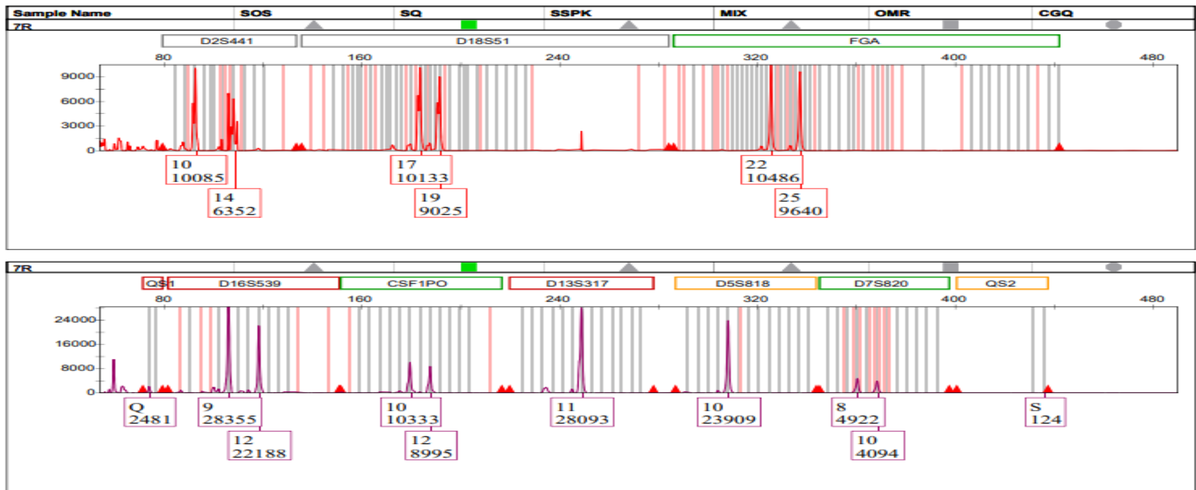


Figure 7: Sample 7R DNA profile results of reference samples

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appliedbiosystems

Project: 20240710_Zulu

GeneMapper™ ID-X 1.6

Sample Name SOS SQ SSPK MIX OMR CGQ
8R
AM TH01 D3S1358 vWA D21S11
X 19747
Y 16437
7 28486
14 16636
18 11796
18 3025
29 4057
31 4127

8R
TPOX DYS391 D1S1656 D12S391 SE33
8 17442
11 16578
11 3172
13 5490
17 3264
18 5404
15.2 5583
17 5095

8R
D10S1248 D22S1045 D19S433 D8S1179 D2S1338
13 3057
16 2916
11 2877
15 1977
13 7263
15 7708
13 4447
15 3965
22 3893
25 3586

Sample Name SOS SQ SSPK MIX OMR CGQ
8R
D2S441 D18S51 FGA
11 5515
14 3783
14 5817
16 5742
20 6221
24 6679

8R
QS1 D16S539 CSF1PO D13S317 D5S818 D7S820 QS2
Q 3529
11 19978
9 7616
12 5851
11 8167
14 7324
12 5794
13 5089
11 2803
12 2236
S 340

Figure 8: Sample 8R DNA profile results of reference samples

.

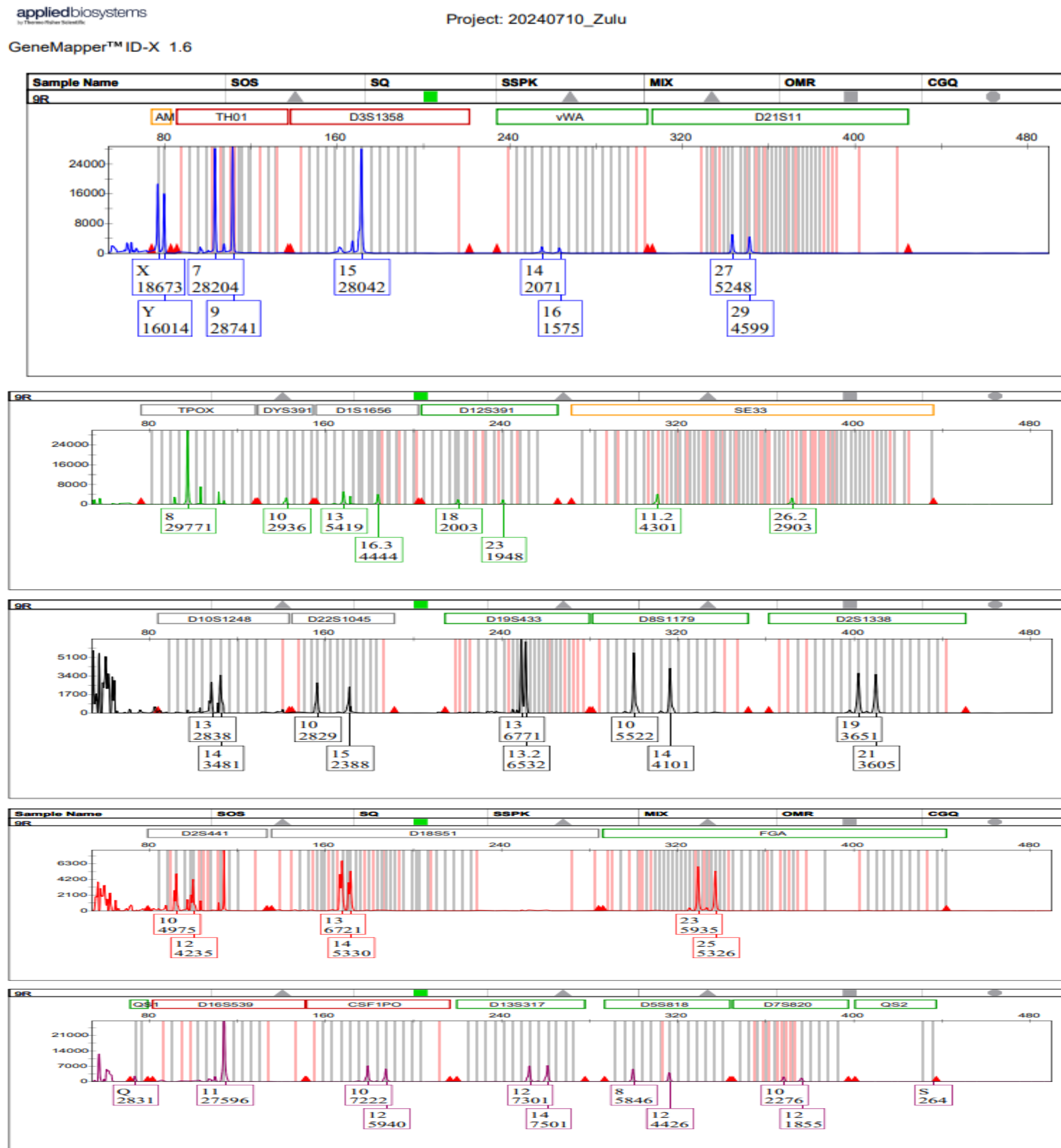

Figure 9: Sample 9R DNA profile results of reference samples.

appliedbiosystems

GeneMapper™ ID-X 1.6

Project: 20240710_Zulu

Sample Name | SOS | SQ | SSPK | MIX | OMR | CGQ

10R

AM TH01 D3S1358 vWA D21S11

X 5550, Y 4573, 7 9627, 8 8763, 15 5858, 16 4712, 16 552, 20 440, 28 2035, 29 1819

10R

TPOX DYS391 D1S1656 D12S391 SE33

8 5053, 9 5039, 10 954, 14 1627, 15.3 1484, 18 1428, 16 1626, 20 1290

10R

D10S1248 D22S1045 D19S433 D8S1179 D2S1338

12 857, 16 755, 14 1220, 14 2426, 13 1558, 15 492, 16 1347, 20 1384

Sample Name | SOS | SQ | SSPK | MIX | OMR | CGQ

10R

D2S441 D18S51 FGA

11 1451, 14 1249, 13 1891, 17 1521, 24 2517, 29 1907

10R

QS1 D16S539 CSF1PO D13S317 D5S818 D7S820 QS2

Q 455, 9 9107, 10 5414, 10 4655, 12 2891, 14 3126, 8 2092, 12 1998, 8 918, 10 982, S 58

Figure 10: Sample 10R DNA profile results of reference samples.

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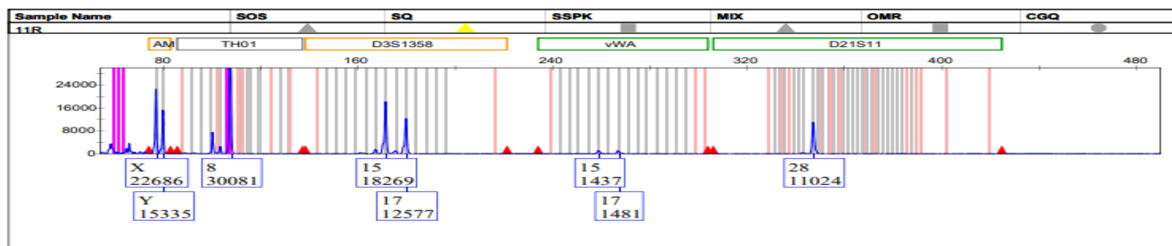
Project: 20240710_Zulu



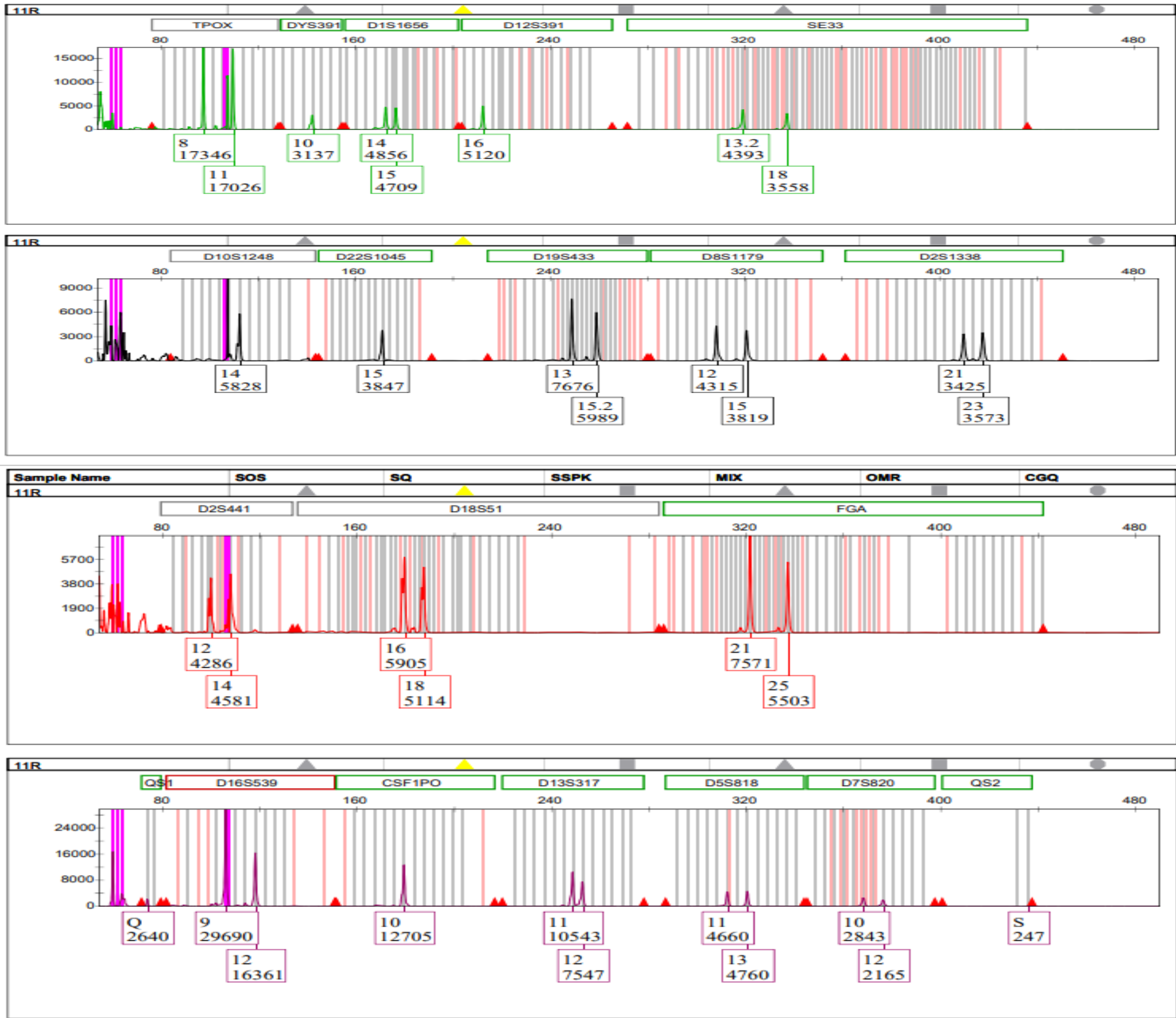
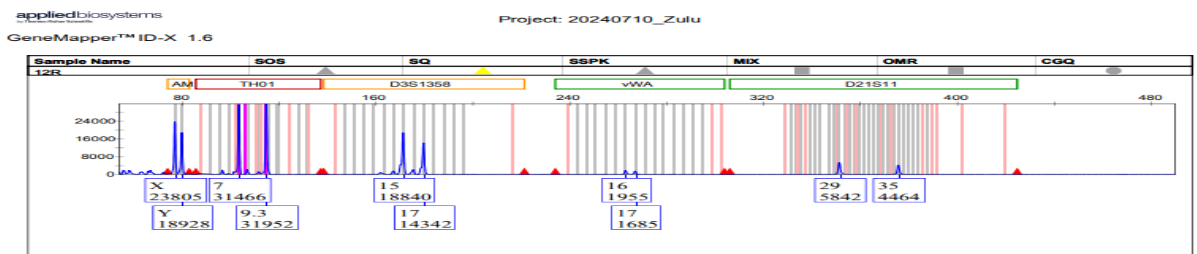


Figure 11: Sample 11R DNA profile results of reference samples.



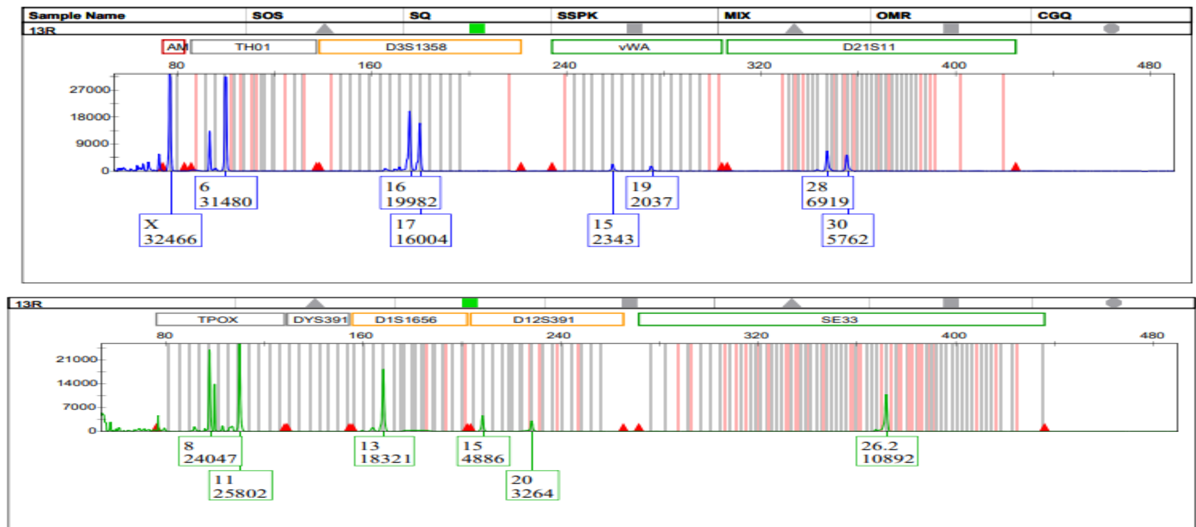
12R
TPOX DYS391 D1S1656 D12S391 SE33
6 23690 | 10 19557 | 10 3863 | 14 6451 | 16 5158 | 17 2728 | 25 1983 | 17 4737 | 28.2 3176

12R
D10S1248 D22S1045 D19S433 D8S1179 D2S1338
14 6392 | 11 2781 | 14 2395 | 14 7844 | 16 7787 | 12 5238 | 14 4906 | 20 4814 | 21 4300

Sample Name SOS SQ SSPK MIX OMR CGQ
12R
D2S441 D18S51 FGA
11 12027 | 19 13243 | 22 8966 | 28 7909

12R
QS1 D16S539 CSF1PO D13S317 D5S818 D7S820 QS2
Q 3406 | 9 31139 | 13 16659 | 12 14111 | 11 10653 | 12 9623 | 11 7577 | 12 6492 | 8 3186 | 10 2649 | S 209

Figure 12: Sample 12R DNA profile results of reference samples.



