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# Forensic Evaluation of 6-Dye Chemistry Kit Composed Of 23 Loi with Casework Samples

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# FORENSIC EVALUATION OF 6-DYE CHEMISTRY KIT COMPOSED OF 23 LOCI WITH CASEWORK SAMPLES

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This thesis is submitted in partial fulfilment of the requirements for the degree of Master of Science in Molecular Biology and Biotechnology

Under the Supervision of Prof. Synan Abu-Qamar

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## Declaration of Original Work

I, Naeema Saleh Al Janaahi, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this thesis entitled "*Forensic Evaluation of 6-Dye Chemistry Kit Composed of 23 Loci with Casework Samples*", hereby, solemnly declare that this thesis is my own original research work that has been done and prepared by me under the supervision of Professor Synan Abu-Qamar in the College of Science at UAEU. This work has not previously been presented or published, or formed the basis for the award of any academic degree, diploma or a similar title at this or any other university. Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my thesis have been properly cited and acknowledged in accordance with appropriate academic conventions. I further declare that there is no potential conflict of interest with respect to the research, data collection, authorship, presentation and/or publication of this thesis.

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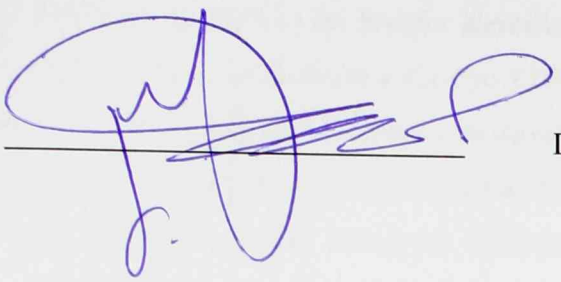
Signature  Date 24/12/2019

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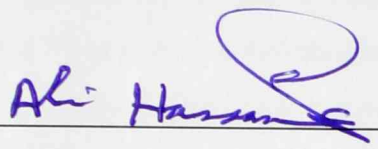
Signature  Date 29/1/2020

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## Abstract

Short Tandem Repeats (STRs) are short nucleotide sequence repeats consisting of 2-8 base pairs (bp), representing approximately 3% of human DNA. Markers of STRs have been widely used as genetic markers in forensic DNA analysis and have proven to be an extremely discriminating method for human identification in forensics. The main objective of this research was to evaluate a six-dye STR multiplex assay (Virifiler™ Plus) composed of 23 autosomal STR loci, one insertion/deletion polymorphic marker on the Y chromosome, Amelogenin and two internal quality control markers (IQCS and IQCL) using biological stains found in different crime scenes. The study investigated the performance of several tests, including: sensitivity, reproducibility, stability, heterozygote balance, precision, mixture study and concordance study. The results showed that the assay was reproducible, sensitive, accurate and robust. Sensitivity testing showed that a full profile could be obtained even with 63 pg of human DNA. Heterozygous allele balance varied between 60-99% for samples with total DNA input ranging from 1 ng-500 pg. It is also suitable for mixture studies which occur when the evidence contains a mixture of DNA coming from several contributors. All alleles of minor contributors were called for ratios of 1:1, 1:3 and 3:1. Overall, the current study demonstrates that this multiplex assay is robust and reliable as an assay for human identification with forensic casework samples, and most importantly is suitable to be used in Dubai Forensic Laboratory and other forensic laboratories worldwide. This study is the first to evaluate Virifiler™ Plus with casework samples.

**Keywords:** Concordance study, heterozygote balance, limit of detection, limit of quantification, mixture study, sensitivity, STRs.

## Title and Abstract (in Arabic)

# تفعيل بصمة وراثية تتكون من 23 موقع جيني لاستخدامها في تطبيقات الأدلة الجنائية


***الملخص***

تعتبر ترددات البصمة الوراثية القصيرة عبارة عن سلسلة قصيرة من القواعد النيتروجينية المتكررة والتي يتراوح طولها ما بين 2-8 قواعد نيتروجينية زوجية، وهي تشكل ما يقارب 3% من تكوين الحمض النووي للإنسان. أصبحت ترددات المواقع الجينية للبصمة الوراثية العلامة الجينية المختارة في التحليل الجنائي للعينات البيولوجية. وأثبتت أنها وسيلة تمييزية للكشف عن هوية الانسان في التحاليل الجنائية. إن الهدف الرئيس من هذه الأطروحة هو تقييم فحص ترددات المواقع الجينية للبصمة الوراثية (Virifiler™ Plus) والتي تتكون من 23 موقع جيني منتشرة على الكروموسومات الرئيسية المكونة للحمض والنووي بالإضافة الى مواقع من الحمض النووي متعددة الأشكال على الكروموسومين (XY) اللذان يحددان جنس الانسان وعلامات مراقبة جودة تحاليل البصمة الوراثية خاصة العينات المرفوعة من مسرح الجريمة. بحثت هذه الدراسة في أداء العديد من الاختبارات بما في ذلك: الحساسية، قابلية إعادة الحصول على نفس النتائج بالتكرار، الثبات، توازن الزيجوت المتغاير، الدقة، دراسة العينات المختلطة التي تنتج من الآثار المحتوية على الحمض النووي لعدة أشخاص ودراسة توافق العينات. برهنت الدراسة بأن هذا الفحص قابل لإعادة الإنتاج وعلى درجة عالية من الحساسية والدقة والقوة. هذا وأظهرت اختبارات الحساسية انه يمكن الحصول على قراءات متكاملة من الحمض النووي باستخدام كمية 63 بيكوغرام من الحمض النووي الآدمي. تراوح توازن الزايجوت المتغاير بين 60-99% للعينات التي تكون كمية الحمض النووي فيها 1 نانوجرام-500 بيكوجرام. يعتبر هذا الفحص مناسبا لدراسة العينات المختلطة. حيث أن جميع أليلات المساهم الثانوي كانت مقروءة بالنسب 1:1، 1:3، و 3:1. تظهر الدراسة، وبشكل عام، أن هذا الفحص مناسب ودقيق لتحديد هوية الانسان في العينات المرفوعة من مسرح الحوادث الجنائية وملائم للاستخدام في المختبر الجنائي بدبي وغيرها من المختبرات الجنائية حول العالم. هذه الدراسة هي أول دراسة لتقييم ( Virifiler™ Plus) واستخدامها لتحاليل البصمة الوراثية للعينات المرفوعة من مسرح الجريمة.



**مفاهيم البحث الرئيسية**: دراسة توافق العينات، توازن الزايجوت المتغاير، علامات مراقبة جودة تحاليل البصمة الوراثية، دراسة العينات المختلطة، الحساسية، ترددات البصمة الوراثية.

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# Dedication

*To my beloved parents and family*

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# List of Abbreviations

| | |
|---|---|
| AT | Analytical threshold |
| AL | Allelic ladder |
| bp | Base pair |
| CE | Capillary electrophoresis |
| CODIS | Combined DNA index system |
| LOD | Limit of detection |
| LOQ | Limit of quantification |
| nt | Nucleotide |
| PCR | Polymerase chain reaction |
| PHR | Peak height ratio |
| RFLP | Restriction fragment length polymorphism |
| RFU | Relative fluorescence unit |
| STR | Short tandem repeats |
| VNTR | Variable number of tandem repeats |

# Chapter 1: Introduction

## 1.1 Literature Review

Advanced molecular biological tools are universally used in DNA analysis to identify information contained in crime scene evidence. These advances have allowed scientists to provide critical information to solve crimes and the criminal justice community to convict the guilty and justify the innocent. Scientific research continues to develop new automated technologies and methods to yield more information from limited samples allowing scientists to optimize time and effort [1].

### 1.1.1 History of Forensic DNA

In 1980s, forensic scientist used statistical interpretation to analyze the biological stains from different type of cases such as homicide and sexual assault that could represent only 10% of the population; Unfortunately, results were not accurate as it ruled out 90% of the population as a possible donor [1].

In 1985, Alec Jefferys discovered a new method to analyze biological stains called DNA typing. It was found that certain regions of DNA contain specific sequences that are next to each other and repeated a number of times. These regions are known as variable number of tandem repeats (VNTR). He also proved the ability of these repeats to differentiate human individuals. Moreover, a technique by Jefferys was developed to examine the length variation of these sequence repeats called restriction fragment length polymorphism (RFLP) analysis. This technique uses multi locus probe and a restriction enzyme that cuts the region of DNA surrounding the VNTRs [2].

Since that time, DNA typing methods has been used in human identity testing in forensic laboratories. Enormous growth has been seen in the use of DNA evidence in crime scene investigation and paternity testing. More than 150 public forensic laboratories and other private paternity testing laboratories are conducting thousands of DNA tests in the United States [2] and in other forensic laboratories around the world.

### 1.1.2 DNA Typing Methods

STR markers analyzed by capillary electrophoresis (CE) represent the gold-standard for forensic DNA analysis. For the past twenty years, STR loci from the human genome have been the genetic markers of choice in forensic DNA analysis in large measure. This is because the multi-allelic nature of STRs produce many possible genotype combinations that can aid human identification and most importantly DNA mixture interpretation. STRs are copied by polymerase chain reaction (PCR) and the analysis is performed by size-based DNA separations using capillary electrophoresis or CE. However, analysis of PCR product length alone fails to capture the potential internal sequence variation that may exist in many STR loci detected via base composition mass spectrometry or through full sequence analysis [3].