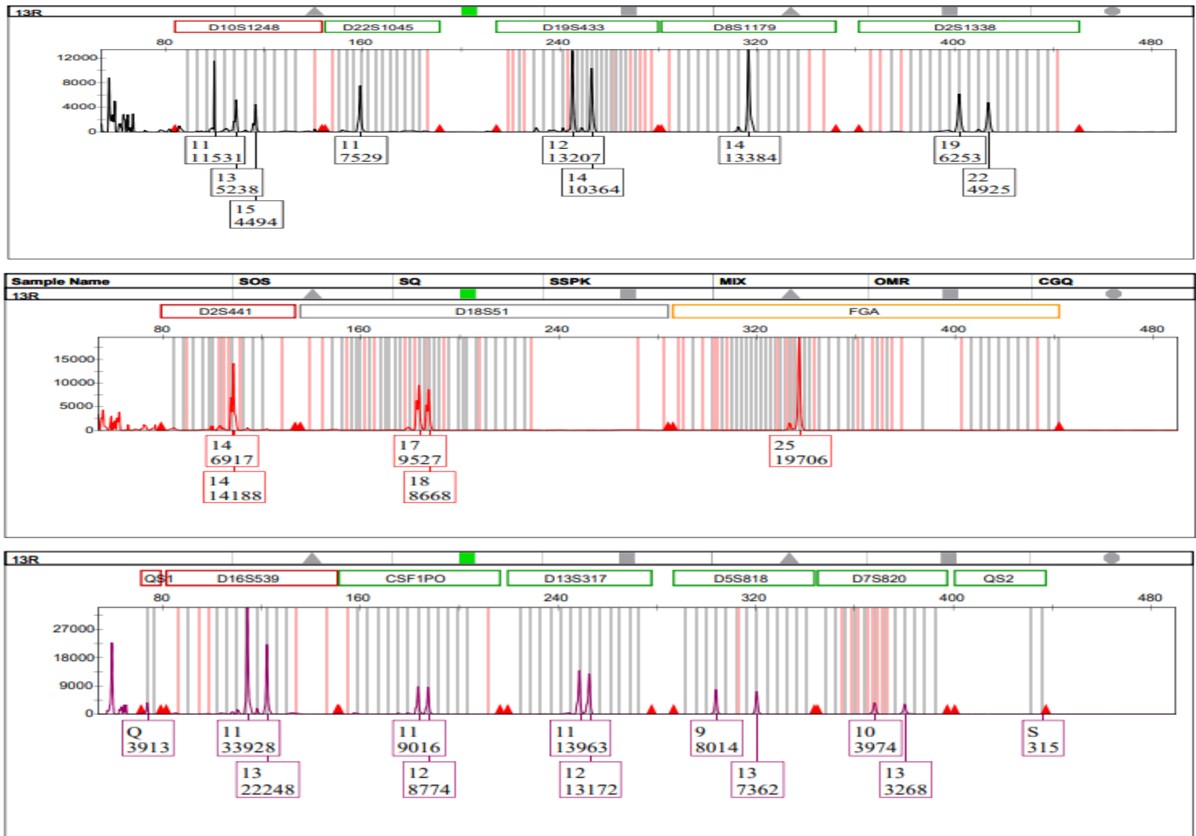
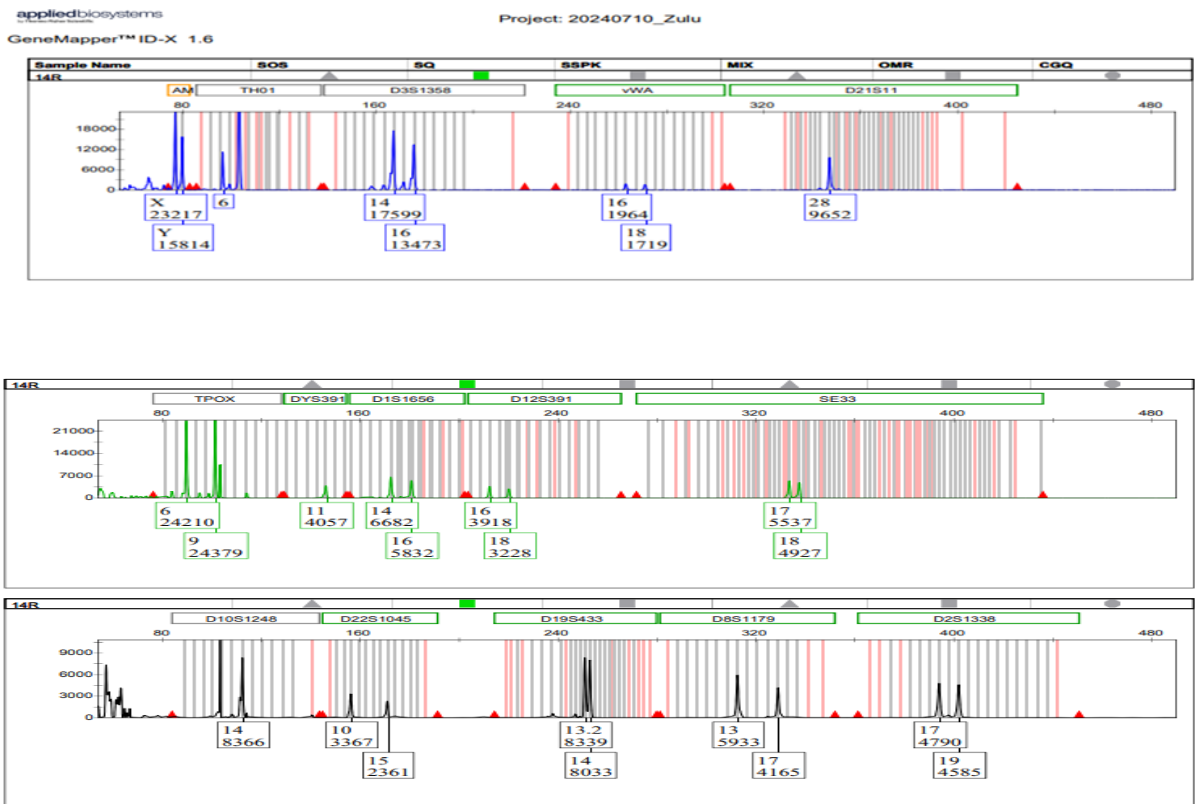


Figure 13: Sample 13R DNA profile results of reference samples.



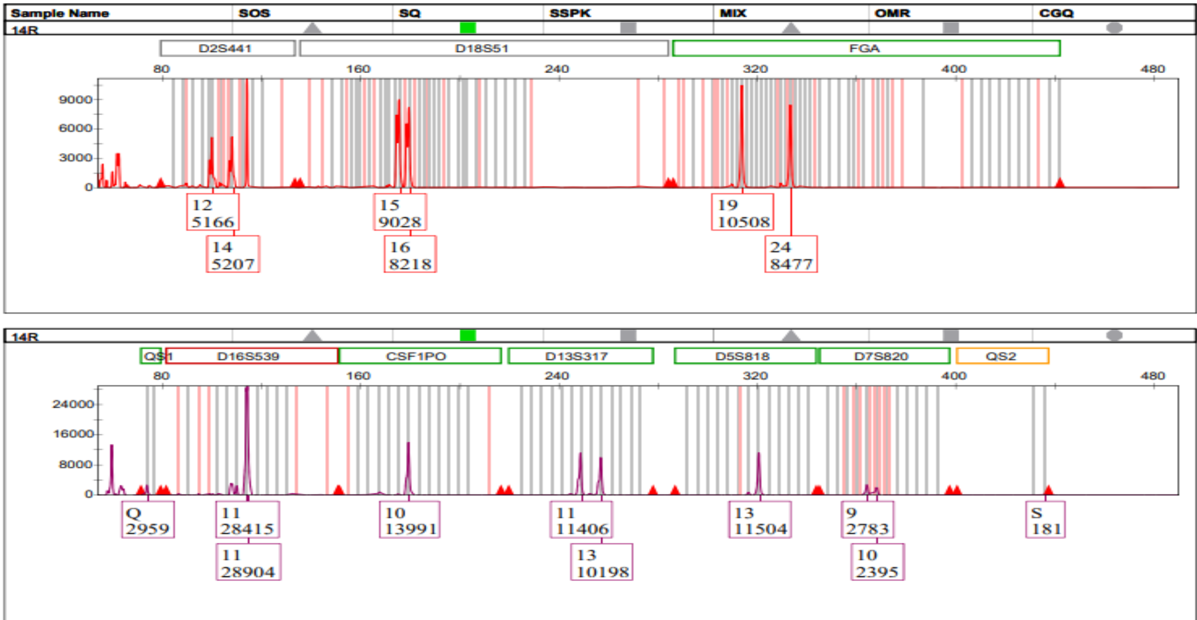
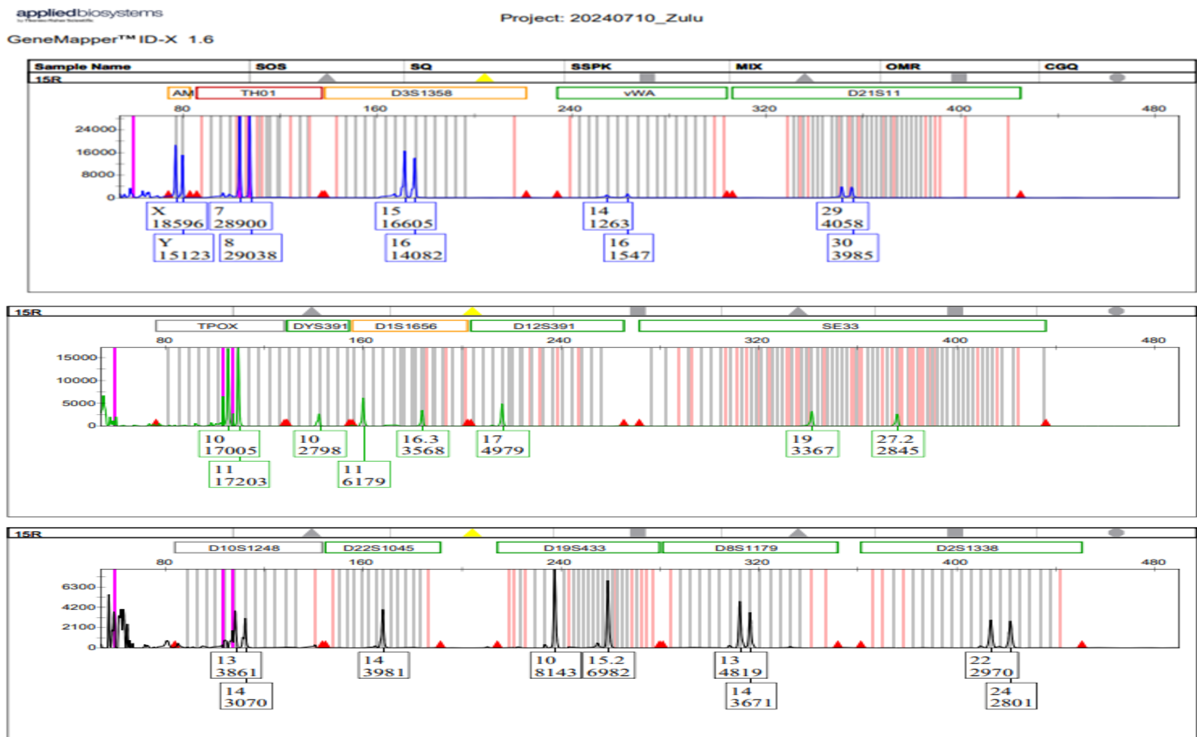


Figure 14: Sample 14R DNA profile results of reference samples.



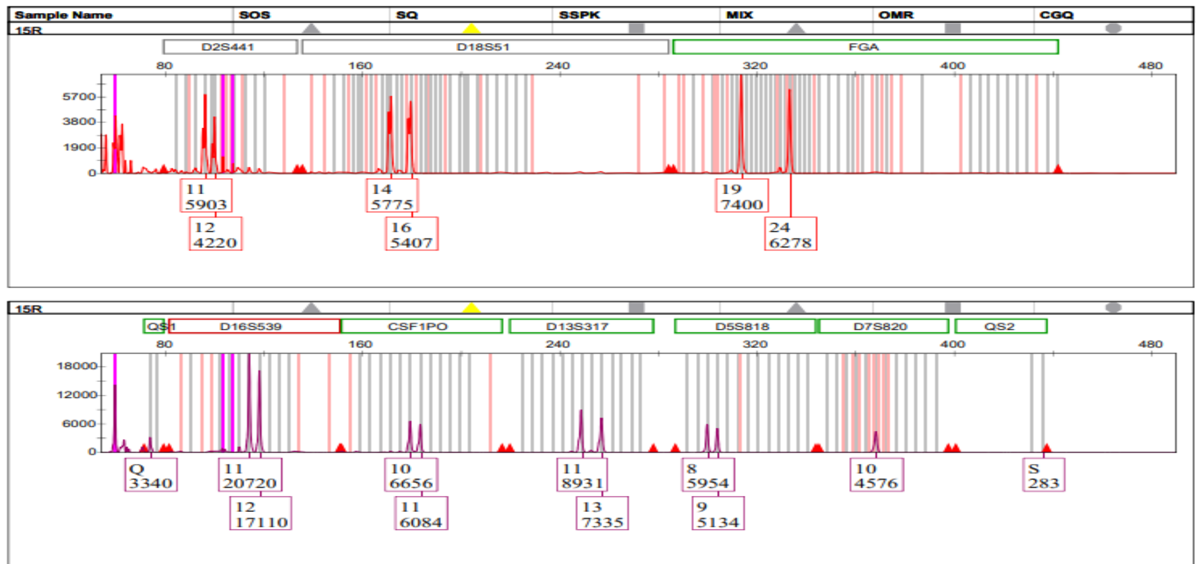
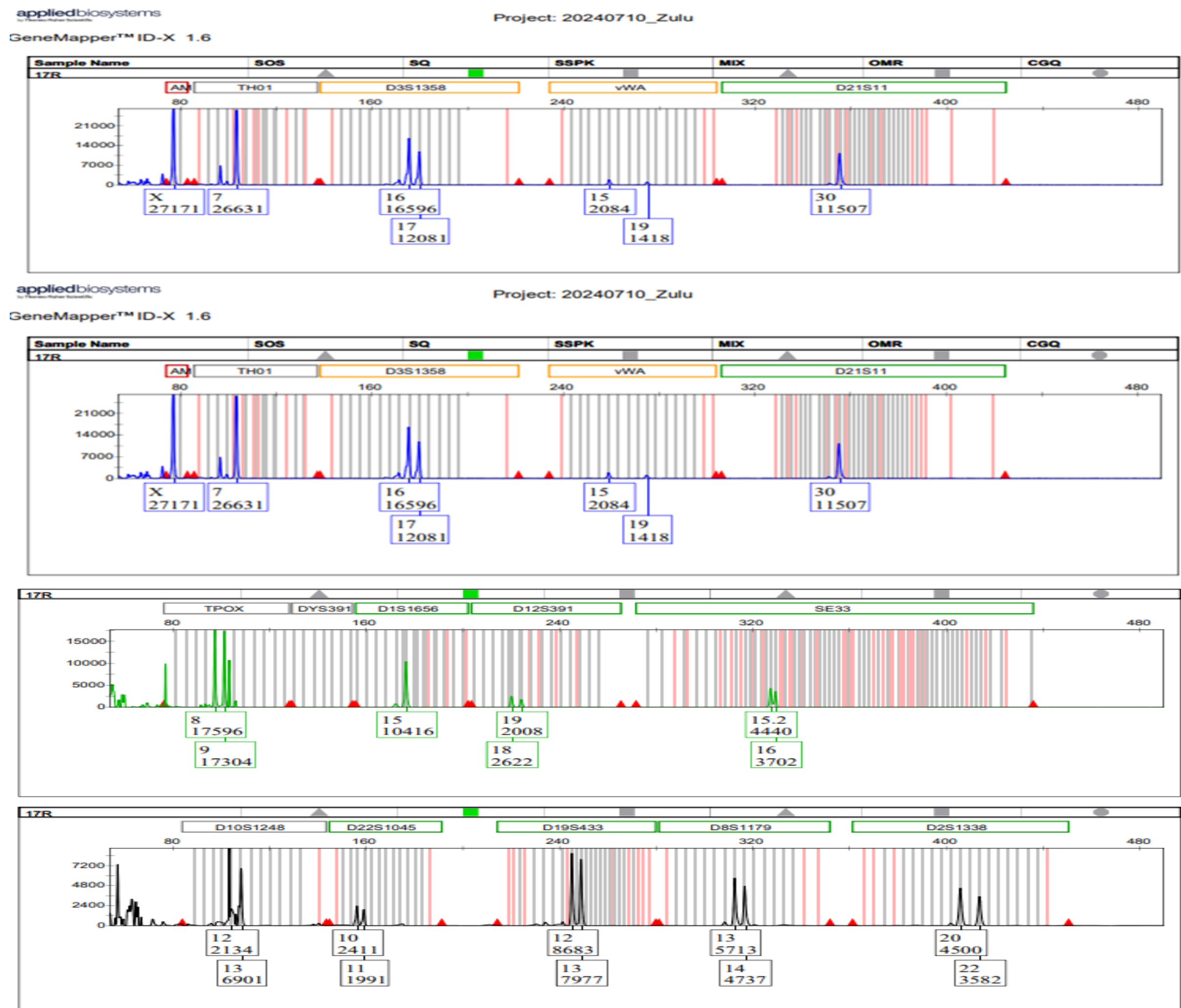


Figure 15: Sample 15R DNA profile results of reference samples.



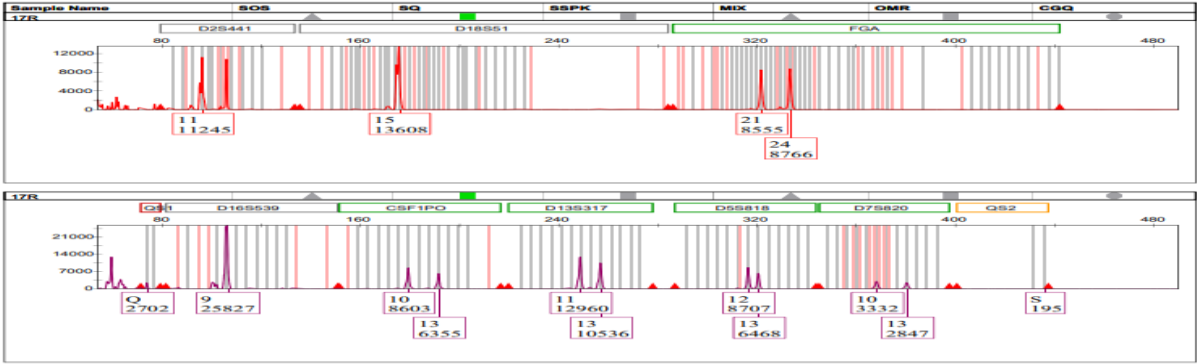


Figure 17: Sample 17R DNA profile results of reference samples.

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Project: 20240710_Zulu

GeneMapper™ ID-X 1.6

Sample Name SOS SQ SSPK MIX OMR CGQ

18R

AM TH01 D3S1358 vWA D21S11

X 29967; 7 29619; 8 29663; 15 19298; 17 14741; 15 3385; 27 6281; 31 5038

TPOX DYS391 D1S1656 D12S391 SE33

9 18869; 10 17925; 13 5113; 16 4671; 21 2156; 22 2051; 21.2 4338; 29.2 2648

D10S1248 D22S1045 D19S433 D8S1179 D2S1338

12 3673; 15 2740; 10 2658; 15 2132; 13 9432; 14 8084; 14 5489; 15 4893; 20 4523; 24 4034

D2S441 D18S51 FGA

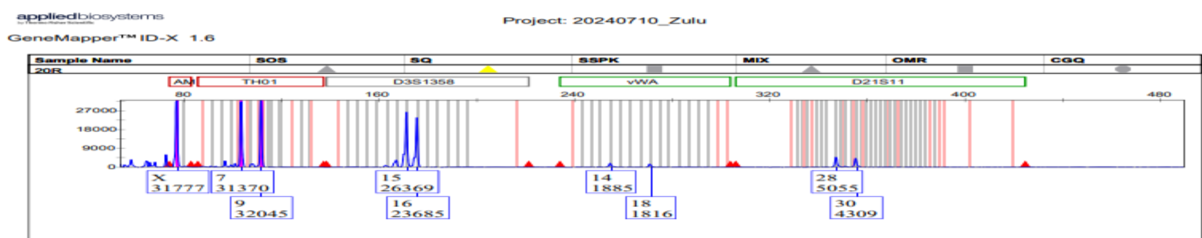
11 5167; 12.3 5857; 15 7157; 20 5745; 22 17660

QS1 D16S539 CSF1PO D13S317 D5S818 D7S820 QS2

Q 1775; 11 29152; 12 13263; 12 9892; 13 10444; 11 13392; 10 2871; 11 2823; S 106

Figure 18: Sample 18R DNA profile results of reference samples.

Figure 19: Sample 19R DNA profile results of reference samples.



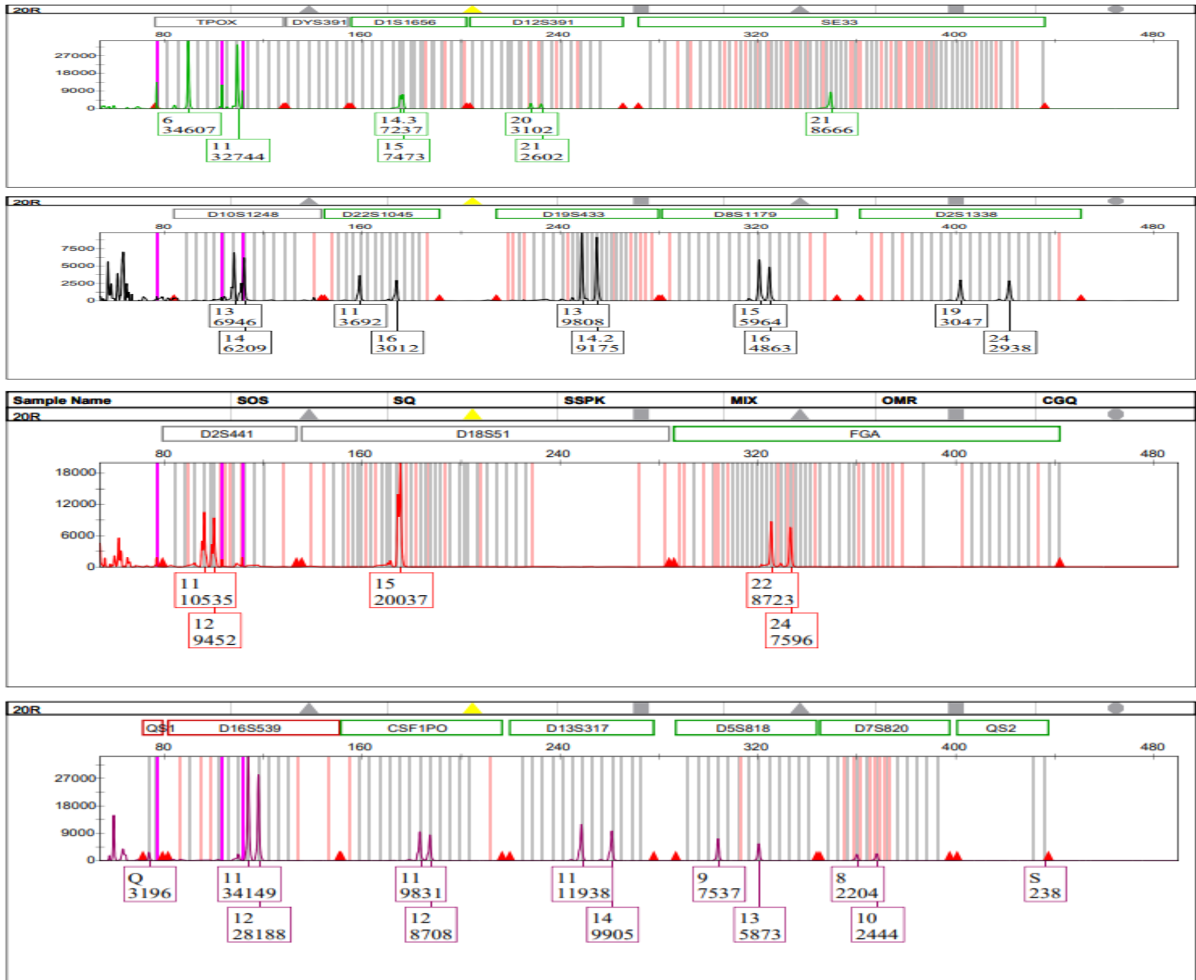


Figure 20: Sample 20R DNA profile results of reference samples.

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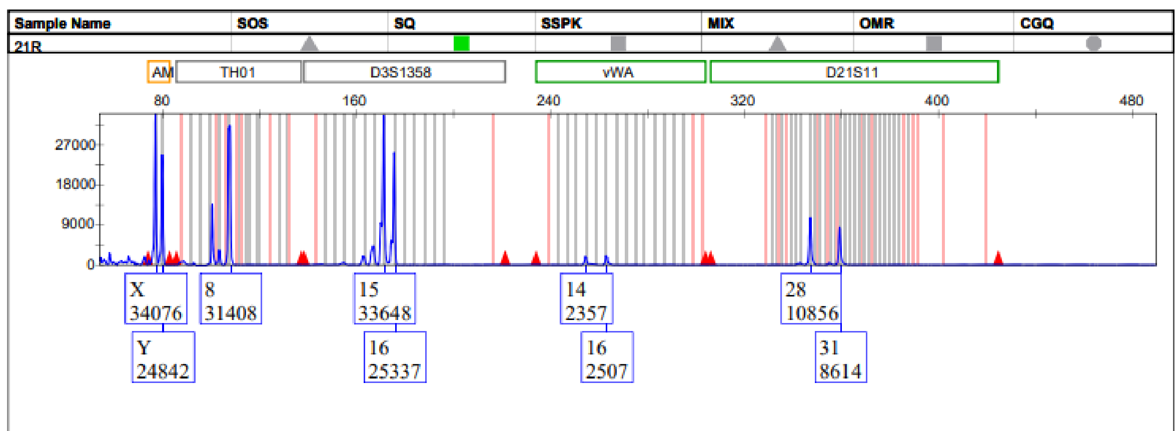
Project: 20240710_Zulu

GeneMapper™ ID-X 1.6



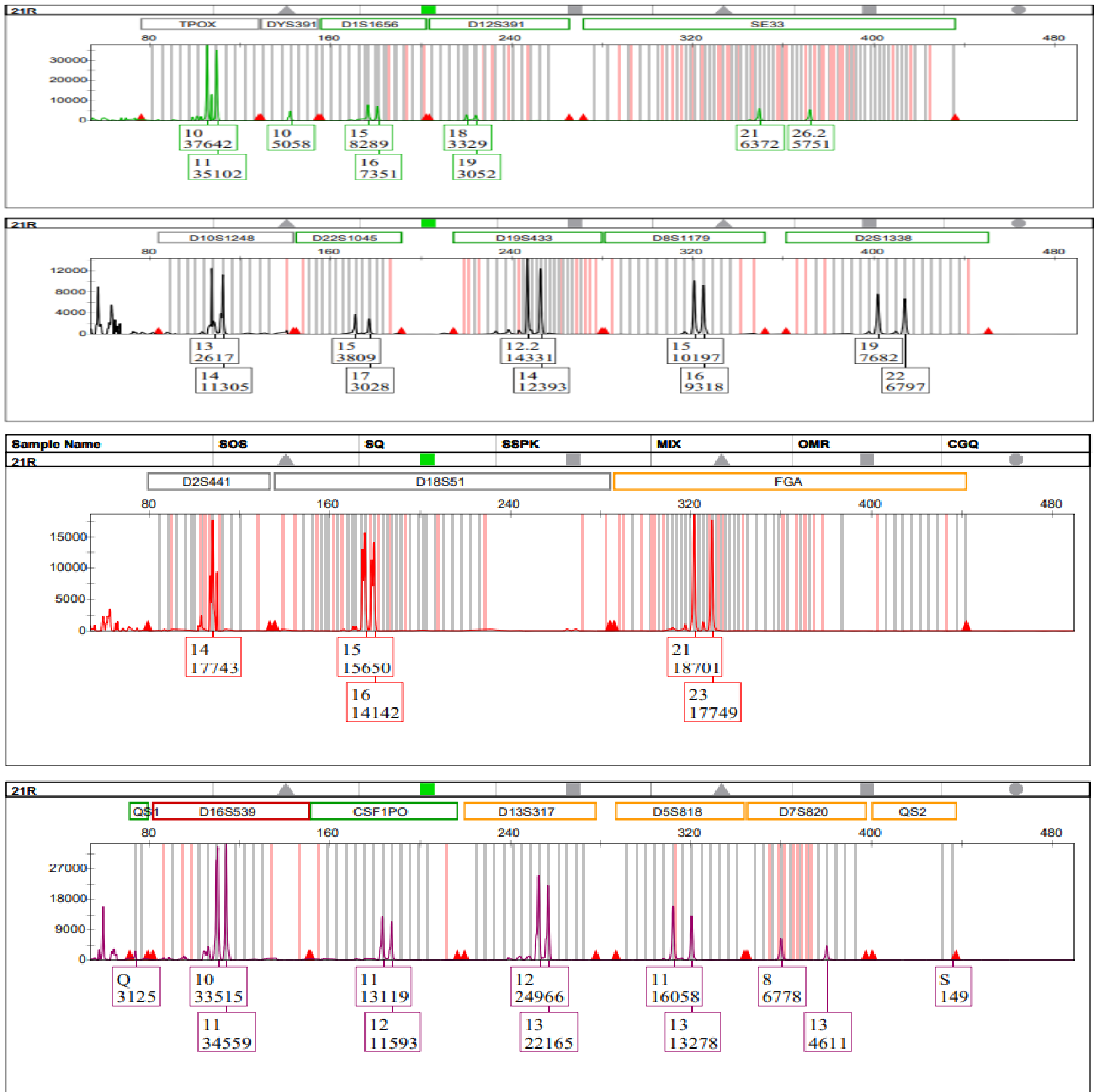


Figure 21: Sample 21 R DNA profile results of reference samples

Appendix 2: Profiles of Casework samples

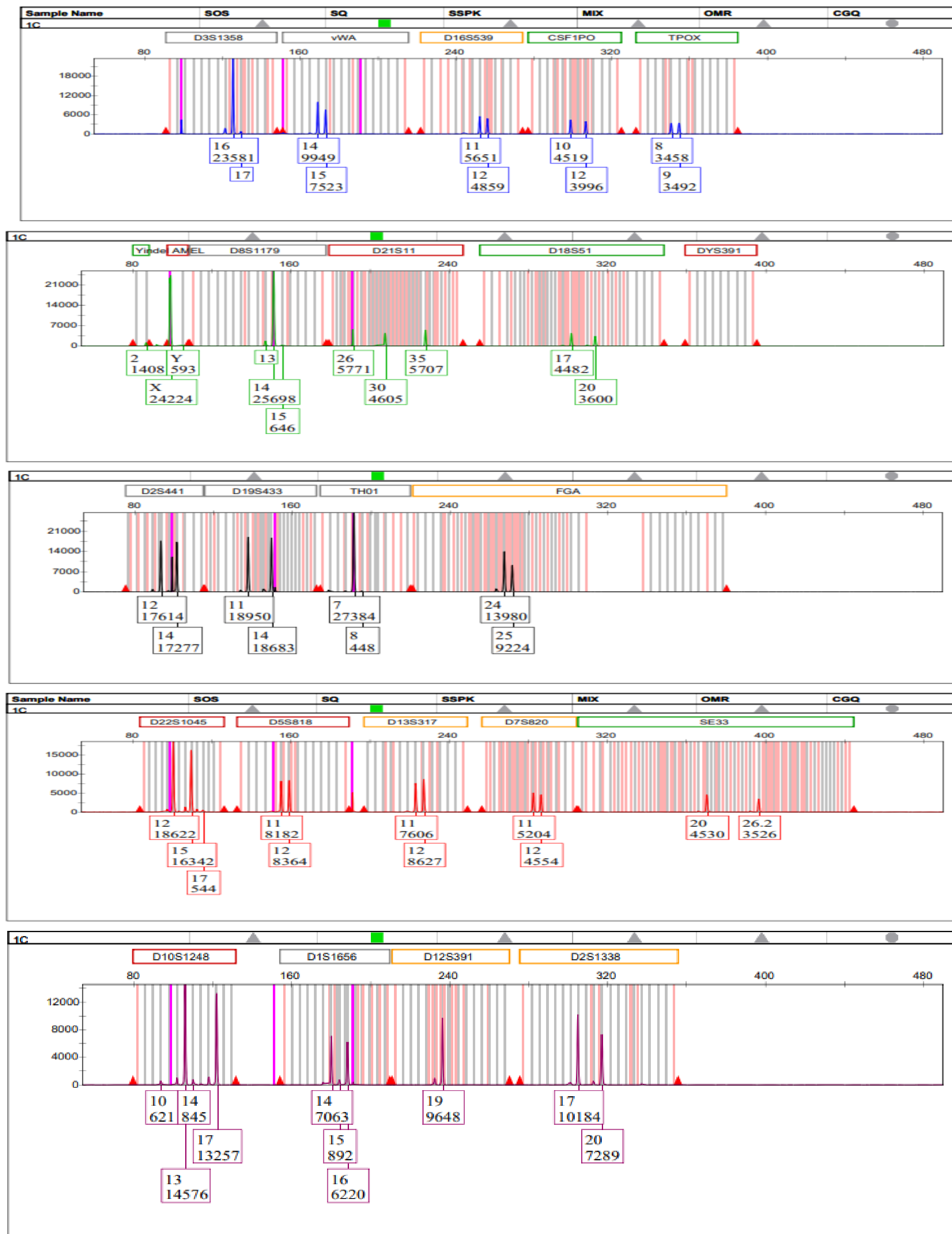


Figure1: Sample 1C DNA profile results of casework samples.

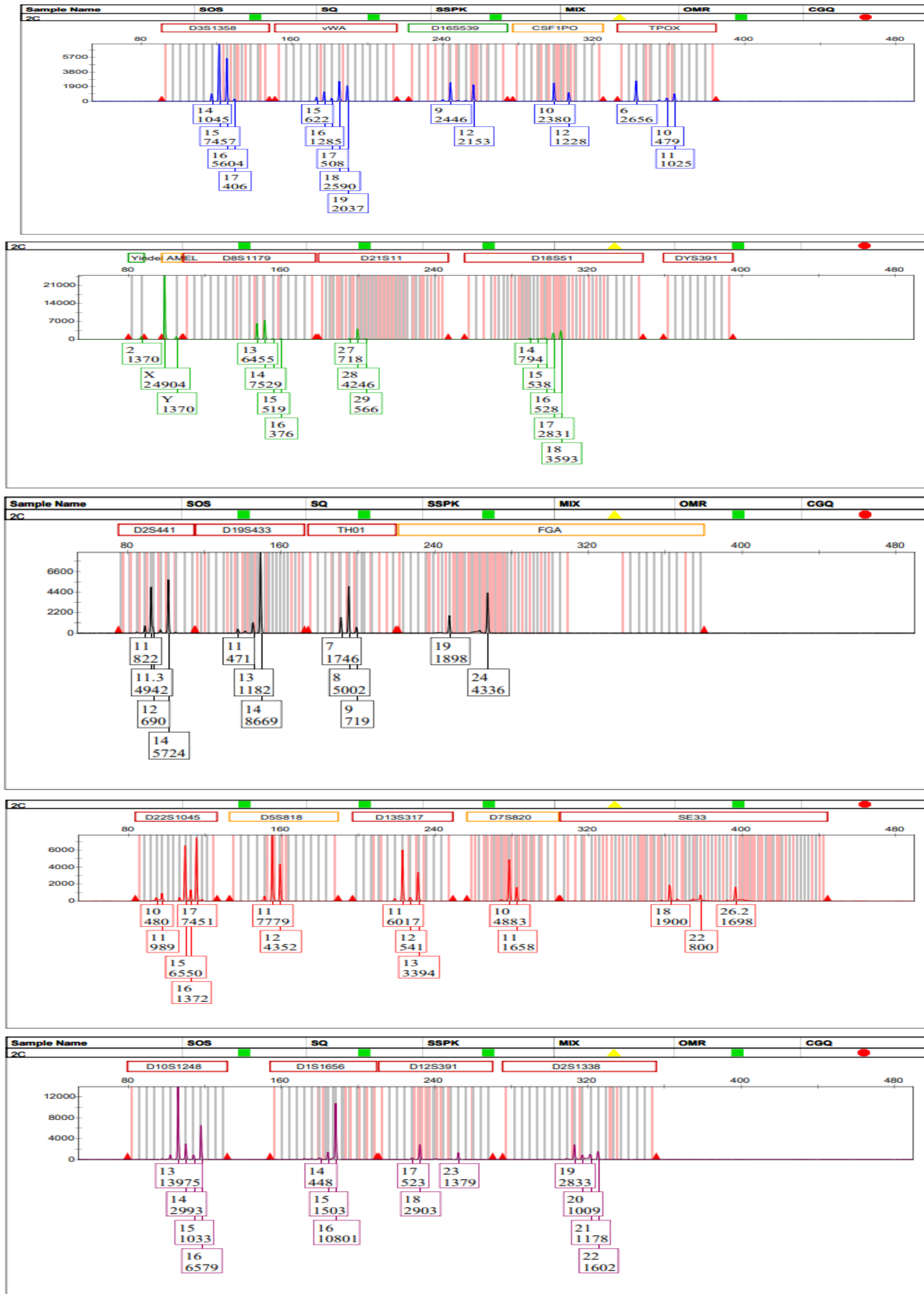
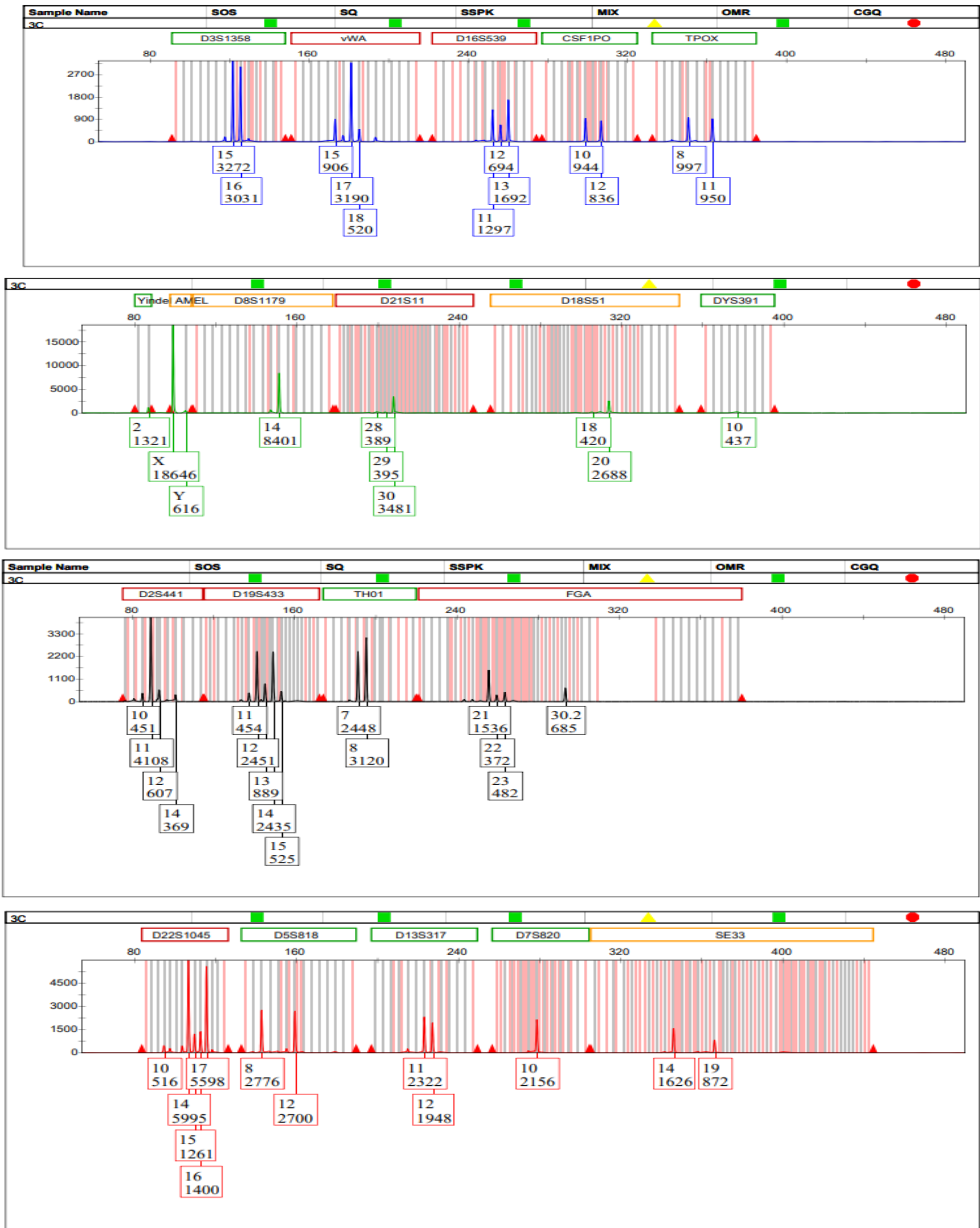


Figure 2: Sample 2C DNA profile results of casework samples.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Project: 20240725_ZULU PROJECT
GeneMapper™ ID-X 1.6
Sample Name
SOS
SQ
SSPK
MIX
OMR
CGQ
3C
D3S1358
vWA
D16S539
CSF1PO
TPOX
15 3272
16 3031
15 906
17 3190
18 520
12 694
13 1692
11 1297
10 944
12 836
8 997
11 950
Yindel
AMEL
D8S1179
D21S11
D18S51
DYS391
2 1321
X 18646
Y 616
14 8401
28 389
29 395
30 3481
18 420
20 2688
10 437
D2S441
D19S433
TH01
FGA
10 451
11 4108
12 607
14 369
11 454
12 2451
13 889
14 2435
15 525
7 2448
8 3120
21 1536
22 372
23 482
30.2 685
D22S1045
D5S818
D13S317
D7S820
SE33
10 516
17 5598
14 5995
15 1261
16 1400
8 2776
12 2700
11 2322
12 1948
10 2156
14 1626
19 872

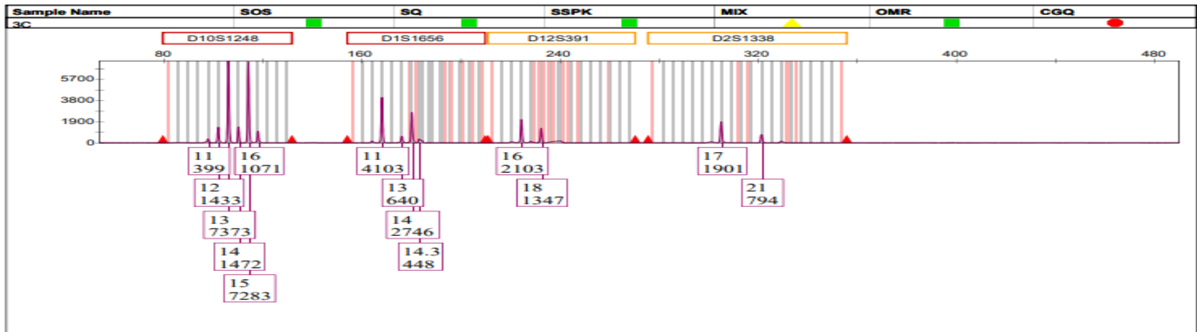

Figure 3: Sample 3C DNA profile results of casework samples.