### 1.1.3 Type of STRs

STR loci can vary in three manners: the repeat unit length (e.g., di-, tri-, tetra-penta-, hexa-nucleotides), the number of repeat units (e.g.10–25 repeats) and in the rigor with which alleles conform to an incremental repeat pattern. In one of the early studies by the UK Forensic Science Service (FSS), STR loci were categorized as simple, compound or complex based on how well alleles conformed to the core repeat pattern [3].

Simple repeats, such as TH01 with an AATG repeat motif, contain repeat units of identical length and sequence, although they may occasionally have non-consensus repeats like the TH01 9.3 allele with a [AATG] 6 ATG[AATG] 3 sequence [3].

Compound repeats comprise two or more adjacent simple repeats, such as TCTA and TCTG in the tetranucleotide STR locus vWA. Complex repeats may contain several repeat blocks of variable unit length as well as variable interv ening sequences with numerous variant alleles, such as the STR loci D21S11 or FGA. Some STR alleles contain partial repeats (e.g., the ATG interspersed between AATG repeats in the TH01 9.3 allele) or other sequence variation that has arisen due to mutation in the repeat region or in the nearby flanking regions [3].

Nomenclature for the designation of length-based STR alleles has been developed over the years by the DNA Commission of the International Society for Forensic Genetics (ISFG) and categorized under simple repeat, variant allele, compound repeat and complex repeat [3]. With simple repeats, the number of repeat units is counted (e.g., TH01 allele 7). While variant alleles are designated by counting the number of full repeats, adding a decimal point, and then counting the number of nucleotides (nt) in the incomplete repeat (e.g., TH01 allele 9.3 with nine full repeats plus three additional nt). For compound repeats, alleles are designated by counting the total number of full repeats (e.g., vWA allele 18 comprised of 1 TCTA + 4 TCTG + 13 TCTA repeats). With complex repeat systems, the typical approach taken is to establish a mathematical relationship to the (nt length of a consensus allele (e.g., D21S11 allele 27). For highly variable systems such as SE33, alleles may be identified according to their relative size compared to an allelic ladder containing sequenced alleles, eventhough it is understood that internal sequence variation is possible [3].

DNA typing of STRs located in the human genome has proven to be an extremely discriminating method for human identification in forensic and paternity applications for decades. It has been widely used for the identification of individuals based on their DNA characteristics [4]. In forensic applications, STR sequence data is expected to increase the effective number of alleles, which may aid mixture interpretation in some cases. More generally, this type of data has shown benefits in characterizing STR mutation rates which contributes to our foundational understanding of these loci [3]. Expansion to additional STRs while retaining connection to legacy STR profile information appears to be the way forward in the United States and Europe [5].

Single nucleotide polymorphism (SNP) can overcome the technical difficulties with the forensic use of STRs. The use of short PCR amplicons (50 nt or less) leads to a successful analysis of low amounts of highly degraded DNA, which is difficult with highly polymorphic STRs because of their repetitive sequence [6]. Furthermore, owing to the very short amplicons that can be employed in SNPs, successful SNP profiling can be obtained from degraded DNA which STR profile cannot be obtained. SNP profiling lacks artefacts that appears in STR profiles and complicates the interpretation of results specially with low amount of DNA [6]. However, challenges are also exist with the use of autosomal SNPs for human individual identification in forensic investigations. Because SNPs are bi-allelic and less polymorphic that multi allelic STRs, they are less informative in the analysis of mixtures of DNA from multiple individuals. Therefore, using higher number of SNPs may help, but the use of tri-allelic SNPs, combined with multiplex genotyping technologies may compensate for this effect [6].

Another forensic approach is using Y-STRs for male identification in mixed stain analysis. Since 1990, haplotypes from sets of male specific Y-STRs have been used for male identification [6]. The new DNA markers promise to improve human identification with the ability to identify two or more male individuals in cases of sexual assault. When male contributors are not shown in autosomal STR profiles as a result of preferential PCR amplification of the excess female contributor [6].

Several studies confirmed that Y-STR analysis plays an important role in different type of cases such as the analysis of: complex mixtures, sexual cases having samples with no spermatozoa [7], samples with multiple contributor mixtures, and mixture samples which shows no male component [8]. Commercial kits have been improved over years, and more Y- STR multiplexes have been developed [9] which provides rich information to be used in constructing phylogenetic trees and the deduction of ethnic origin [10].

### 1.1.4 Trace DNA Definition

It is important to understand what is exactly meant by the term trace DNA. Nowadays, trace DNA collected from crime scene has become a large part of the average forensic laboratories' workload. Remarkably low DNA amounts (<100 pg) have been successfully analyzed to obtain profiles from a wide range of sample types. Touched objects constitute the most common source of trace DNA, but any type of biological material present in low amounts may be considered as trace, including minute blood deposits and saliva residue on partially consumed food. In addition to supplementing existing analysis techniques in serious crime cases, trace DNA can allow investigation of volume crime cases such as burglary, vehicle theft and run over, where DNA evidence had not previously been considered usable. However, despite

the widespread use of trace DNA, at present there are very few specific validated methods. This has led to controversy in the use of trace DNA, and particularly the low copy number amplification technique. It has been established that the use of existing methodology that specifically developed for high-copy number samples, leads to significant levels of artefacts with trace DNA, including allele drop-out and drop-in, stutter, and allelic/locus imbalance. To minimize these artefacts, there are numerous modifications that can be made to existing methods to increase the success of trace DNA analysis. These include reduced extraction volumes, increased cycle number, reduced PCR volume, and increased injection time for CE [1].

Recent researches have introduced techniques such as post-PCR purification, whole genome amplification and molecular crowding which can increase success rates with trace DNA significantly. Moreover, each step starts from sample collection, extraction, amplification and fragment detection that can be optimized to trace DNA. Indeed, the use of trace DNA analysis techniques with high sensitivity must bring an increasing awareness of the potential for contamination, both within the laboratory and at crime scenes, especially with DNA traces from cold cases, which were not collected or stored with highly sensitive DNA detection techniques. Although trace DNA continues to be used within forensic biology, a wide range of practices may need to be modified to ensure accuracy and reliability [1].

### 1.1.5 DNA Inhibitors

The most common cause of PCR failure is the presence of PCR inhibitors in the samples, especially when dealing with adequate copies of DNA. It causes a great challenge for scientist analyzing biological remains recovered from the environment. Casework samples are in a high risk of containing different type of PCR inhibitory

compounds which interfere with downstream DNA typing success, resulting in imbalanced, partial or negative DNA profiles. Theoretically, the activity of the inhibitors may affect every component of PCR reaction including binding to the template DNA, the nt, the amplification primers, $Mg^{2+}$ and the Taq polymerase [11].

Degree of inhibition could have different effect, severe inhibition can lead to the loss of alleles from the larger STR loci, or complete false-negative results, which is a pattern similar to severe template degradation that can mistakenly attributed. While a slight to moderate inhibition which can result in a minor loss of alleles and misestimating of the affected sample's DNA quantity, it has potential consequences for downstream applications such as STR analysis. Generally, only larger loci are lost when the Taq is affected by the inhibitors. However, alleles may be lost regardless of amplicon size when inhibitors bind the DNA, based on where in the template the inhibitor binds [12, 13].

PCR inhibitors associated with casework samples could be humic acid in soil, hematin in red blood cells, humic substances in soil, melanin in hair and skin, myoglobin in tissue, bile salts and complex polysaccharides in feces, collagen in soft tissue and bone, polysaccharides in plants [13, 14]. Moreover, STR typing is limited by the quality of human DNA obtained from forensic casework samples that can be influenced by environmental factors which may cause different degrees of degradation, that have a negative impact on the amplification process especially of STR systems with large amplicons [15]. Amplifying of mini STRs, which provides shorter length markers, in addition of increasing the amount of classical STRs marker showed obtaining more reliable results [11].

### 1.1.6 DNA Database

Forensic scientists have become able to identify individuals with a significant discrimination power by combining DNA profiling results at several independent STR loci. The STR technology is more powerful with the establishment of centralized forensic DNA databases in many countries around the world. This is because it provides valuable information for direct matching of DNA profiles of individuals to those of DNA samples collected at crime scenes. After nearly three decades of operation, the size of the centralized DNA database continues to grow [12]. In order to reduce the number of adventitious matches and to increase international compatibility as well as the power of discrimination for criminal and missing person cases, the Federal Bureau of Investigation (FBI) expanded the combined DNA Index system (CODIS) core loci from the existing 13 to 20 [5].

### 1.1.7 Six-dye STR Kits

In 2012, a group of scientists started the first beta-testing with a six-dye STR kit prototype containing 24 loci (GlobalFiler$^{TM}$ PCR Amplification Kit) developed by Life Technologies in response to the CODIS Core Loci Working Group's recommendation to expand the CODIS Core Loci; followed by a validation study of these 24 markers in 2014 [16]. Investigator1 24plex QS Kit and Investigator1 24plex GO! Kit are other examples of six- dye STR kit containing 24 loci, developed by QIAGEN [17]. Another group of scientists proved that with the advancement of the six-dye chemistry, it is now possible to expand the number of STR systems analyzed within a single reaction, by examining the performance of another six-dye, multiplex containing 27 loci the (PowerPlex1 Fusion 6C System) developed by Promega [18].