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GeneMapper™ ID-X 1.6

Sample Name SOS SQ SSPK MIX OMR CGQ
4C
D3S1358 vWA D16S539 CSF1PO TPOX
80 160 240 320 400 480
540
360
180
0
14 378
17 603
17 359
19 401
9 690
10 325
10 462
12 492
9 369
10 193

4C
Yindel AMEL D8S1179 D21S11 D18S51 DYS391
80 160 240 320 400 480
1500
1000
500
0
2 1934
X 1810
Y 661
12 978
15 973
27 241
31 328
14 367
15 162
16 176
10 509

Sample Name SOS SQ SSPK MIX OMR CGQ
4C
D2S441 D19S433 TH01 FGA
80 160 240 320 400 480
1500
1000
500
0
11 240
12 1325
14 290
15 247
12 1349
13 1393
13.2 174
14 168
9 1826
19 179
24 729
23 1003

4C
D22S1045 D5S818 D13S317 D7S820 SE33
80 160 240 320 400 480
1200
800
400
0
15 882
16 539
17 224
12 454
13 373
11 289
12 1206
9 430
12 1014
20 334
26.2 195

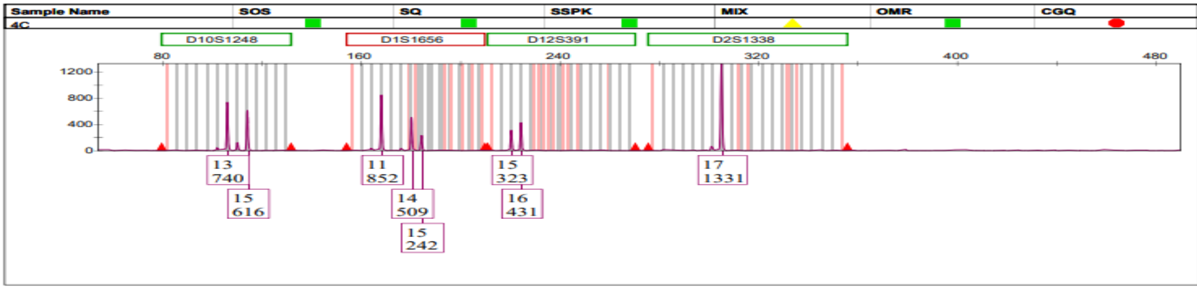
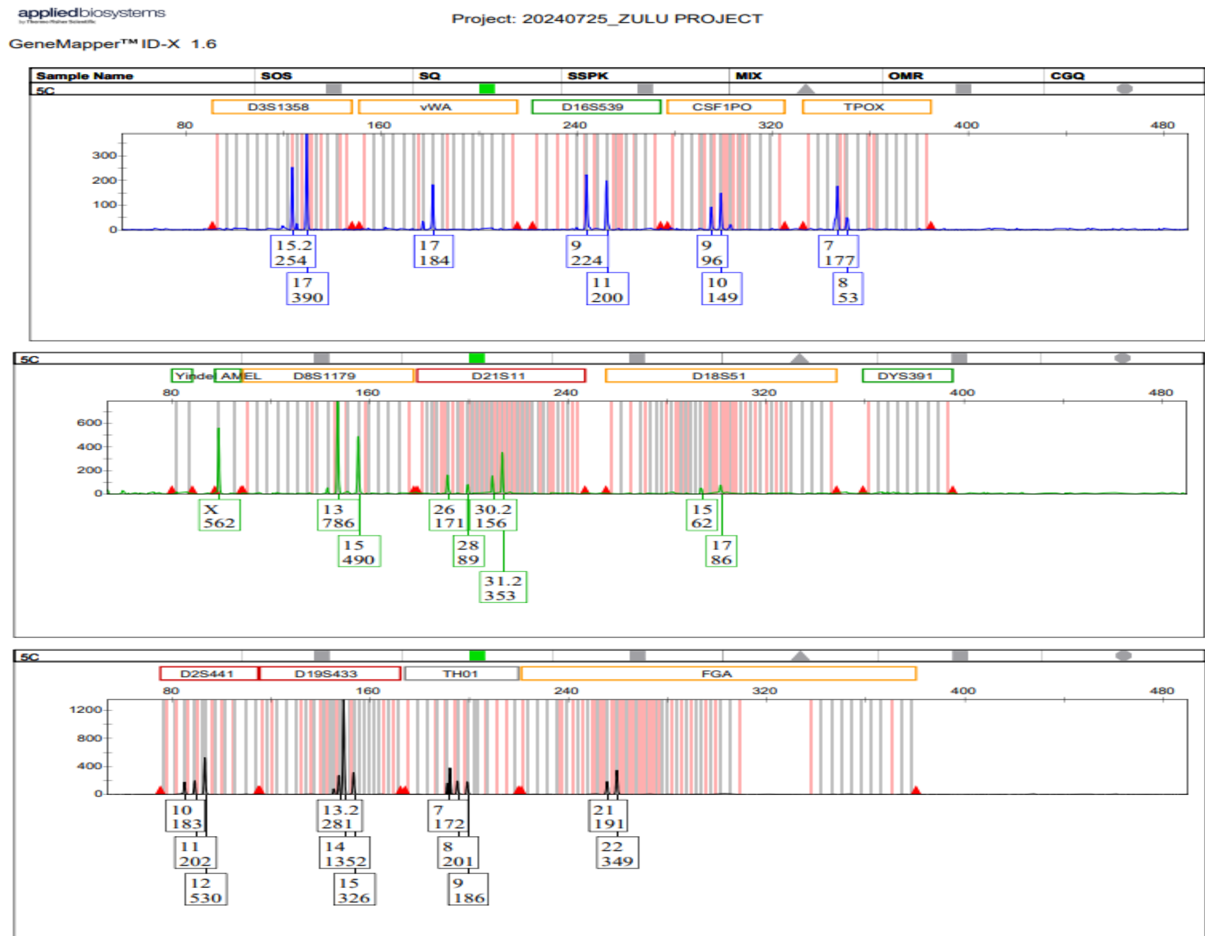


Figure 4: Sample 4C DNA profile results of casework samples.



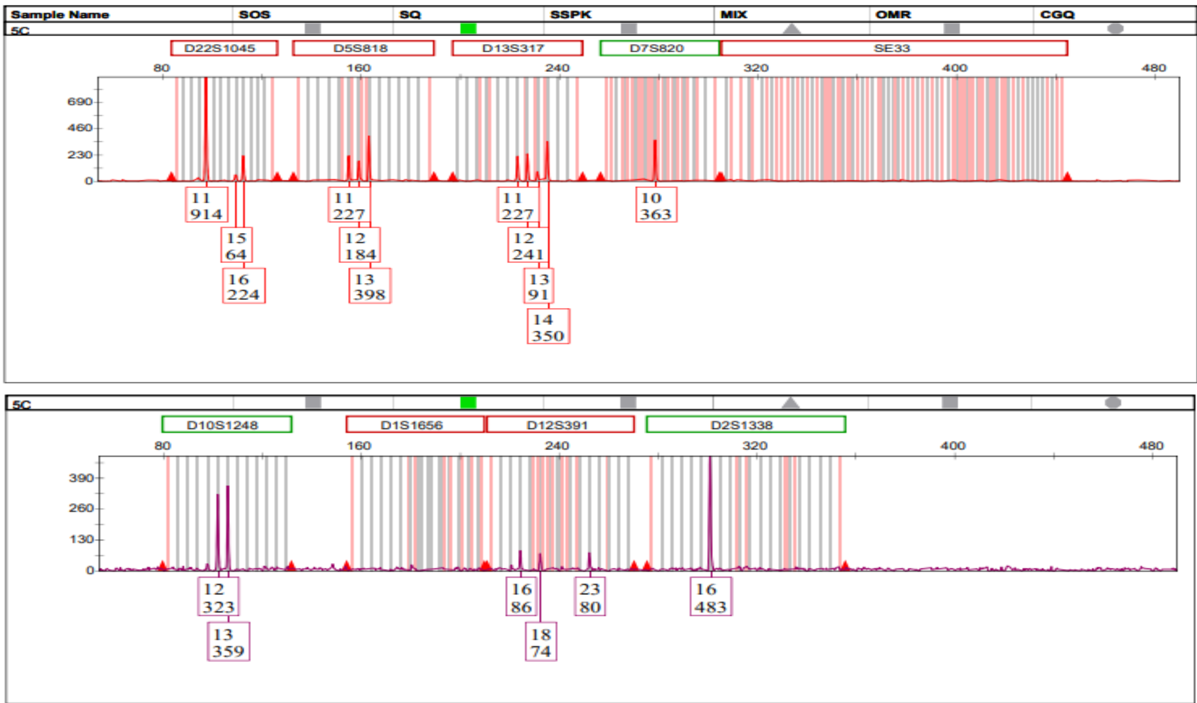
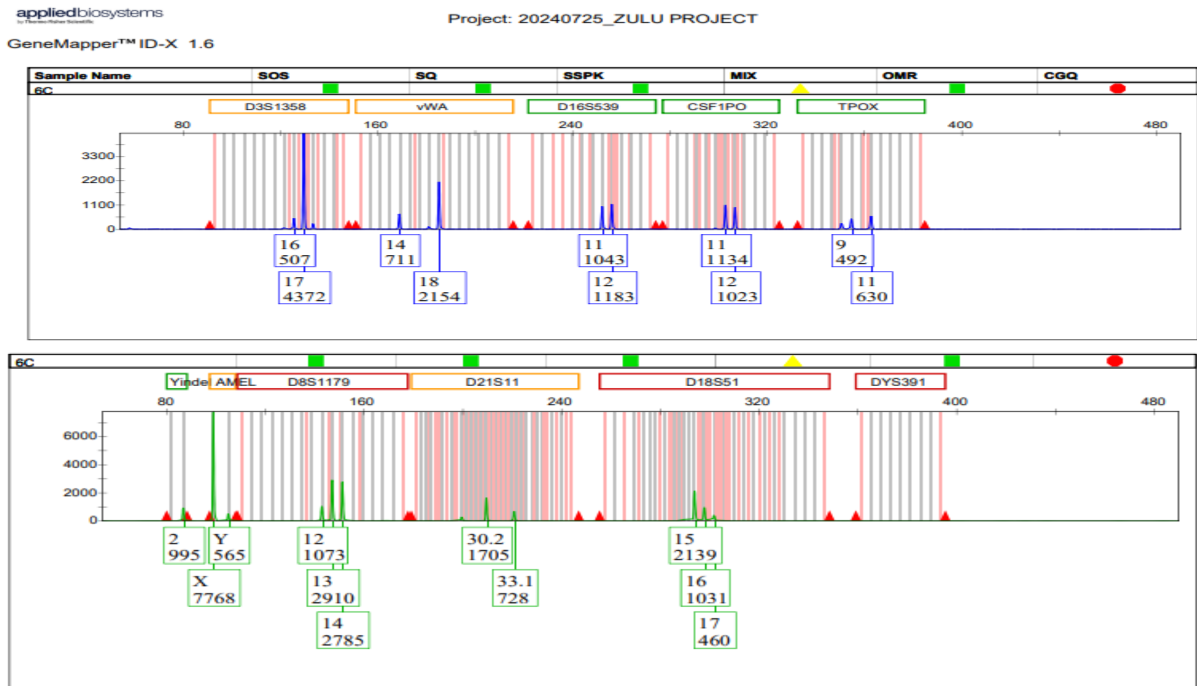


Figure 5: Sample 5 C DNA profile results of casework samples.

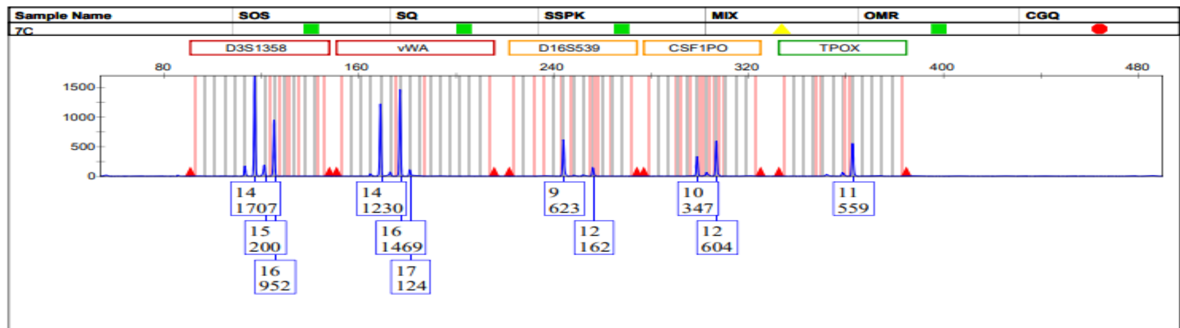


6C
D2S441 D19S433 TH01 FGA
80 160 240 320 400 480
2400 1600 800 0
11 3181
14 1505
11 1244
13 772
15.2 2000
7 1431
8 1612
22 2095
24 755

Sample Name SOS SQ SSPK MIX OMR CGQ
6C
D22S1045 D5S818 D13S317 D7S820 SE33
80 160 240 320 400 480
3900 2600 1300 0
10 4865
11 1008
17 1872
16 611
10 762
12 3936
13 3446
12 3099
13 1370
8 2534
12 890
12.2 1587
18 926
27.2 1600

6C
D10S1248 D1S1656 D12S391 D2S1338
80 160 240 320 400 480
3300 2200 1100 0
11 523
12 1093
13 1198
14 3320
15 1273
12 1004
14 1016
15 3835
16 2185
15 420
18 353
23 367
16 1255
19 4309

Figure 6: Sample 6 C DNA profile results of casework samples.



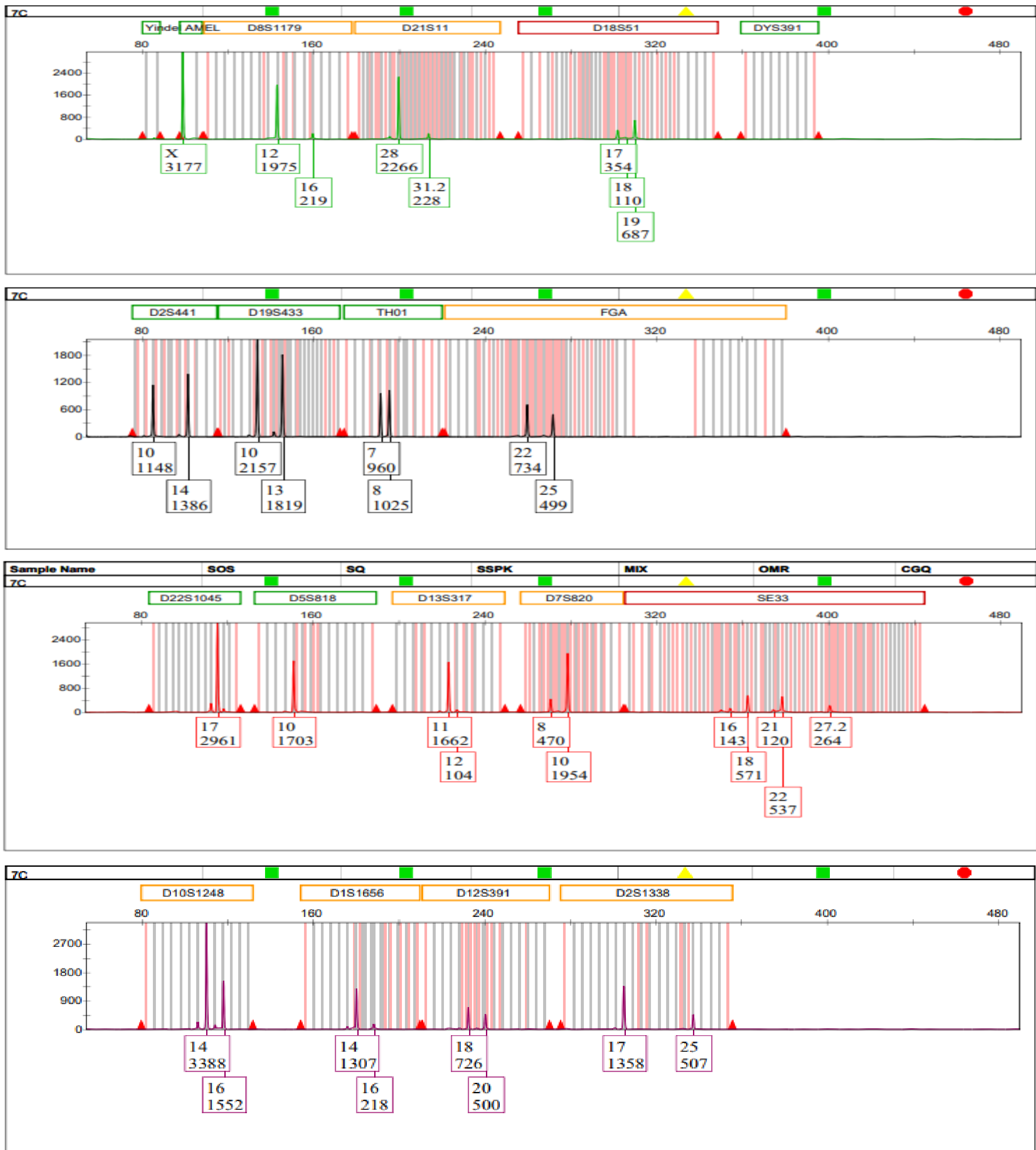


Figure 7: Sample 7 C DNA profile results of casework samples.