It is worth mentioning that the six-dye kits allow analyses of a larger volume of input DNA (*i.e.*, 15 µL instead of 10 µL). The fact that they possess 8-10 mini-STR systems with amplicon sizes of less than 220 bp further improves their information recovery from heavily degraded samples [19].

In this study, an evaluation of a six-dye STR multiplex assay (VeriFiler$^{TM}$ Plus) was conducted; this assay is composed of the following markers:

- 23 autosomal STR loci (D3S1358, vWA, D16S539, CSF1PO, TPOX, D8S1179, D21S11, D18S51, D2S441, D19S433, TH01, FGA, D22S1045, D5S818, D13S317, D7S820, D10S1248, D1S1656, D12S391, D2S1338, D6S1043, Penta D, Penta E).
- 1 insertion/deletion polymorphic marker on the Y chromosome (Y indel) and Amelogenin (sex-determining marker)
- 2 internal Quality control markers (IQCL & IQCS)

Evaluation of the assay with casework samples was performed using a 3500 genetic analyzer to detect the different fragment size of alleles. This study included the analysis of different parameters and aspects; sensitivity study to determine the lowest amount of DNA required to produce a complete electropherogram, determination of analytical threshold which is the lowest relative fluorescence units (RFU) value at which DNA can be distinguished from noise, determination of stochastic threshold which is the threshold at which the analyst can be confident that if one peak for a heterozygote is above this threshold, then its sister allele will be present and should be above the analytical threshold, heterozygous balance which is the relative ratio of two alleles at a given locus, determined by dividing the peak height of an allele with a lower RFU value by the peak height of an allele with a higher RFU value, precision

and accuracy, reproducibility study is being able to obtain the same result under the same condition, genotype concordance is also conducted to assess the success rate of the assay, DNA mixtures studies is conducted to define and mimic the range of detectable mixture ratios, including detection of major and minor components, and stability study to assess the tolerance and the robustness of the assay in the presence of common type of inhibitors that could bear in casework samples.

### 1.2 Statement of the Problem

DNA samples collected from a crime scene showed to be the key element in solving crimes. However, the traces of DNA samples could be affected by the environmental factors which may lead to the loss of the evidence. This study aimed to evaluate the use of Verifiler™ Plus, a six-dye multiplex assay, with casework samples. This study will be beneficial for DNA forensic laboratories in UAE as well as other laboratories, since it enhances the success rate of obtaining positive results from challenging forensic samples.

### 1.3 Hypothesis and Objectives

This research study hypothesize that if Verifiler™ Plus is used with casework samples, success rate of obtaining positive results will increase.

The objectives of this study are:

- To measure the sensitivity, precision, accuracy and reproducibility of the Verifiler™ Plus assay,
- To study mixture sensitivity and inhibition resistance, and
- To perform a concordance study between GlobalFiler and this assay.

# Chapter 2: Methods

## 2.1 Research Design

### 2.1.1 Human DNA Samples

- AmpFLSTR™ Control DNA 007 sourced from Thermo Fisher Scientific (South San Francisco, CA), and set of three control DNA SRM 2391c purchased from National institute of standard techniques (NIST).
- A set of 32 consented samples, 16 blood samples and 16 buccal swabs was prepared. Samples were extracted using PrepFiler™ Express Forensic DNA Extraction Kit (Thermo Fisher Scientific) and the AutoMate Express™ Nucleic Acid Extraction System (Thermo Fisher Scientific). The DNA was quantified using the Quantifiler™ Human DNA Quantification Kit on an Applied Biosystems™ 7500 Real-Time PCR System (Thermo Fisher Scientific).
- Pre-extracted casework samples, previously analyzed using GlobalFiler™ PCR Amplification Kit were used.

### 2.1.2 Pre PCR Sample Preparation

#### 2.1.2.1 Sensitivity Experiments and Determination of Stochastic Thresholds

A serial dilution of Control DNA 007 (Thermo Fisher Scientific, South San Francisco, CA, USA) was amplified using the six-dye multiplex assay (Verifiler™ Plus). Four replicate reactions were performed and analyzed with the following inputs: 1000, 750, 500, 250, 125, 63, 32, 16 and 8 pg DNA. Non-template controls (NTCs) were run. Amplified product was electrophoresed on the 3500 instrument and analyzed with GeneMapper™ ID-X 1.4 software. 11 PCR negative controls from multiple

injections were analyzed across the six channels in the range 60–450 base pairs to determine the analytical threshold.

#### 2.1.2.2 Peak Height Ratio

Data from sensitivity study was used to calculate the heterozygote balance (HB) in each dye individually and then all together.

#### 2.1.2.3 Reproducibility and Allele Call Accuracy

A total of 34 allelic ladder from two different instruments across multiple injections were analyzed, and the average base pair (bp) size and standard deviation (SD) of each allele in the allelic ladder was calculated.

#### 2.1.2.4 DNA Mixture Study

DNA mixtures was prepared using two male DNA control provided with standard SRM 2391c (component B and C) in ratios of 1:1, 3:1, 7:1, 10:1, 15:1 and vice versa. Mixtures were amplified in triplicate. Electrophoreses was performed for the amplified product on the 3500 instrument and analyzed with GeneMapper™ ID-X 1.4 software.

#### 2.1.2.5 Stability Study

Hematin porcine (Sigma) was prepared at a concentration of 0.6 mM and added to the PCR component in the following amount: 1µl, 1.5 µl, 3.125 µl, 6.25 µl, 9.5 µl and 12.5 µl. Humic acid (Sigma) was dissolve in water at a concentration of: 200 ng/µl, 150 ng/µl, 100 ng/µl, 80 ng/µl, 60 ng/µl. Test was performed in triplicates.

### 2.1.2.6 Concordance Study

Standard Reference material SRM-2391c derived from 2 single male donor and 1 female donor (component A, B and C) produced and certified by the National Institute of Standards and Technology (MD, USA) were amplified, and allele calls were analyzed against the certified STR allele values. A total of 32 consented samples (Ref: DPSC-2019-EA-018), 16 blood samples and 16 buccal swabs were amplified, and allele calls were analyzed against profiles from the same set of samples previously analyzed using GlobalFiler$^{TM}$ Amplification kit.

### 2.1.2.7 Casework Samples

A total of 8 bone samples (Bn), 7 swabs of bottle (Bt), 5 cigarette butts (CB), 1 plug, 1 blood from the floor along with a human tissue sample (Ref: DPSC-2019-EA-018), previously analyzed using GlobalFiler$^{TM}$ Amplification kit, were used to test the applicability of the multiplex on casework samples. Controls were amplified along with casework samples; amplification reaction was performed with final concentrations mentioned in Table 1. PCR was programmed as recommended by manufacturer.

Table 1: Amount of sample needed for PCR reaction

| **Sample** | **Add** |
|---|---|
| **Negative control** | 17.5 µL of nuclease-free water |
| **Test Sample** | 17.5 µL of DNA (500 pg) |
| **Positive control** | Combined, then added to the reaction tube:<br>• 5 µL of DNA Control 007 (0.1 ng/µL)<br>• 12.5 µL of nuclease-free water |
| **Master Mix** | 5.0 µL |
| **Primer set** | 2.5 µL |

## 2.2 PCR Amplification and Thermal Cycling Conditions

The Master Mix and Primer Set were vortexed for 3 seconds and the tubes were centrifuged briefly. Total of 5.0 µL of Master Mix and 2.5 µL of Primer set per sample were pipetted into 1.5 ml tube and the reaction mixture was vortexed for 3 seconds, then centrifuged briefly and 7.5 µL of reaction mixture was dispensed into each MicroAmp™ tube. Samples were prepared and added to the appropriate tube (the final reaction volume is 25 µL). The sample input amount volume was adjusted as needed to reach DNA input amount of 500 pg. The solution was mixed until it was homogenous and each tube was centrifuged at 3,000 rpm for approximately 30 seconds in a tabletop centrifuge.

The thermal cycling conditions was programmed as recommended by manufacturer (Table 2). Sample tubes were loaded into the thermal cycler, the heated cover was closed, then the run was started.

Table 2: Thermo cycler program for the assay

| Initial incubation | First stage (2cycles) | | Second stage (27 cycles) | | Final extension | Final hold |
|---|---|---|---|---|---|---|
| | **Denature** | **Anneal/ extend** | **Denature** | **Anneal/ extend** | | |
| **Hold** | Cycle (29 Cycle) | | | | Hold | Hold |
| **95°C** | 96°C | 62°C | 96°C | 59°C | 60°C | 4°C |
| **1 min** | 10 sec | 90 sec | 10 sec | 90 sec | 5 min | ∞ |

## 2.3 Capillary Electrophoresis (CE)

9.5 µL of Hi-DiTM Formamide, 0.5 µL of GeneScan$^{TM}$-600 LIZ size standard (both from Life Technologies) and 1 µL of amplified sample was added into each well. Samples were denatured for 3 min at 95° C. CE was performed on the 3500 (8-

capillary) genetic analyzer (Life Technologies). Data were collected using the 3500 Data Collection Software v.2.0 and HID files generated with the 3500 Data Collection Software v.2.0 was analysed using GeneMapper1 ID-X Software v1.4.

# Chapter 3: Results

## 3.1 Sensitivity Experiments and Determination of Thresholds

Scientist conduct sensitivity tests for number of reasons including the need to determine the lowest amount of DNA required to produce a complete electropherogram. Different amount of control DNA 007 (Thermo Fisher Scientific) was used for sensitivity study.

To assess this test the percentage of allele call was calculated. Full STR profiles (100% of allele call) were obtained from control DNA 007 (Thermo Fisher Scientific) samples ranging from 1000 pg down to 63 pg (Figure 1), and drop out was detected starting from 32 pg recovering 95% of expected alleles.

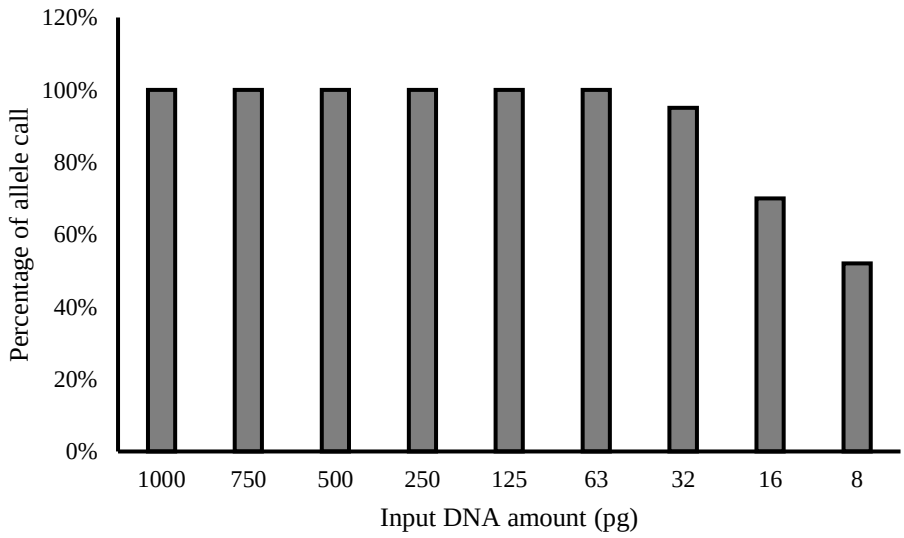


Figure 1: Percentage of allele call detected in sensitivity test of template DNA 007 ranging from 1000-8 pg, n = 27

Figure 1 illustrates the percentage of allele call resulted from the following DNA input of control DNA 007: 1000, 750, 500, 250, 125, 63, 32, 16 and 8 pg. 70% and 52% of all expected alleles were recovered at 16 pg and 8 pg respectively.

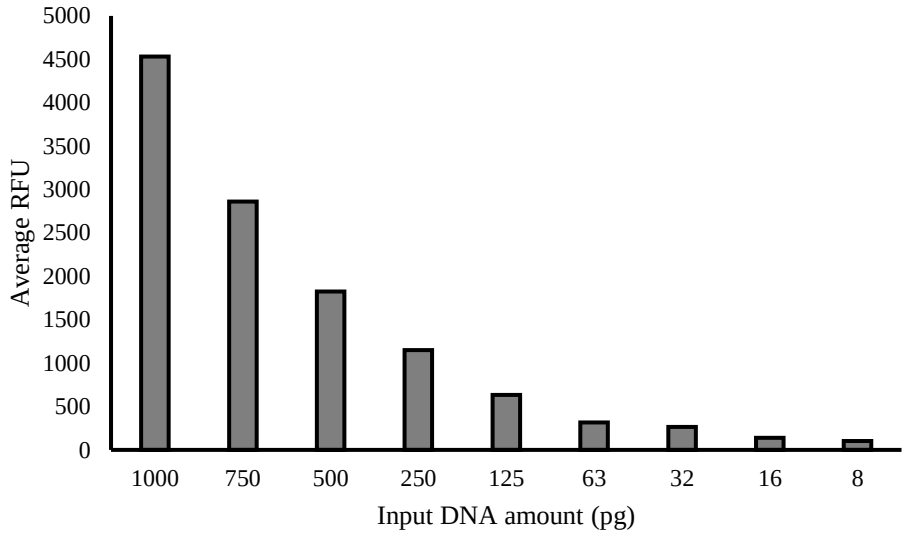


Figure 2: Average peak height of control DNA 007 ranging from 1000-8 pg in sensitivity test, n = 27

Overall, as the DNA input decrease, the average peak height of the alleles also decrease (Figure 2); indicating decrease of the quality and the completeness of the profiles. This result shows well correlation between the signal height and the serially diluted template DNA.

Table 3: Illustration of allele dropout in template DNA input ranging from 1000-8 pg

| Marker | DNA Template Amount | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1000 pg | 750 pg | 500 pg | 250 pg | 125 pg | 63 pg | 32 pg | 16pg | 8pg |
| D3S1358 | | | | | | | | | |
| vWA | | | | | | | | | |
| D16S539 | | | | | | | | | |
| CSF1PO | | | | | | | | | |
| D6S1043 | | | | | | | | | |
| Yindel | | | | | | | | | |
| AMEL | | | | | | | | | |
| D8S1179 | | | | | | | | | |
| D21S11 | | | | | | | | | |
| D18S51 | | | | | | | | | |
| D5S818 | | | | | | | | | |
| D2S441 | | | | | | | | | |
| D19S433 | | | | | | | | | |
| FGA | | | | | | | | | |
| D10S1248 | | | | | | | | | |
| D22S1045 | | | | | | | | | |
| D1S1656 | | | | | | | | | |
| D13S317 | | | | | | | | | |
| D7S820 | | | | | | | | | |
| Penta E | | | | | | | | | |
| Penta D | | | | | | | | | |
| TH01 | | | | | | | | | |
| D12S391 | | | | | | | | | |
| D2S1338 | | | | | | | | | |
| TPOX | | | | | | | | | |

Note: Green wells represent full allele call, yellow wells represent allele drop out.

The number of dropouts increase with decreasing the input amount of DNA (Table 3). Profiles generated from 32 pg of DNA input showed two allele dropouts, detected in D3S1358 (Blue Dye) and D8S1179 (Green Dye). For 16 pg of DNA input, allele dropout was detected in the Blue Dye (CSF1PO, D6S1043), Green Dye (D18S51, D5S818), Yellow Dye (D2S441, D10S1248) and in the Purple Dye (D12S391). Total of 11 allele dropout was detected in the 5 dyes of profiles generated from 8 pg DNA template (Table 3). No locus dropout was detected. A number of pull up peaks was also detected in samples with DNA input > 500 pg, and were clearly

distinguishable from the true allele peaks, which points to the optimum range to be determined at 125 - 500 pg. All the alleles in those profiles remained balanced and was not affected with the pull up peaks.

Analytical threshold (AT) is the minimum height requirement, above which detected peaks can be reliably distinguished from background noise; peaks above this threshold are generally not considered noise and are either artifacts or true alleles. It was calculated using the following formula: AT= 2 x (Max. PH – Min. PH) [22]. To calculate AT two different set of samples were used to calculate an acurate AT for casework samples.

The first set is composed of 11 negative controls from multiple injections, where the analysis of baseline noise was conducted, and the AT calculated from these set was (36.8 RFU) (Table 4) which is concicerd to be very low AT and might cause an increase in the baseline noise if a mass of DNA amount (>1 ng) was amplified. Alot of pull ups to be called specially with strong DNA input samples. However this low threshold can be usefull with casework sampls of low DNA amount.

Table 4: Analytical threshold (AT) calculated from negative control samples

| Dye | Max. PH | Min. PH | AT= 2 * (Max. PH – Min. PH), | Average AT |
|---|---|---|---|---|
| **blue** | 19 | 2 | 34 | **36.8** |
| **Green** | 20 | 4 | 32 | |
| **yellow** | 26 | 1 | 50 | |
| **red** | 17 | 2 | 30 | |
| **Purple** | 22 | 3 | 38 | |

Max. PH: Maximum peak height; Min. PH: Minimum peak height

The second set composed of positive control samples from sensitivity study. Calls for all true alleles and artefactes were removed, and the minimum and maximum peak heigt of the identified baseline noise was calculated in each dye channel. The average AT for all dyes is 76.8 RFU (Table 5), so the overall analytical threshold was set to 80 RFU.

Table 5: Analytical threshold (AT) calculated from sensitivity study

| Dye | Max. PH | Min. PH | AT= 2 * (Max. PH – Min. PH) | Average AT |
|---|---|---|---|---|
| **Blue** | 30 | 1 | 58 | **76.8** |
| **Green** | 33 | 3 | 60 | |
| **Yellow** | 37 | 2 | 70 | |
| **Red** | 60 | 3 | 114 | |
| **Purple** | 45 | 4 | 82 | |

Max. PH: Maximum peak height; Min. PH: Minimum peak height

The stochastic threshold (ST) is the threshold at which the analyst can be confident that if one peak for a heterozygote is above this threshold, then its sister allele will be present and should be above the analytical threshold; it is the peak height value above which it is reasonable to assume that, at a given locus, allelic dropout of a sister allele has not occurred. This can be described as the limit of quantification (LOQ). The stochastic threshold was calculated using the following formula: Average peak height + (3 x SD). It was calculated by examining the peak height of heterozygote loci where one sister allele has dropped below the analytical threshold. The highest peak height recorded was 179 RFU and the overall stochastic threshold was set to 180 RFU (Table 6).