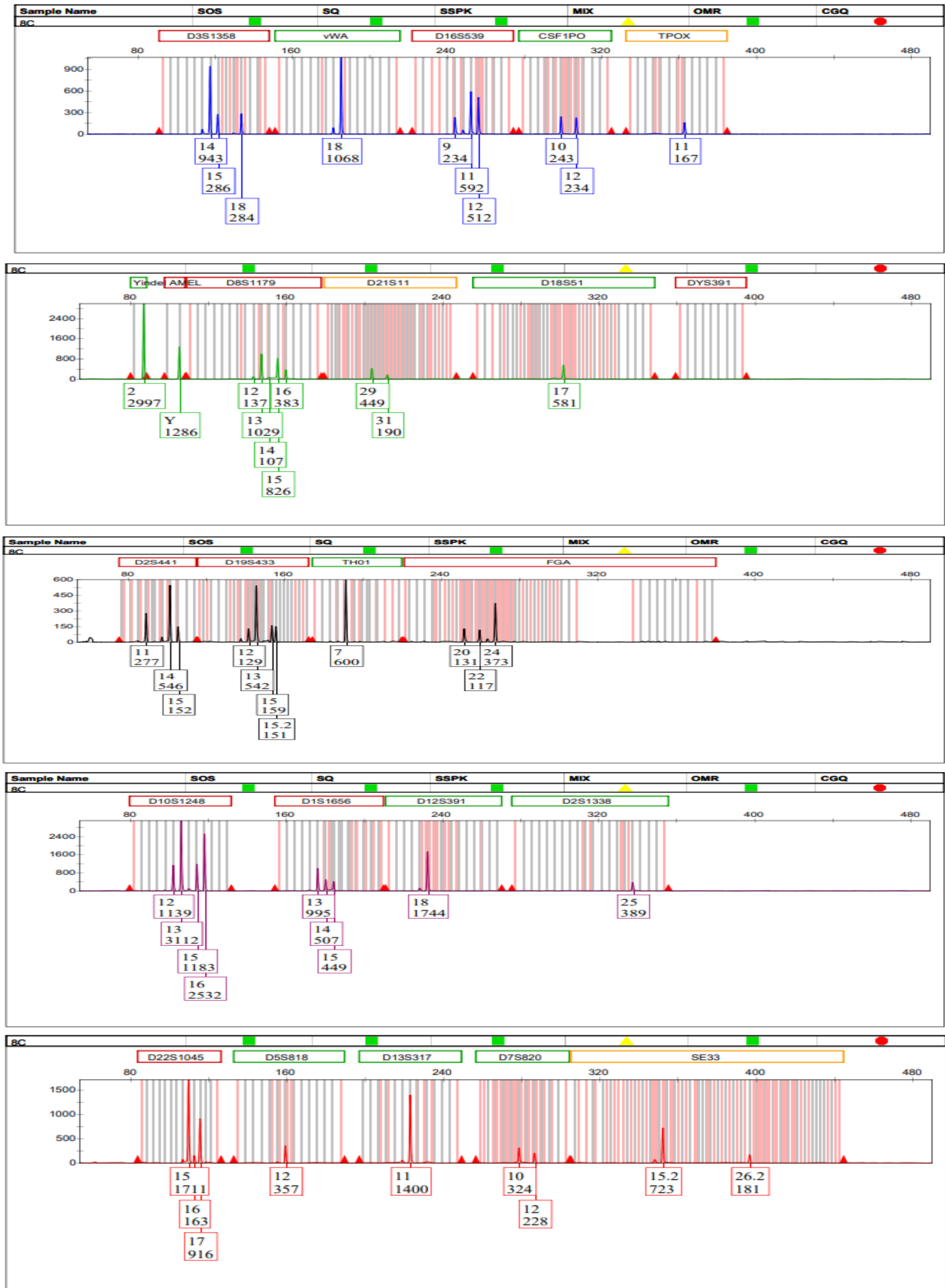


Figure 8: Sample 8 C DNA profile results of casework samples.

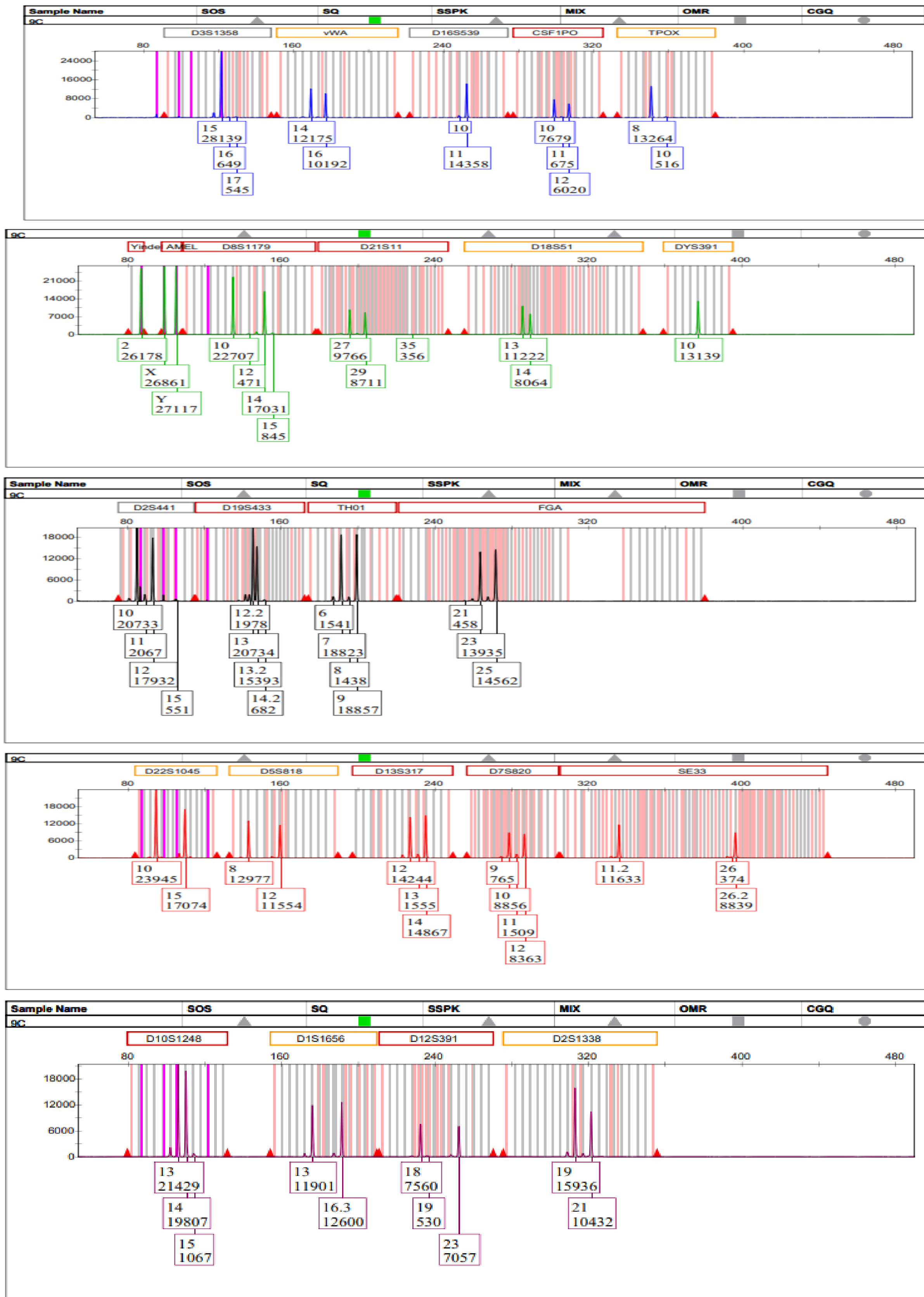


Figure 9: Sample 9C DNA profile results of casework samples.

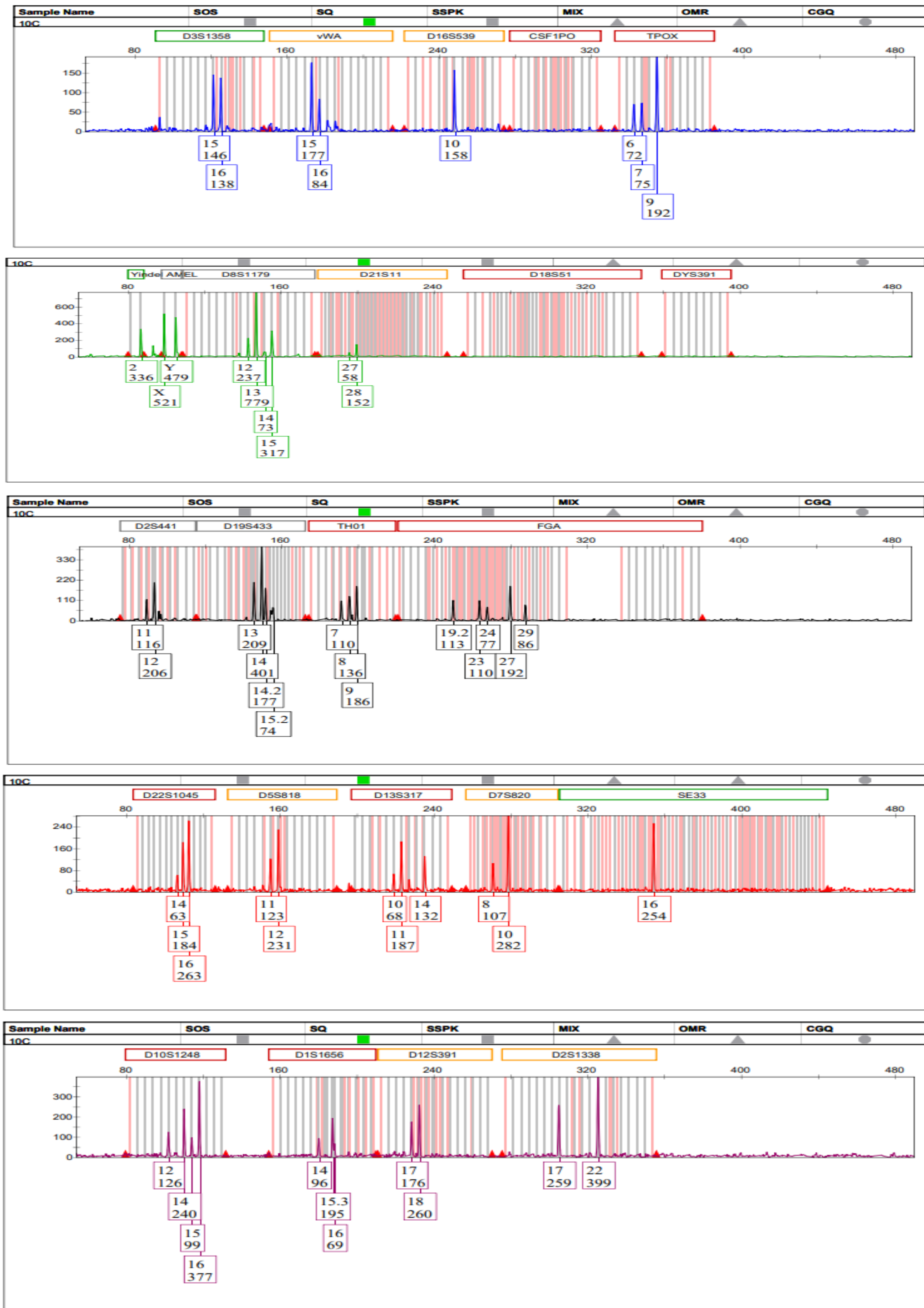


Figure 10: Sample 10C DNA profile results of casework samples.

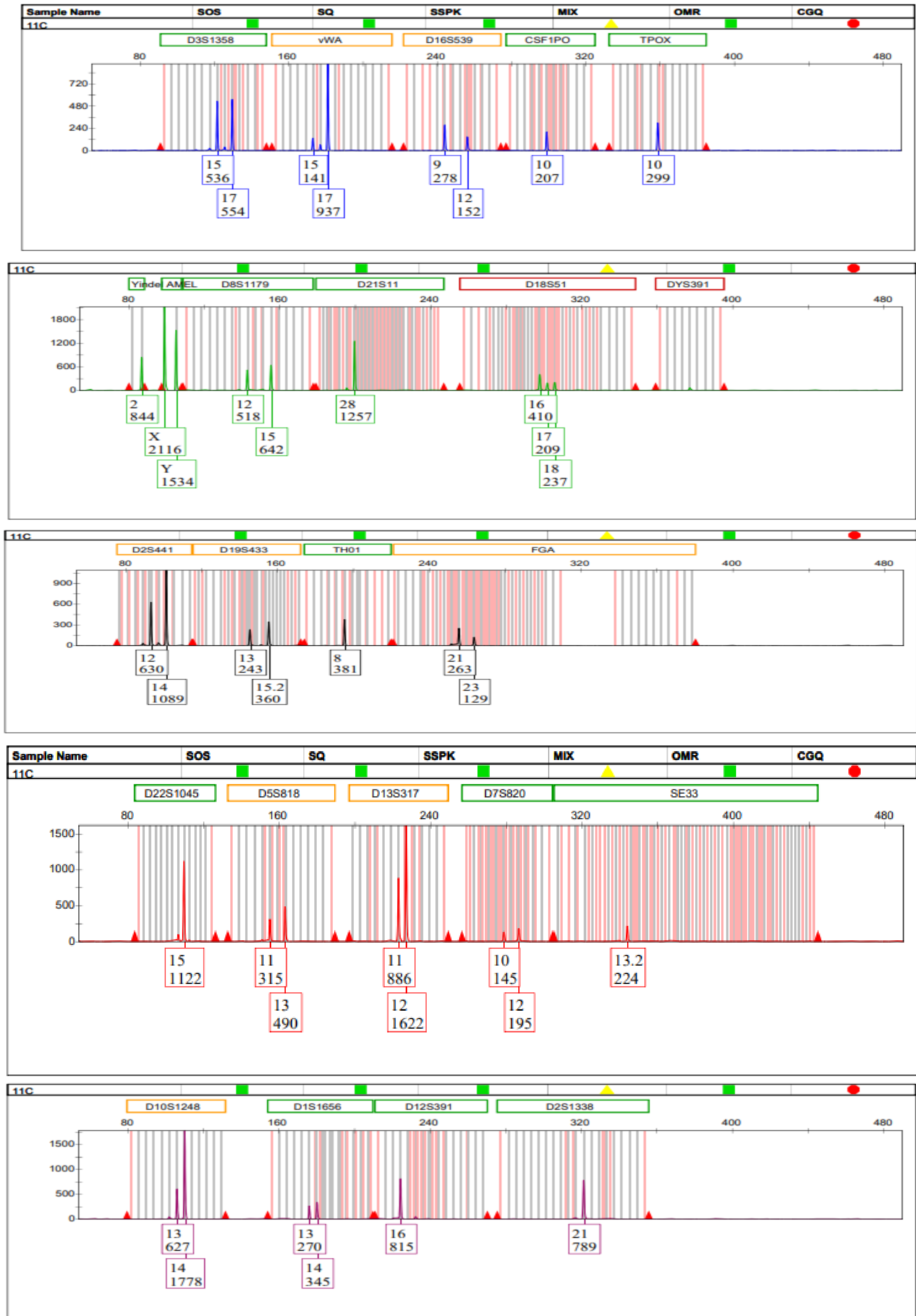


Figure11: Sample 11C DNA profile results of casework samples.

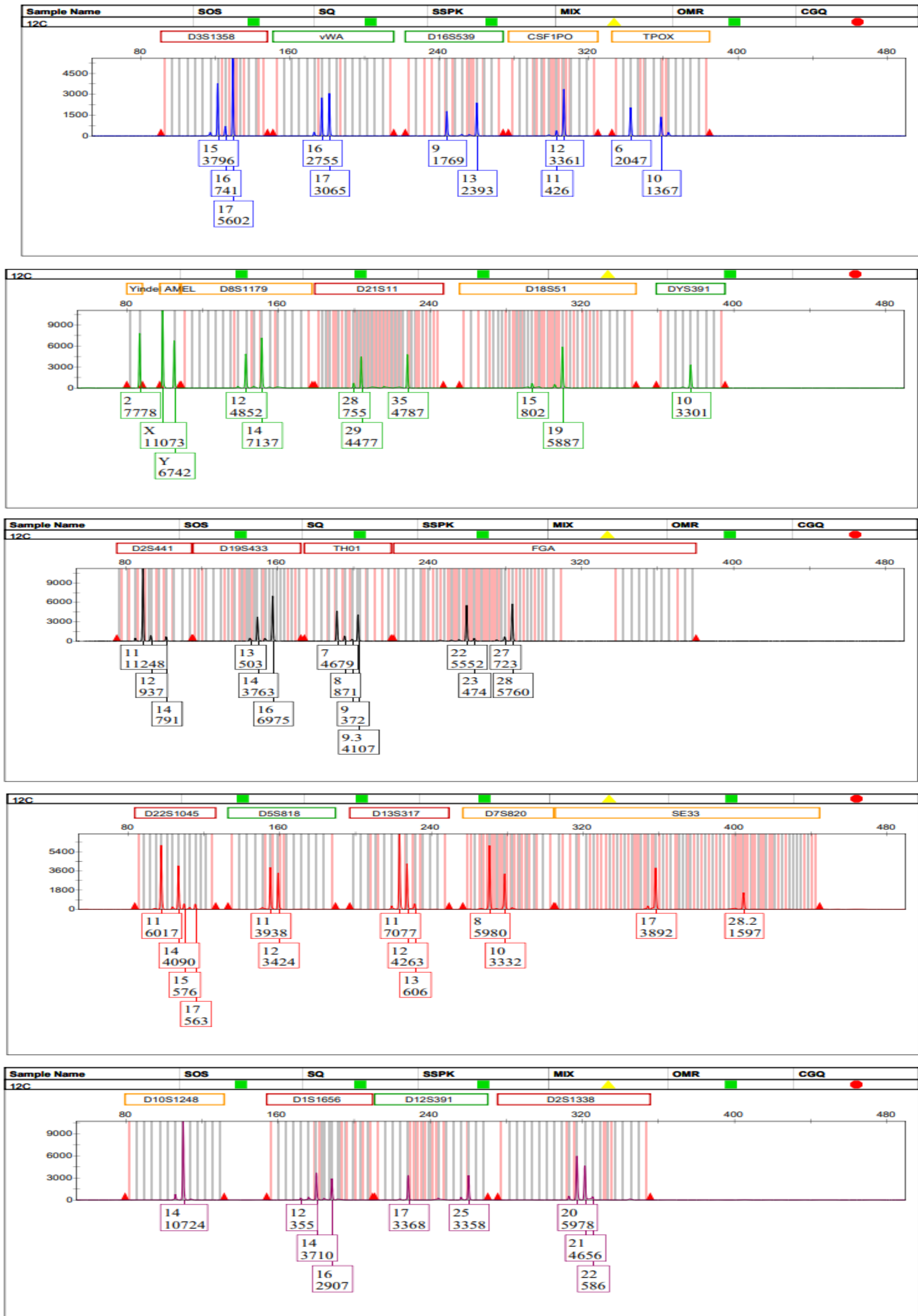


Figure12: Sample 12C DNA profile results of casework samples.