Table 6: Stochastic threshold (ST) calculation

| Average in all dye channel | SD | ST= Average Peak Height + (3* SD) | ST |
|---|---|---|---|
| **113.5** | 22.14 | 179.9 | 180 |

SD: Standard deviation.

### 3.2 Heterozygote Balance (HB)

The HB is the relative ratio of two alleles at a given locus, as determined by dividing the peak height of an allele with a lower RFU value by the peak height of an allele with a higher RFU value, and then multiplying this value by 100 to express the PHR as a percentage. HB is used as an indication of which alleles may be heterozygous pairs and also in mixture deconvolution.

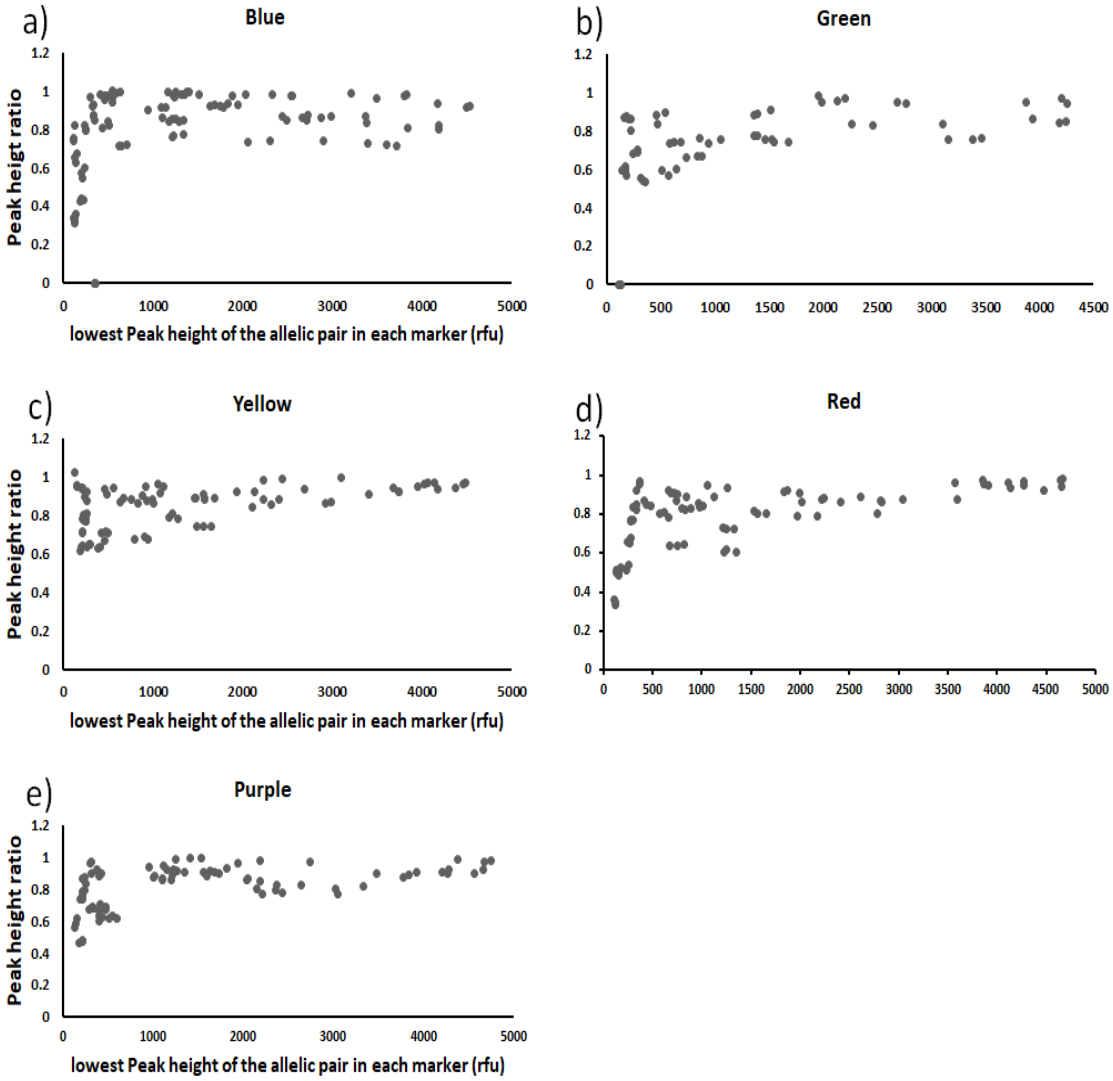


Figure 3: Peak height ratio for each dye channel observed in sensitivity study (a) blue dye channel, (b) green dye channel, (c) yellow dye channel, (d) red dye channel, (e) purple dye channel

HB was calculated individually for each dye (Figure 3). According to the recommendations of ENFSI [23, 24] the peak balance ratio of heterozygote alleles should be > 60%. PHR was below 60% when the peak height of the allelic pair was lower than 250 RFU in the blue, green, red and purple dye (Figure 3 a, b, d, e) showing that heterozygous peak height ratios decreased towards lower template amounts. All the alleles recorded 60% PHR in the yellow dye (Figure 3c).

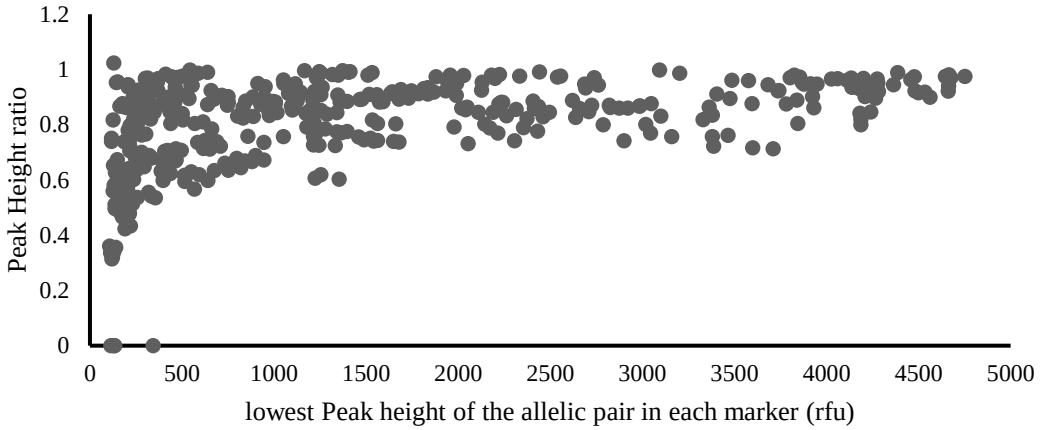


Figure 4: Heterozygote balance average in all dye channels

The overall peak height ratio in all markers is illustrated in Figure 4. The average peak height ratio varied between 99- 60% which meets the recommendation of ENFSI. Overall, HB increase toward higher allele peak heights.

Peak height ratio comparison was done between DNA profiles with 500 pg DNA input, which is considered to be the intermediate amount of DNA input, and DNA profile with 16 pg DNA input (low amount of DNA) using t-test. A p- value = $5.56343E^{-09}$ was obtained which is significantly less than α (0.05), and t-stat = 6.761035004 (Table 7). This result provides strong evidence that the amount of input DNA affects the peak height ratio balance producing lower ratios toward lower DNA input. This result might help the scientist to make correct decision about profiles generated from low template DNA samples.

Table 7: T-test comparing HB between 16 pg and 500 pg DNA input

| | 16 pg DNA | 500 pg DNA |
|---|---|---|
| **Mean** | 0.603072681 | 0.840966 |
| **Variance** | 0.03296552 | 0.006652 |
| **Observations** | 32 | 32 |
| **Pooled Variance** | 0.019808752 | |
| **Hypothesized Mean Difference** | 0 | |
| **df** | 62 | |
| **t Stat** | -6.761035004 | |
| **P(T<=t) one-tail** | 2.78172E-09 | |
| **t Critical one-tail** | 1.669804163 | |
| **P(T<=t) two-tail** | 5.56343E-09 | |
| **t Critical two-tail** | 1.998971517 | |

### 3.3 Reproducibility

Reproducibility is the test of being able to obtain the same result under the same condition. To test for reproducibility of genotyping results across different sites and instruments, 500 pg as input DNA (Table 8) of Control DNA 007 was amplified in 6 replicates, and the amplified product was analyzed at two sites.

Table 8: Allele call of control DNA 007 replicates

| **Marker** | **Allele call of each sample** | | | | | |
|---|---|---|---|---|---|---|
| | site A | | | site B | | |
| | 1st injection | 2nd injection | 3rd injection | 4th injection | 5th injection | 6th injection |
| **D3S1358** | 15,16 | 15,16 | 15,16 | 15,16 | 15,16 | 15,16 |
| **vWA** | 14,16 | 14,16 | 14,16 | 14,16 | 14,16 | 14,16 |
| **D16S539** | 9,10 | 9,10 | 9,10 | 9,10 | 9,10 | 9,10 |
| **CSF1PO** | 11,12 | 11,12 | 11,12 | 11,12 | 11,12 | 11,12 |
| **D6S1043** | 12,14 | 12,14 | 12,14 | 12,14 | 12,14 | 12,14 |
| **Yindel** | 2 | 2 | 2 | 2 | 2 | 2 |
| **AMEL** | XY | XY | XY | XY | XY | XY |
| **D8S1179** | 12,13 | 12,13 | 12,13 | 12,13 | 12,13 | 12,13 |
| **D21S11** | 28,31 | 28,31 | 28,31 | 28,31 | 28,31 | 28,31 |
| **D18S51** | 12,15 | 12,15 | 12,15 | 12,15 | 12,15 | 12,15 |
| **D5S818** | 11 | 11 | 11 | 11 | 11 | 11 |
| **D2S441** | 14,15 | 14,15 | 14,15 | 14,15 | 14,15 | 14,15 |
| **D19S433** | 14,15 | 14,15 | 14,15 | 14,15 | 14,15 | 14,15 |
| **FGA** | 24,26 | 24,26 | 24,26 | 24,26 | 24,26 | 24,26 |
| **D10S1248** | 12,15 | 12,15 | 12,15 | 12,15 | 12,15 | 12,15 |
| **D22S1045** | 11,16 | 11,16 | 11,16 | 11,16 | 11,16 | 11,16 |
| **D1S1656** | 13,16 | 13,16 | 13,16 | 13,16 | 13,16 | 13,16 |
| **D13S317** | 11,11 | 11,11 | 11,11 | 11,11 | 11,11 | 11,11 |
| **D7S820** | 7,12 | 7,12 | 7,12 | 7,12 | 7,12 | 7,12 |
| **Penta E** | 7,12 | 7,12 | 7,12 | 7,12 | 7,12 | 7,12 |
| **Penta D** | 11,12 | 11,12 | 11,12 | 11,12 | 11,12 | 11,12 |
| **TH01** | 7,9.3 | 7,9.3 | 7,9.3 | 7,9.3 | 7,9.3 | 7,9.3 |
| **D12S391** | 18,19 | 18,19 | 18,19 | 18,19 | 18,19 | 18,19 |
| **D2S1338** | 20,23 | 20,23 | 20,23 | 20,23 | 20,23 | 20,23 |
| **TPOX** | 8,8 | 8,8 | 8,8 | 8,8 | 8,8 | 8,8 |

### 3.4 Allele Call Accuracy and Size Precision

To measure the degree of variation in each fragment size, a total of 34 replicates of allelic ladder from multiple injections was run on two 3500 Genetic analyzer instruments.

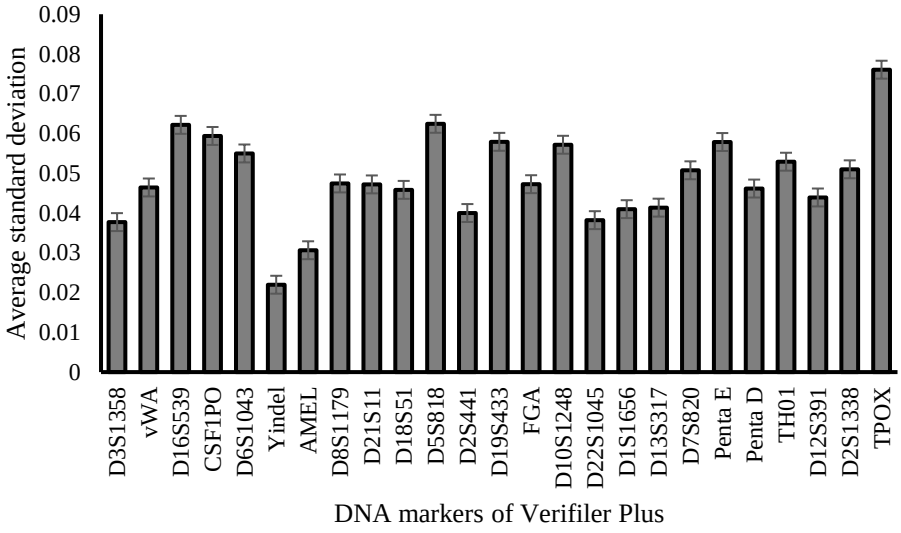


Figure 5: Average SD of allele size in each marker, n= 34

The average SD of all allele size (bp) in every loci between all replicates varied from 0.02 to 0.08 bp (Figure 5), which is within the recommended ± 0.5 bp window for genotyping as it is mentioned by ENFSI (2010) [23].

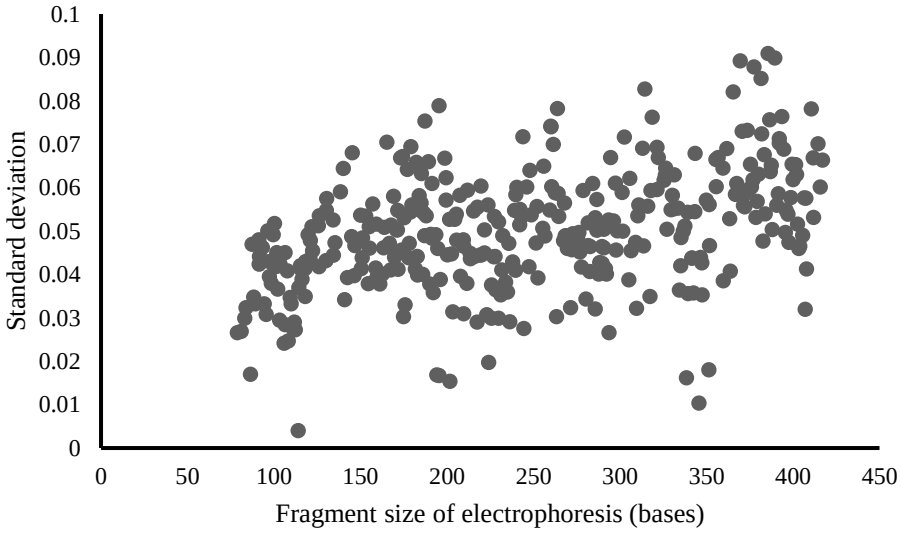


Figure 6: Sizing precision testing of allelic ladder across 12 injections, n= 34

No dropout was observed in any allelic ladder. Size variation increases with larger fragment size (Figure 6). Average length and SDs were determined for each allelic ladder fragment across all dye channels (Figures 7-11).

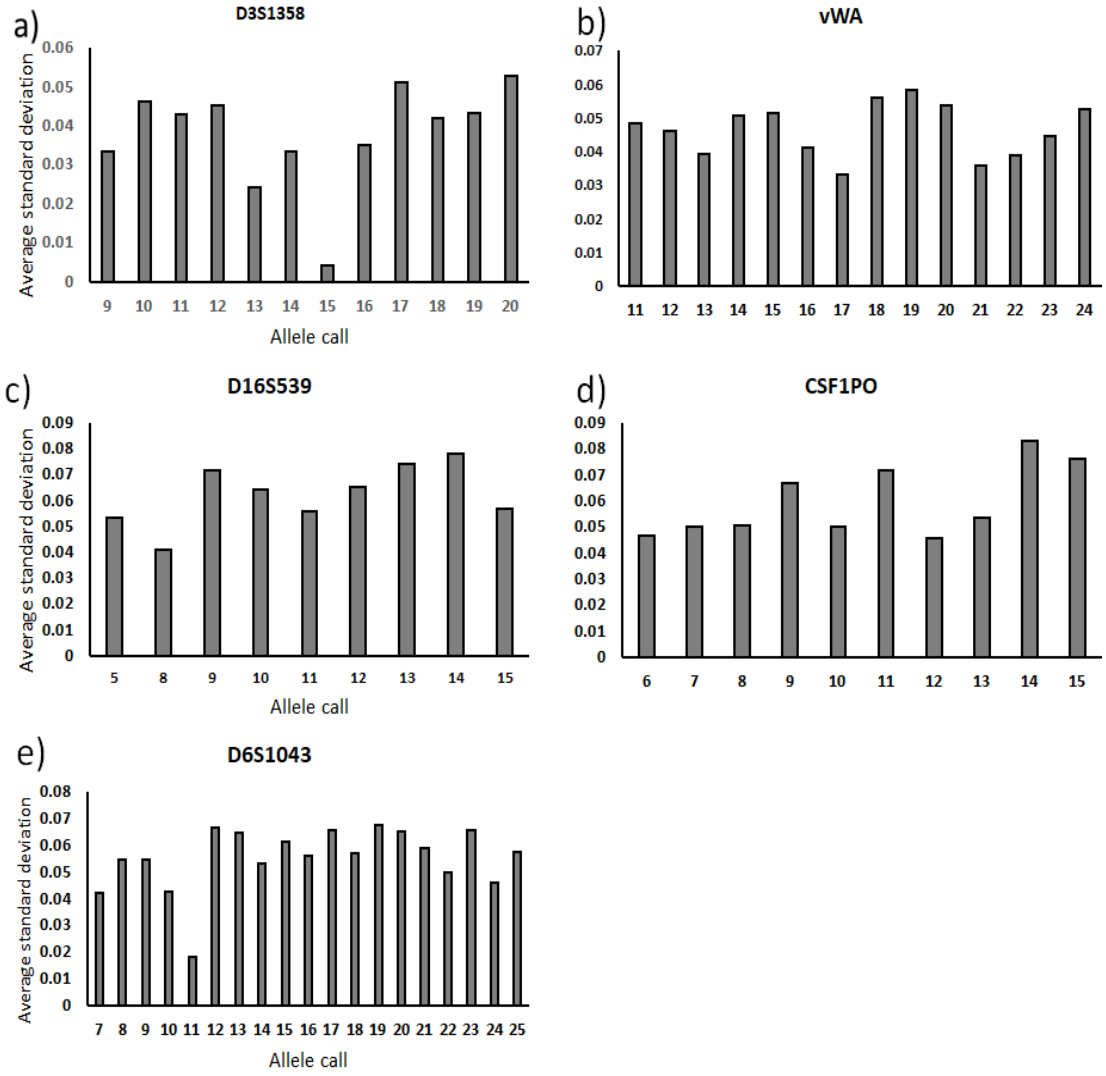


Figure 7: Allele range and detection size precision for each marker in the blue dye

Figure 7a illustrates the minimum SD which was observed for D3S1358 allele 15. While average SD of each fragment size varies between 0.02 bp to 0.08 bp among the 5 markers in the blue dye channel (Figure 7).