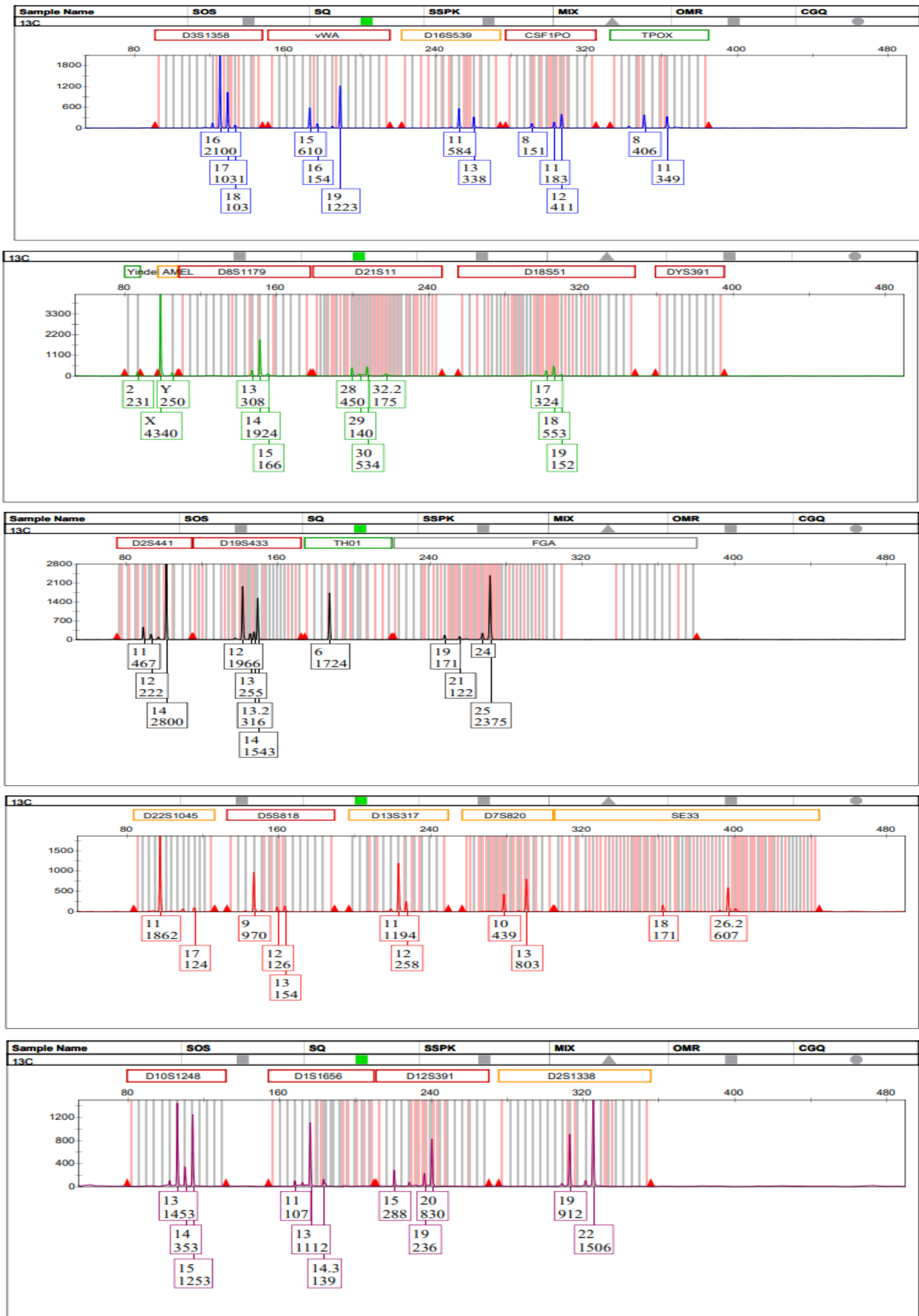


Figure13: Sample 13C DNA profile results of casework samples.

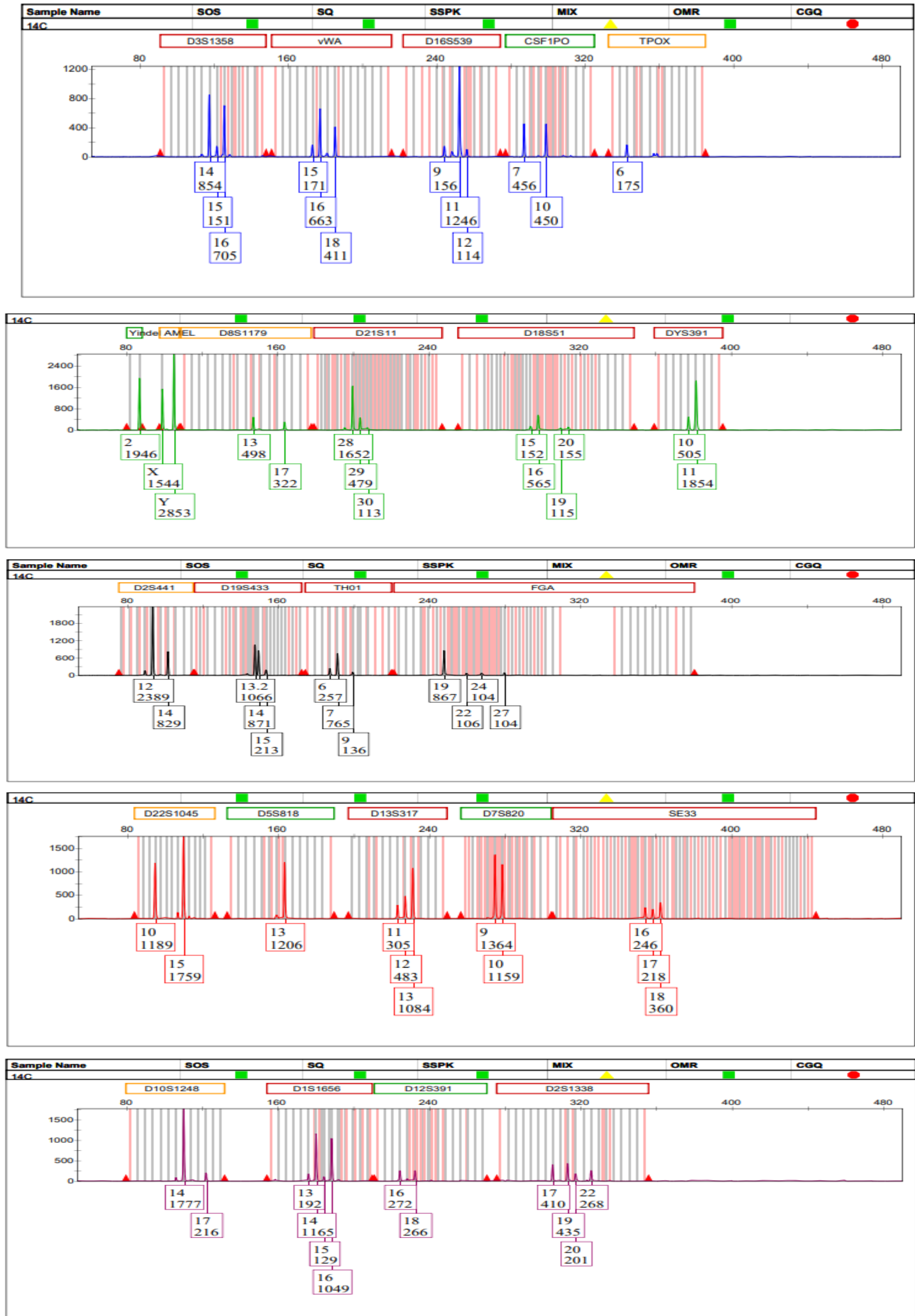


Figure14: Sample 14 C DNA profile results of casework samples.

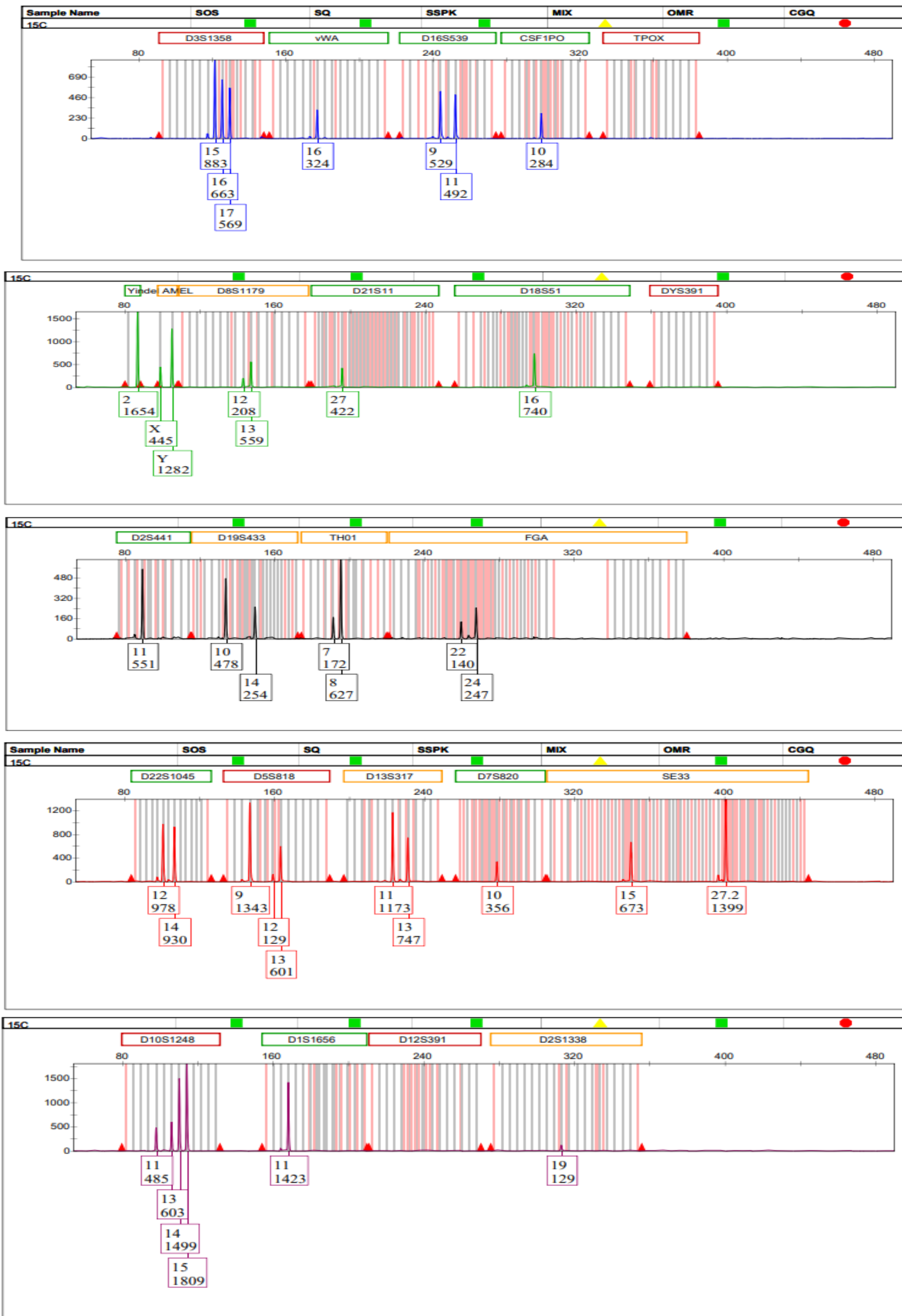


Figure15: Sample 15 C DNA profile results of casework samples.

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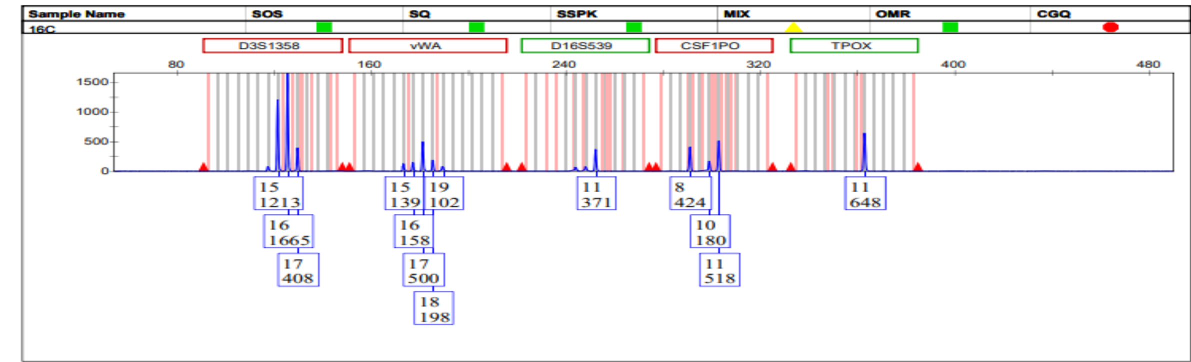

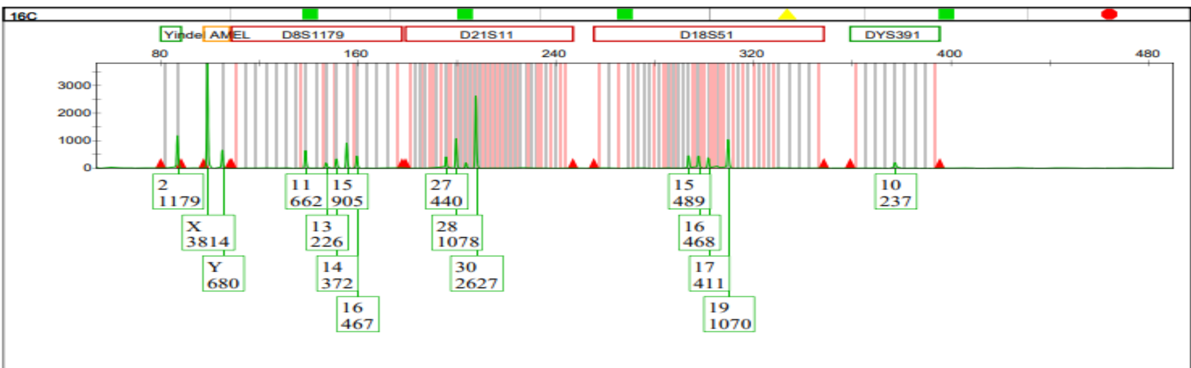

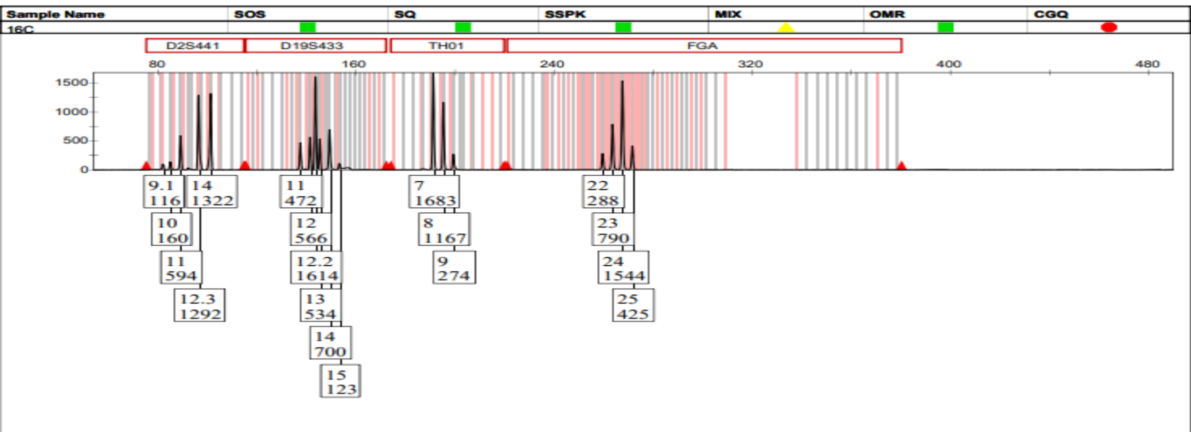

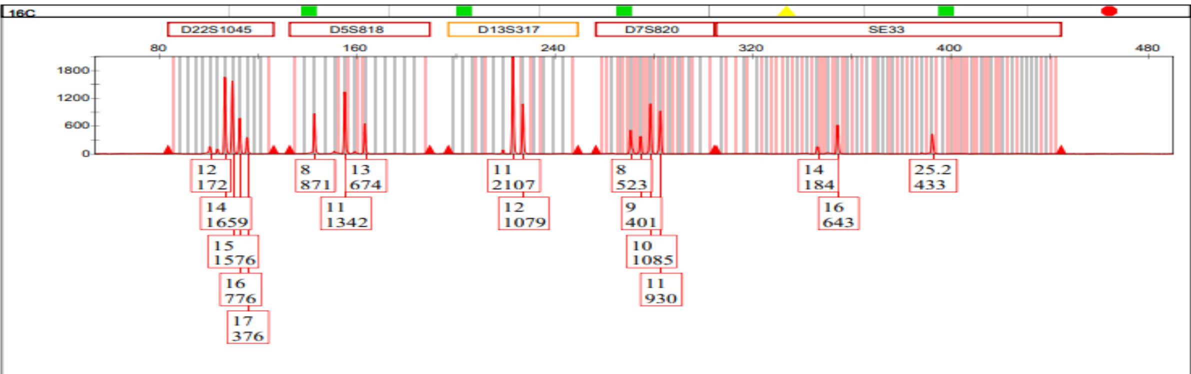

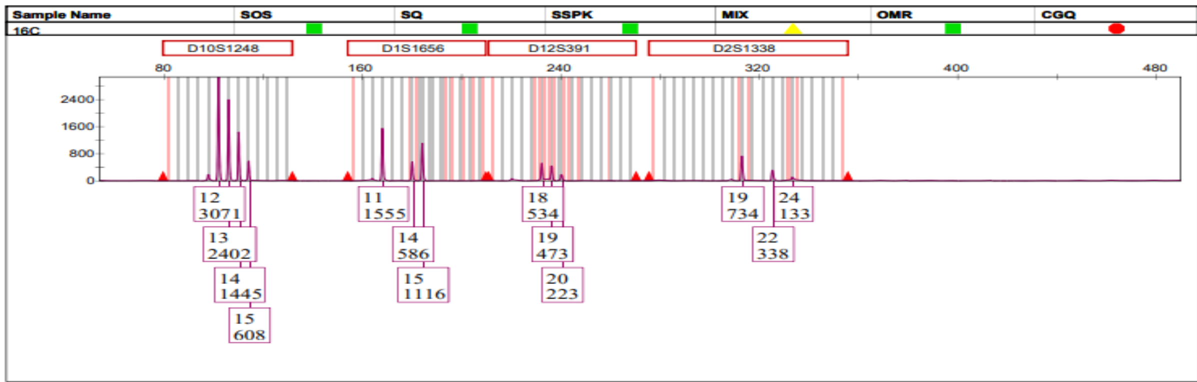

Figure16: Sample 16 C DNA profile results of casework samples.