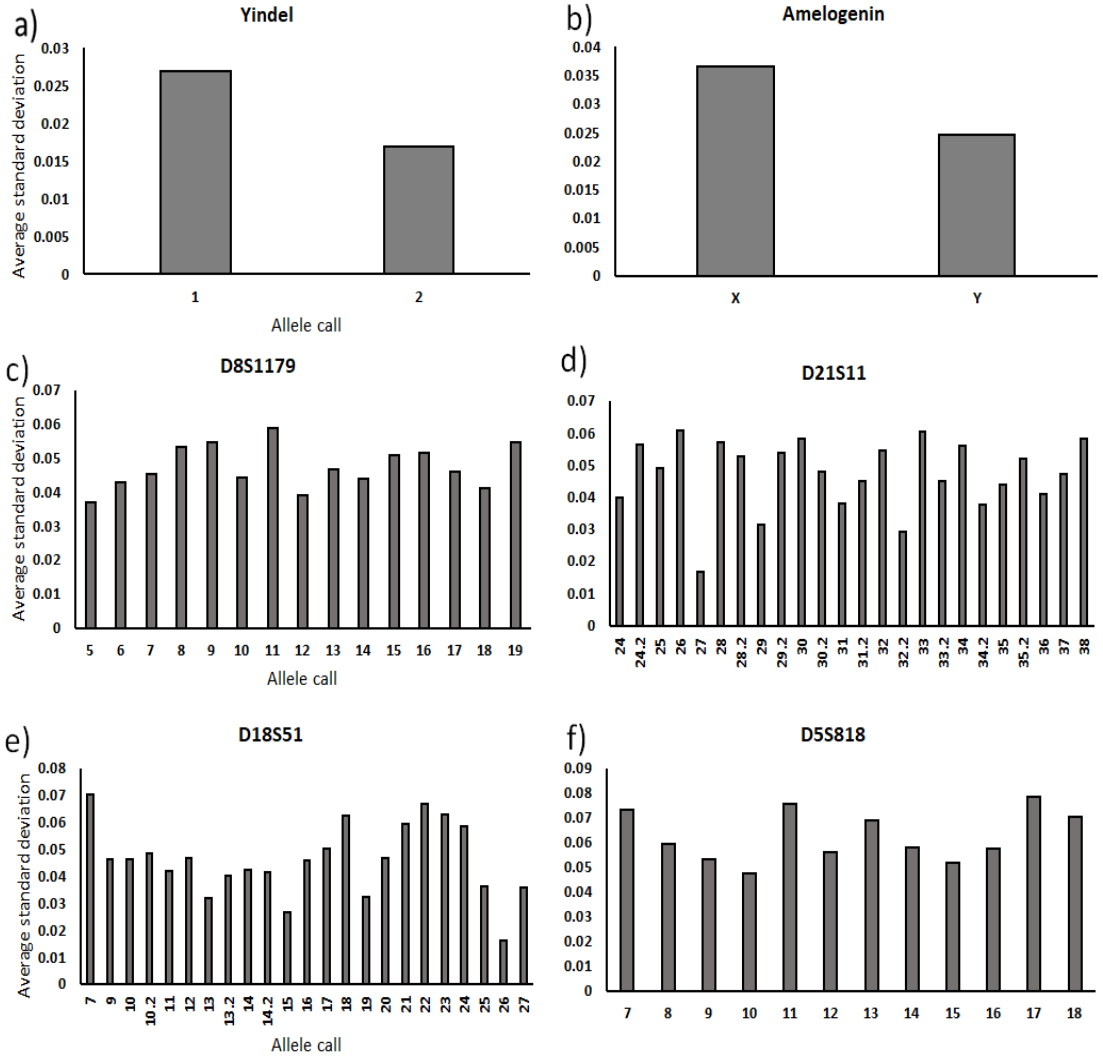


Figure 8: Allele range and detection size precision for each marker in the green dye

Figure 8 illustrates the average SD of each fragment size in 6 markers of green dye channel which varies between 0.02 bp to 0.08 bp.

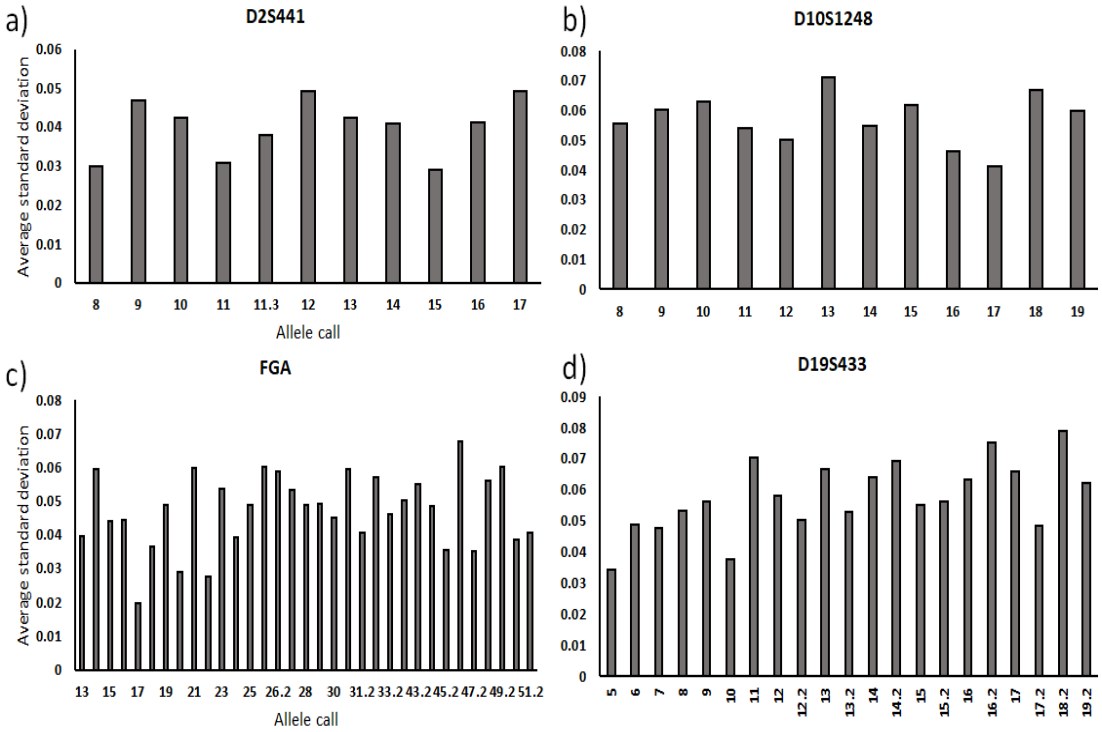


Figure 9: Allele range and detection size precision for each marker in the yellow dye

Figure 9 illustrates the average SD of each fragment size in 4 markers of yellow dye channel which varies between 0.03 bp and 0.08 bp.