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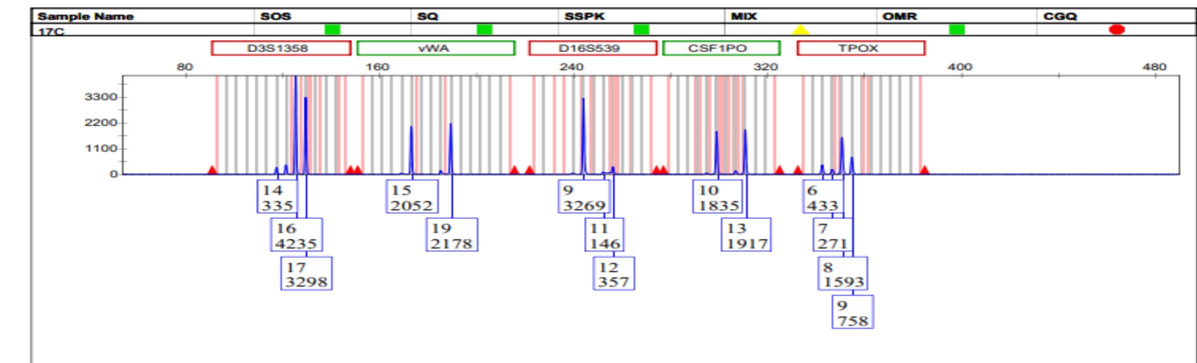
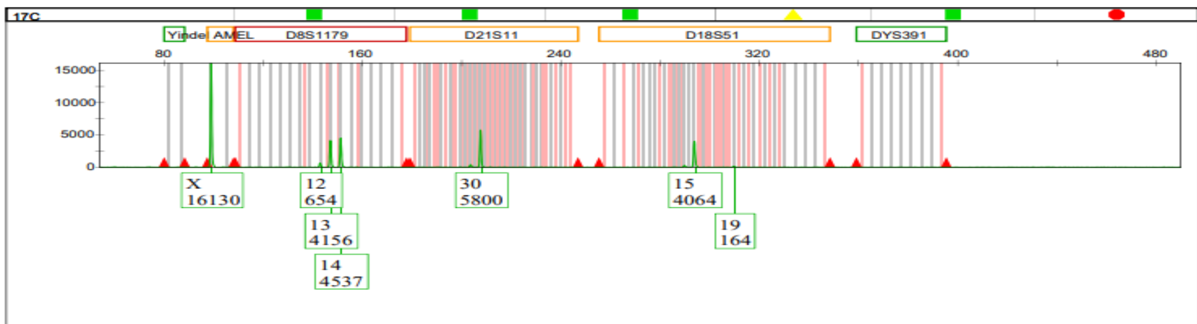
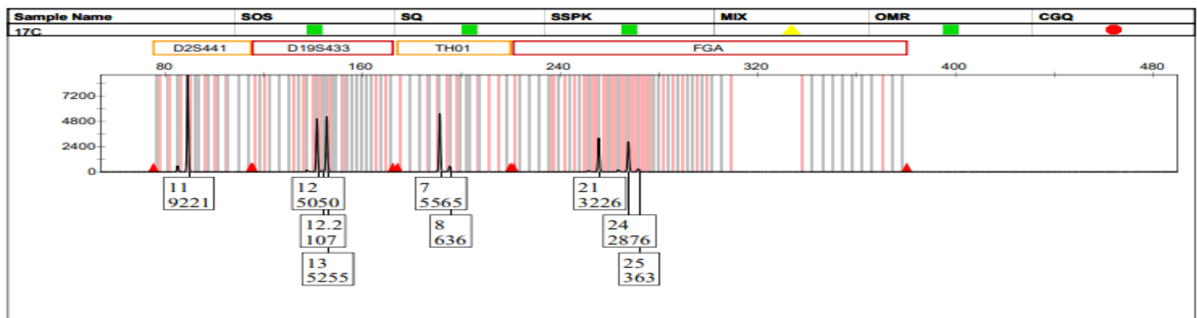
GeneMapper™ ID-X 1.6







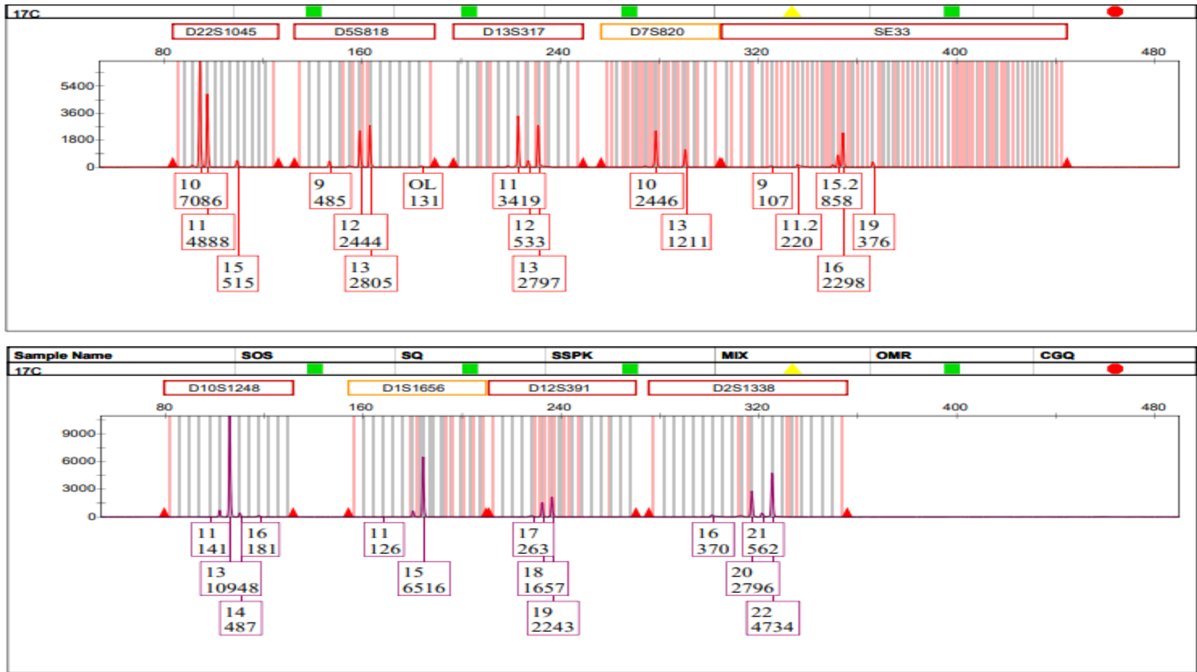


Figure17: Sample 17C DNA profile results of casework samples.

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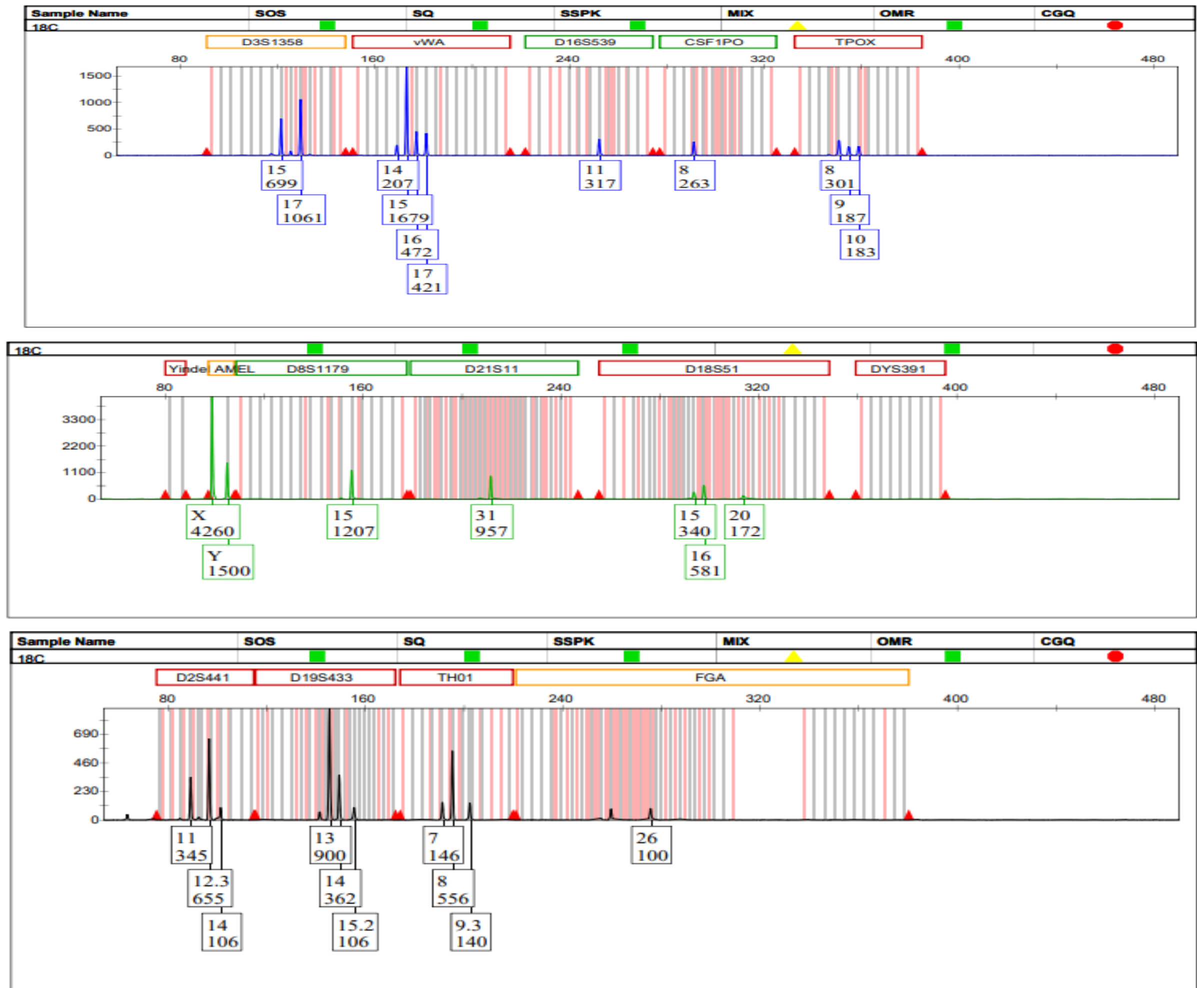

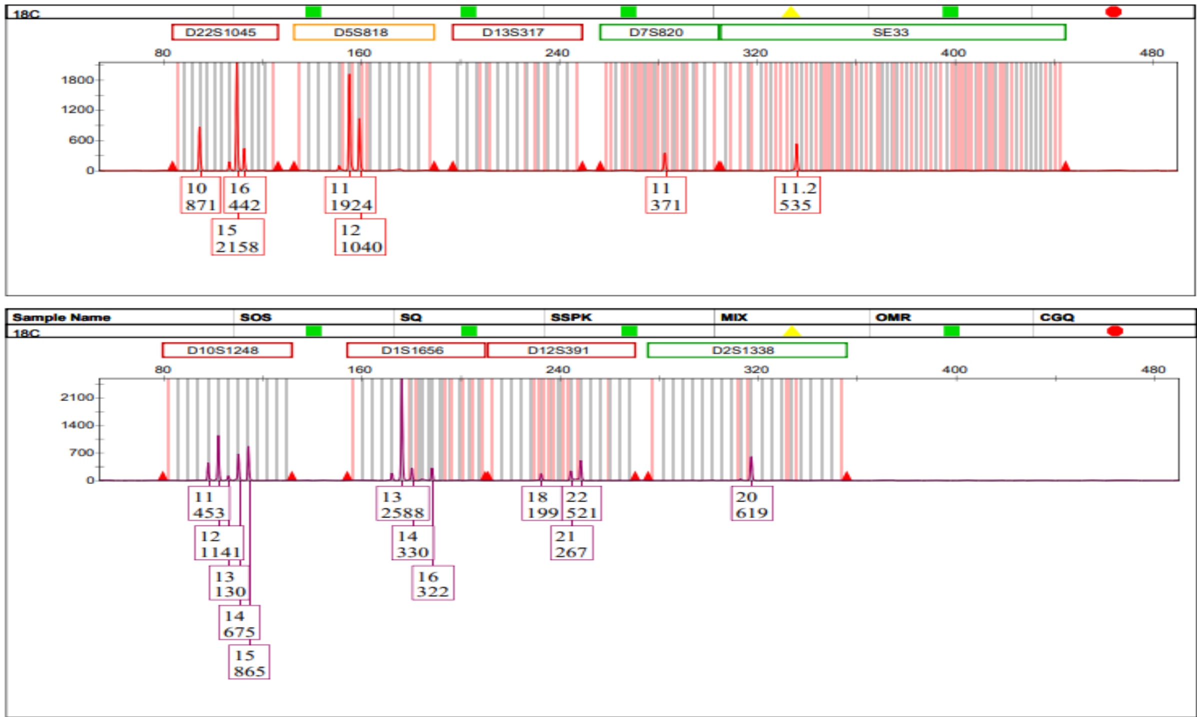

Figure18: Sample 18C DNA profile results of casework samples.

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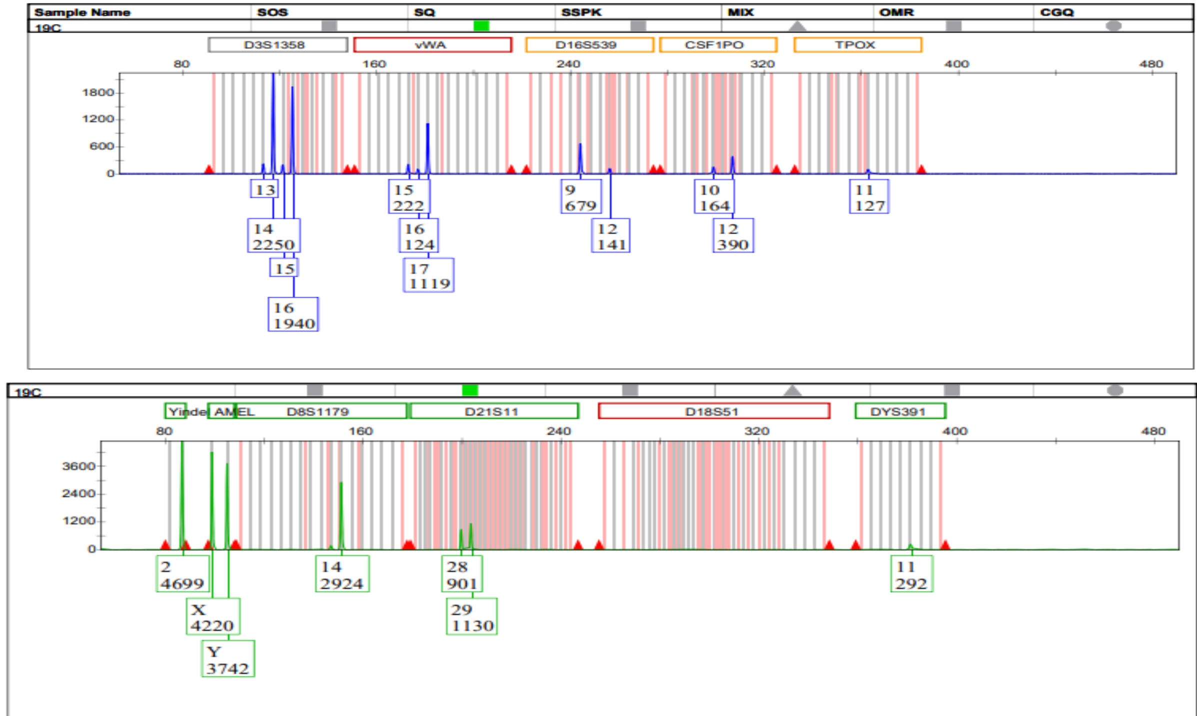
GeneMapper™ ID-X 1.6



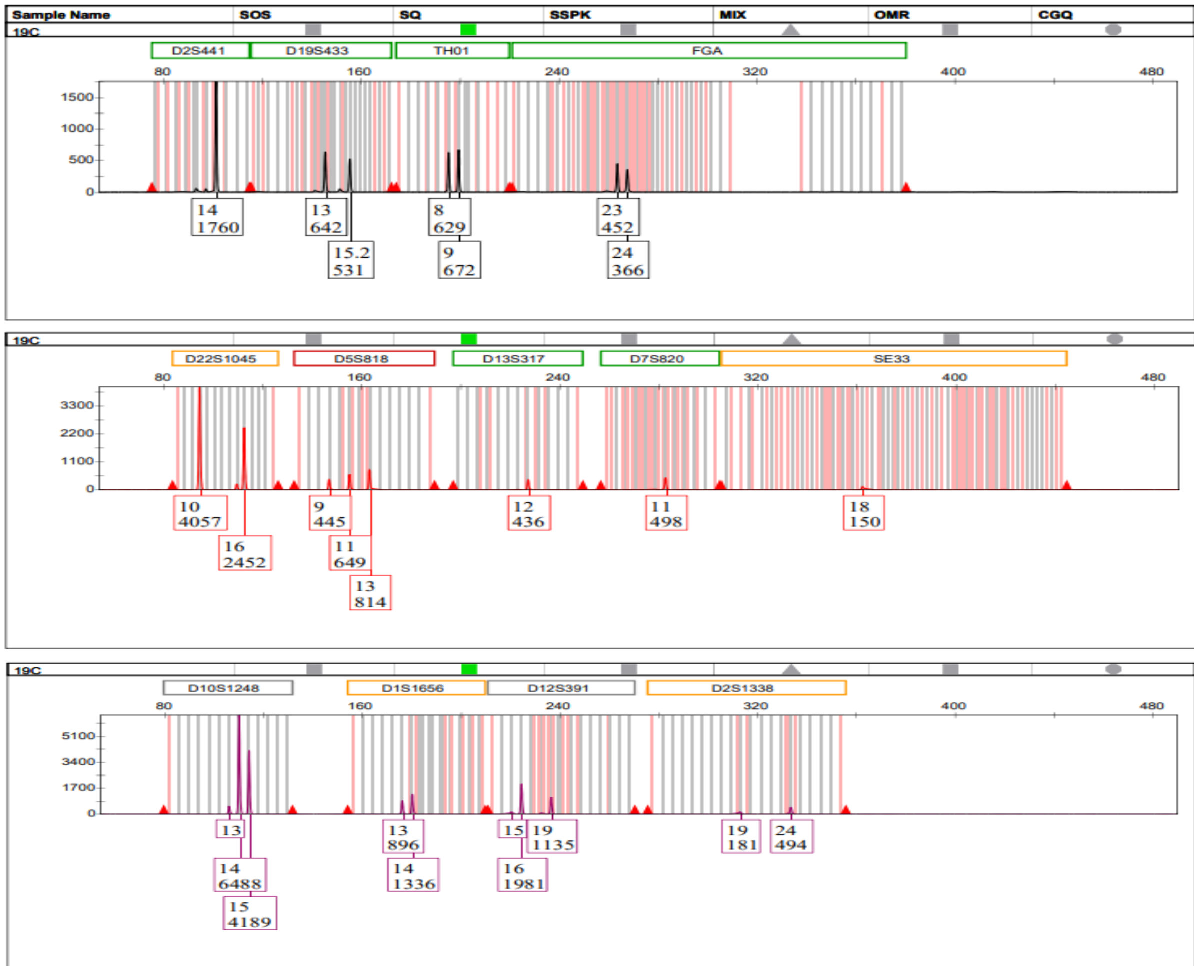


Figure 19: Sample 19 C DNA profile results of casework samples.

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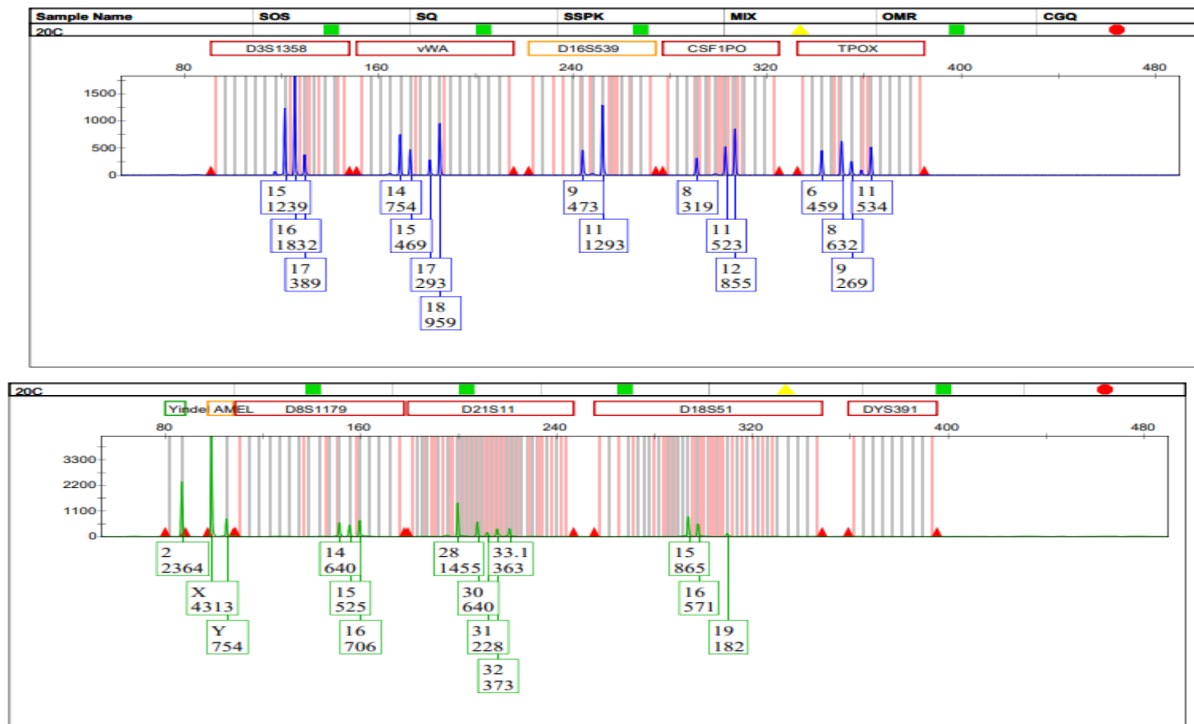
Project: 20240725_ZULU PROJECT

GeneMapper™ ID-X 1.6



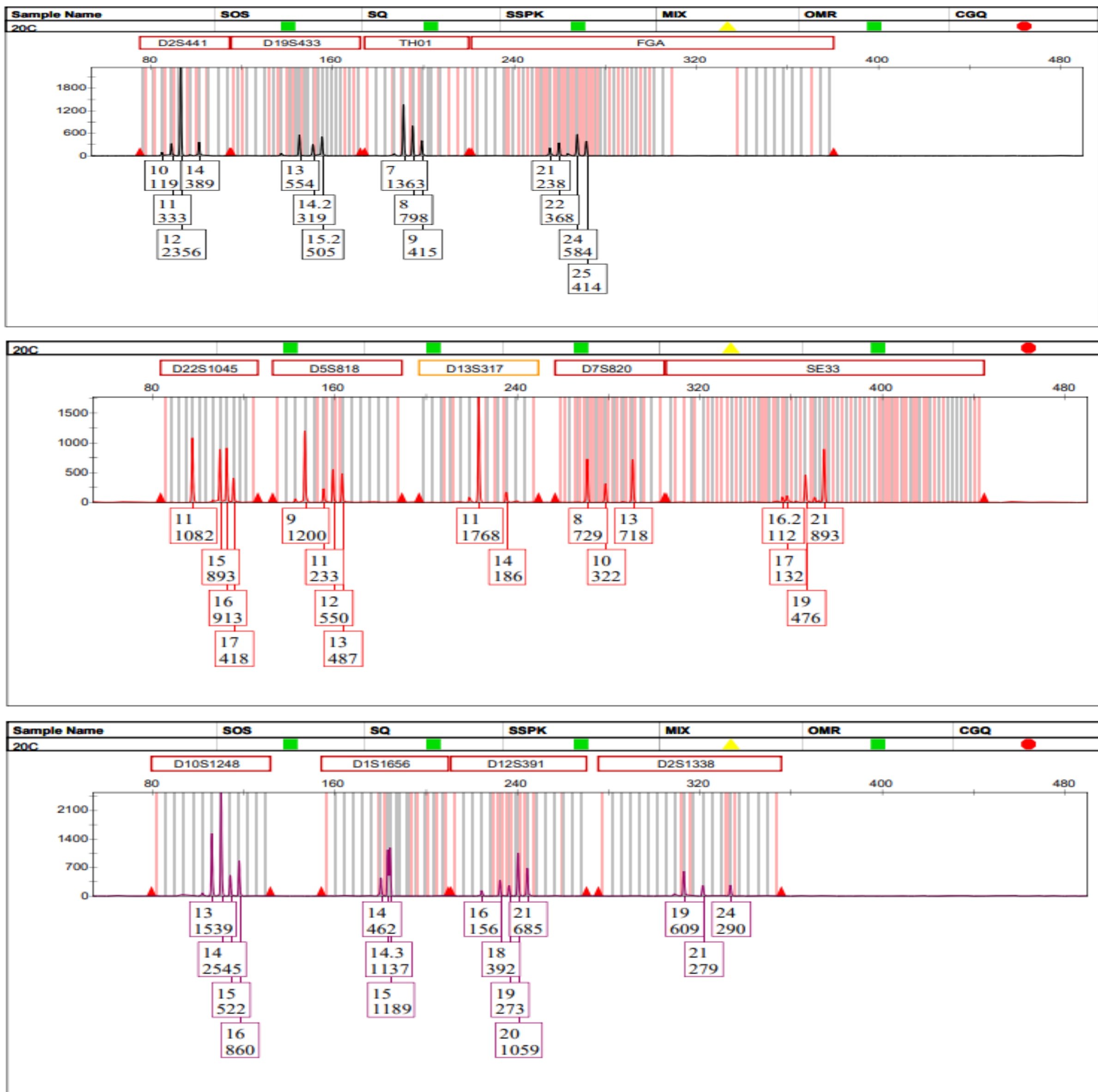


Figure 20: Sample 20 C DNA profile results of casework samples.

DNA PROFILES FEMALE PARTICIPANTS

SAMI	AM		TH01		D3S1358		vWA		D21S11		TPOX		DYS391	
1C	XX,XY		7,8		16,17		14,15		26,30,35		8,9			
1R	X	X	6	8	16	16	14	15	30	35	8	9		
2C	XX,XY		7,8,9		14,15,16,17		15,16,17,18,19		27,28,29		6,10,11			
2R	X	X	7	8	15	16	18	19	28	28	6	11		
3C	XX,XY		7,8		15,16		15,17,18		28,29,30		8,11		10	
3R	X	X	7	8	15	16	17	17	30	30	8	9		
5C	X,X,		7,8,9		15.2,17		17		26,28,30.2,31.2		7,8			
5R	X	X	7	9	16	16	16	20	28	31.2	7	8		
6C	XX,XY		7,8		16,17		14,18		30.2,33.1		9,11			
6R	X	X	7	8	17	17	14	18	28	30.2	8	9		
7C	X,X		7,8		14,15,16		14,16,17		28,31.2		11			
7R	X	X	7	8	14	16	14	16	28	28	11	11		
13C	XX,XY		6,6		16,17,18		15,16,19		28,29,30,32.2		8,11			
13R	X	X	6	6	16	17	15	19	28	30	8	11		
17C	X,X		7,8		14,16,17		15,19		30, 30		6,7,8,9			
17R	X	X	7	7	16	17	15	19	30	30	8	9		
18C	XX,XY		7,8,9.3		15,17		14,15,16,17		31		8,9,10			
18R	X	X	7	8	15	17	15	15	27	31	9	10		
20C	XX,XY		7,8,9		15,16,17		14,15,17,18		8,30,31,32,33.		6,8,9,11			
20R	X	X	7	9	15	16	14	18	28	30	6	11		

SAMI	D1S1656		D12S391		SE33		D10S1248		D22S1045		D19S433		D8S1179	
1C	14,15,16		19,19		20,26.2		10,13,14,17		12,15,17		11,14		13,14,15	
1R	14	16	19	19	20	26.2	13	17	12	15	11	14	14	14
2C	14,15,16		17,18,23		18,22,26.2		13,14,15,16		10,11,15,16,17		11,13,14		13,14,15,16	
2R	16	16	18	23	18	26.2	13	16	15	17	14	14	13	14
3C	11,13,14,14.3		16,18		14,19		1,12,13,14,15,1		10,14,15,16,17		11,12,13,14,15		14,14	
3R	11	14	16	18	14	19	13	15	14	17	12	14	14	14
5C			16,18,23				12,13		11,15,16		13.2,14,15		13,15	
5R	16	17	16	23	14	19	13	13	11	15	13.2	14	13	15
6C	12,14,15,16		15,18,23		12.2,18,27.2		11,12,13,14,15		10,11,16,17		11,13,15.2		12,13,14	
6R	15	16	15	17	12.2	27.2	12	14	10	11	11	15.2	13	14
7C	14,16		18,2		16,18,21,22,27.2		14,16		17,17		10,13		12,16	
7R	14	14	18	20	18	22	14	16	17	17	10	13	12	12
13C	11,13,14.3		15,19,20		18,26.2		13,14,15		11,17		12,13,13.2,14		13,14,15	
13R	13	13	15	20	26.2	26.2	13	15	11	11	12	14	14	14
17C	11,15		17,18,19		9,11.2,15.2,16,19		11,13,14,16		10,11,15		12,12.2,13		12,13,14	
17R	15	15	18	19	15.2	16	12	13	10	11	12	13	13	14
18C	13,14,16		18,21,22		11.2		11,12,13,14,15		10,15,16		13,14,15.2		15	
18R	13	16	21	22	21.2	29.2	12	15	10	15	13	14	14	15
20C	14,14.3,15		16,18,19,20,21		16.2,17,19,21		13,14,15,16		11,15,16,17		13,14.2,15.2		14,15,16	
20R	14.3	15	20	21	21	21	13	14	11	16	13	14.2	15	16

Appendix 3: Summary Table of Female Participants DNA Profile

SAMPLE	AM		D2S1338		D2S441		D18S51		FGA		D16S539		CSF1PO		D13S317		D5S818		D7S820	
1C	XX,XY		17, 20		12,14		17, 20		24,25		11,12		10,12		11,12		11,12		11,12	
1R	X	X	17	20	12	14	17	20	24	25	11	12	10	12	11	12	11	12	11	12
2C	XX,XY		19,20,21,22		11,11.312,14		,15,16,17,		19,24		9,12		10,12		11,12,13		11,12		10,11	
2R	X	X	19	22	12	14	17	18	19	24	9	12	10	12	11	13	11	12	10	11
3C	XX,XY		17,21		10,11,12,14		18, 20		21,22,23,30.2		11,12,13		10,12		11,12		8,12		10, 10	
3R	X	X	17	21	11	11	20	20	21	30.2	11	13	10	12	11	12	8	12	10	10
5C	X,X,		16		10,11,12		15,17		21,22		9,11		9,1		11,12,13,14		11,12,13		10	
5R	X	X	16	24	10	12	17	18	18.2	21	8	11	9	11	12	14	11	13	8	10
6C	XX,XY		16,19		11,14		15,16,17		22,24		11,12		11,12		12,13		10,12,13		8,12	
6R	X	X	19	19	11	14	15	16	22	24	11	12	11	12	12	13	13	13	8	12
7C	X,X		17,25		10,14		17,18,19		22,25		9,12		10,12		11,12		10, 10		8, 01	
7R	X	X	17	25	10	14	17	19	22	25	9	22	10	12	11	11	10	10	8	10
13C	XX,XY		19,22		11,12,14		17,18,19		19,21,24,25		11,13		8,11,12		11,12		9,12,13		10,13	
13R	X	X	19	22	14	14	17	18	25	25	11	13	11	12	11	12	9	13	10	13
17C	X,X		16,20,21,22		11,11		15,19		21,24,25		9,11,12		10,13		11,12,13		9,12,13		10,13	
17R	X	X	20	22	11	11	15	15	21	24	9	9	10	13	11	13	12	13	10	13
18C	XX,XY		20		11,12.3,14		15,16,20		26		11,11		8				11,12		11	
18R	X	X	20	24	11	12.3	15	20	22	22	11	11	12	12	12	13	11	11	10	11
20C	XX,XY		19,21,24		10,11,12,14		15,16,19		21,22,24,25		9,11		8,11,12		11,14		9,11,12,13		8,10,13	
20R	X	X	19	24	11	12	15	15	22	24	11	12	11	12	11	14	9	13	8	10

Appendix 3: Summary Table of Female Participants DNA Profile

															DNA PROFILES FOR MALE PARTICIPANTS													
SAMP	AM		TH01		D3S1358		vWA		D21S11		TPOX		DYS391		D1S1656		D12S391		SE33		D10S1248		D22S1045		D19S433		D8S1179	
4C	XX,XY		9,9		14,17		17,19		27,31		9, 10		10		11,14,15		15,16		20,26.2		13,15		15,16,17		12,13,13.2,14		12,15	
4R	X	Y	9	9	14	17	17	19	27	31	9	9	10		11	14	15	16	20	26.2	13	15	15	16	12	13	12	15
8C	XX,XY		7,7		14,15,18		18,18		29,31		11				13,14,15		18,18		15.2,26.2		12,13,15,16		15,16,17		12,13,15,15.2		12,13,14,15,16	
8R	X	Y	7	7	14	18	18	18	29	31	8	11	11		13	17	18	18	15.2	17	13	16	11	15	13	15	13	15
9C	XY		6,7,8,9		15,16,17		14,16		27,29,35		8, 10		10		13,16.3		18,19,23		11.2,26,26.2		13,14,15		10,15		12.2,13,13.2,14.2		10,12,14,15	
9R	X	Y	7	9	15	15	14	16	27	29	8	8	10		13	16.3	18	23	11.2	26.2	13	14	10	15	13	13,2	10	14
10C			7,8,9		15,16		15,16		27,28		6,7,9				14,15.3,16		17,18		16		12,14,15,16		14,15,16		13,14,14.2,15.2		12,13,14,15	
10R	X	Y	7	8	15	16	16	20	28	29	8	9	10		14	15.3	18	18	16	20	12	16	14	14	14	14	13	15
11C	XY		8,8		15,17		15,17		28,28		10				13,14		16,16		13.2		13,14		15,15		13,15.2		12,15	
11R	X	Y	8	8	15	17	15	17	28	28	8	11	10		14	15	16	16	13.2	18	14	14	15	15	13	15.2	12	15
12C	XY		7,8,9,9.3		15,16,17		16,17		28,29,35		6, 10		10		12,14,16		17,25		17,28.2		14,14		11,14,15,17		13,14,16		12,14	
12R	X	Y	7	9.3	15	17	16	17	29	35	6	10	10		14	16	17	25	17	28.2	14	14	11	14	14	16	12	14
14C	XY		6,7,9		14,15,16		15,16,18		28,29,30		6		10,11		13,14,15,16		16,18		16,17,18		14,17		10,15		13.2,14,15		13,17	
14R	X	Y	6	6	14	16	16	18	28	28	6	9	11		14	16	16	18	17	18	14	14	10	15	13.2	14	13	17
15C	XY		7,8		15,16,17		16		27						11				15,27.2		11,13,14,15		12,14		10,14		12,13	
15R	X	Y	7	8	15	16	14	16	29	30	10	11	10		11	16.3	17	17	19	27.2	13	14	14	14	10	15.2	13	14
19C	XY		8,9		13,14,15,16		15,16,17		28,29		11		11		13,14		15,16,19		18		13,14,15		10,16		13,15.2		14	
19R	X	Y	8	9	14	16	15	17	28	29	8	11	11		13	14	16	19	18	20	14	15	10	16	13	15.2	14	14

Appendix 4: Summary Table of Male Participants DNA Profile

SAMPLE	AM		D2S1338		D2S441		D18S51		FGA		D16S539		CSF1PO		D13S317		D5S818		D7S820	
4C	XX,XY		17		11,12,14,15		14,15,16		19,23,24		9, 10		10,12		11,12		12,13		9,12	
4R	X	Y	17	22	12	15	14	16	23	24	9	10	10	12	12	12	12	13	9	12
8C	XX,XY		25,25		11,14,15		17		20,22,24		9,11,12		10,12		11		12		10,12	
8R	X	Y	22	25	11	14	14	16	20	24	11	11	9	12	11	14	12	13	11	12
9C	XY		19,21		10,11,12,15		13,14		21,23,25		10,11		10,11,12		12,13,14		8,12		9,10,11,12	
9R	X	Y	19	21	10	12	13	14	23	25	11	11	10	12	12	14	8	12	10	12
10C			17,22		11,12				19.223,24,27,29		10				10,11,14		11,12		8, 10	
10R	X	Y	16	20	11	14	13	17	24	29	9	10	10	10	12	14	8	12	8	10
11C	XY		21		12,14		16,17,18		21,23		9,12		10,1		11,12		11,13		10,12	
11R	X	Y	21	23	12	14	16	18	21	25	9	12	10	10	11	12	11	13	10	12
12C	XY		20,21,22		11,12,14		15,19		22,23,27,28		9,13		12,11		11,12,13		11,12		8, 10	
12R	X	Y	20	21	11	11	19	19	22	28	9	13	12	12	11	12	11	12	8	10
14C	XY		17,19,20,22		12,14		5,16,19,2		19,22,24,27		9,11,12		7,1		11,12,13		13,13		9, 10	
14R	X	Y	17	19	12	14	15	16	19	24	11	11	10	10	11	13	13	13	9	10
15C	XY		19		11		16		22,24		9,11		10		11,13		9,12,13		10, 10	
15R	X	Y	22	24	11	12	14	16	19	24	11	12	10	11	11	13	8	9	10	10
19C	XY		19,24		14,14				23,24		9,12		10,12		12		9,11,13		11	
19R	X	Y	19	24	14	14	17	18	23	24	9	12	10	12	8	12	11	13	10	11

Appendix 4: Consent Form

Participant number: __ __ __

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

   (Tick the appropriate box)

☐ The use of my DNA and information to be used for this study as approved by Research Ethics Committee.

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

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

____

Name of participant

____ __________________________

Signature of participant Date

____ __________________________

Signature of Witness Date