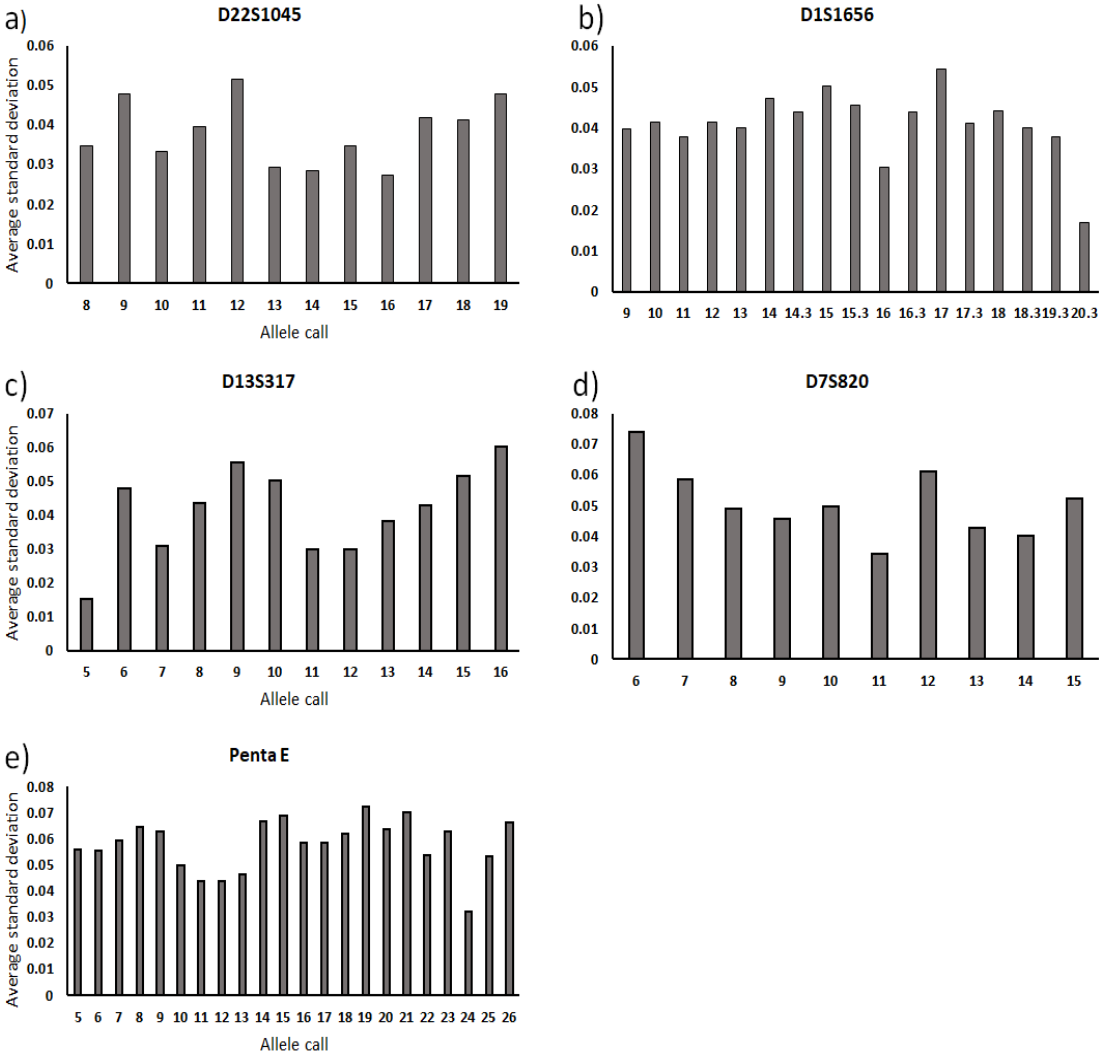


Figure 10: Allele range and detection size precision for each marker in the red dye

Figure 10 illustrates the average SD of each fragment size varies between 0.02 bp to 0.08 bp among the 5 markers in the blue dye channel.

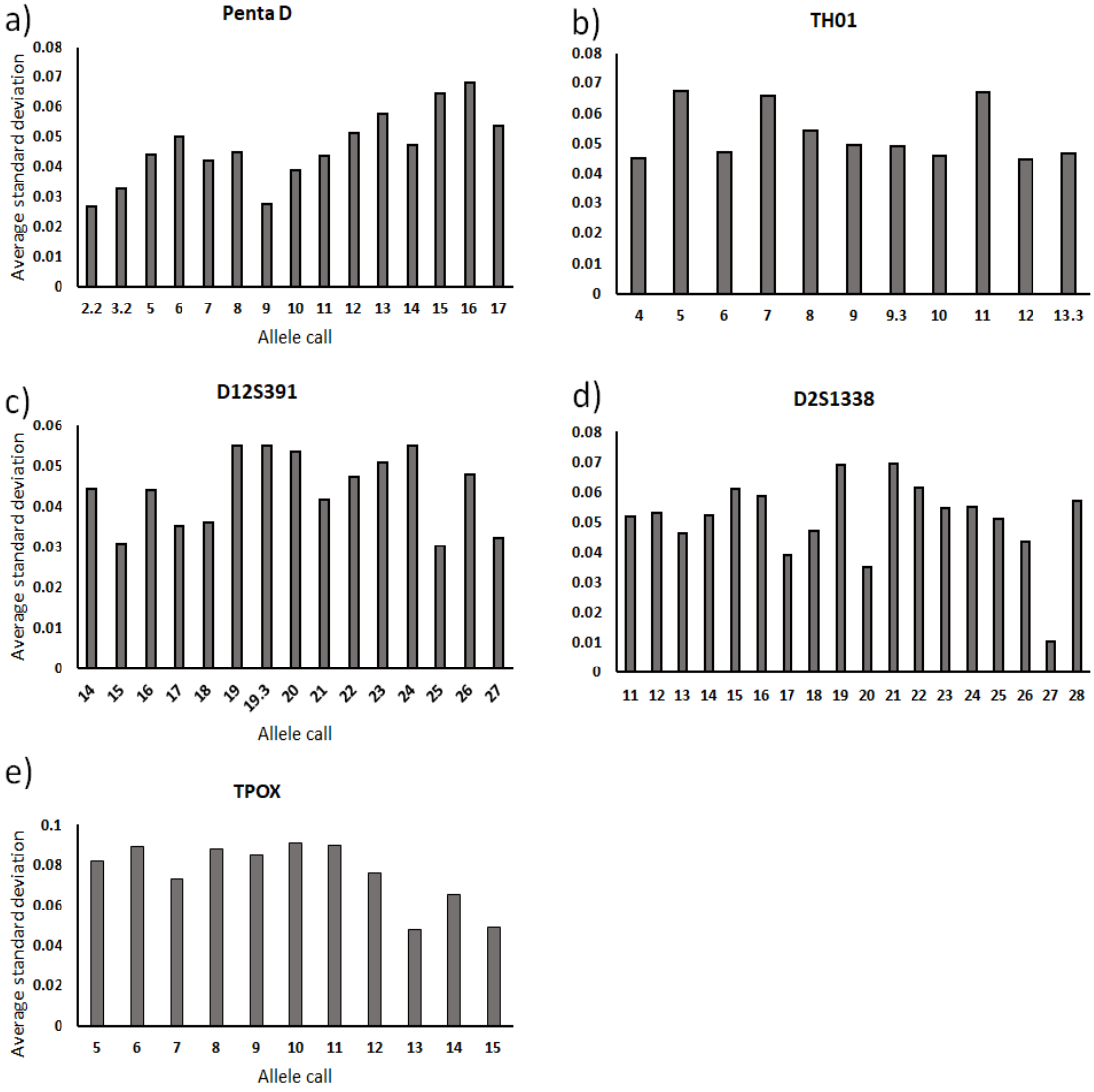


Figure 11: Allele range and detection size precision for each marker in the purple dye

Figure 11c illustrates the maximum SD which was observed for TPOX allele 10 in the purple dye channel. While the average SD of each fragment size in this dye channel varies between 0.02 bp and 0.09 bp (Figure 11).

ANOVA test was performed to compare the average size of each fragments in allelic ladder used in the precision study. Results are summarized in Table 9. F- value is less than F-critical, confirming that there is no significant difference between the mean value of fragment size in the selected allelic ladders. P-value =1 which is greater

than the confidence interval α= 0.05, which also shows that there is no significant difference between the mean value.

Table 9: ANOVA test comparing 11 allelic ladders (AL) from one instrument

| **SUMMARY** | | | | | | |
|---|---|---|---|---|---|---|
| **Groups** | Count | Sum | Average | Variance | | |
| **AL1** | 362 | 89148.79 | 246.2674 | 9122.487 | | |
| **AL2** | 362 | 89166.62 | 246.3166 | 9125.084 | | |
| **AL3** | 362 | 89168.78 | 246.3226 | 9120.824 | | |
| **AL4** | 362 | 89161.4 | 246.3022 | 9120.676 | | |
| **AL5** | 362 | 89152.5 | 246.2776 | 9119.444 | | |
| **AL6** | 362 | 89152.62 | 246.278 | 9117.448 | | |
| **AL7** | 362 | 89156.5 | 246.2887 | 9120.728 | | |
| **AL8** | 362 | 89157.27 | 246.2908 | 9121.239 | | |
| **AL9** | 362 | 89155.43 | 246.2857 | 9118.195 | | |
| **AL10** | 362 | 89157.66 | 246.2919 | 9119.563 | | |
| **AL11** | 362 | 89165.3 | 246.313 | 9124.789 | | |
| | | | | | | |
| **ANOVA** | | | | | | |
| **Source of Variation** | SS | df | MS | F | P-value | F crit |
| **Between Groups** | 1.12356 | 10 | 0.112356 | 1.23E-05 | 1 | 1.833081 |
| **Within Groups** | 36219302 | 3971 | 9120.952 | | | |
| | | | | | | |
| **Total** | 36219303 | 3981 | | | | |

Average SD of the fragment size was calculated for the 34 allelic ladders from multiple injection in two different instruments and the results (Table 10) support the finding in Table 9. F- value (0.000231) is less than F-critical (1.43732) and *P* value = 1, proving the consistency and the accuracy fragment migration among different injections.

Table 10: ANOVA test comparing 34 allelic ladders (AL) from 2 instruments

| **SUMMARY** | | | | |
|---|---|---|---|---|
| **Groups** | Count | Sum | Average | Variance |
| **AL 1** | 362 | 89148.79 | 246.2674 | 9122.487 |
| **AL 2** | 362 | 89166.62 | 246.3166 | 9125.084 |
| **AL 3** | 362 | 89168.78 | 246.3226 | 9120.824 |
| **AL 4** | 362 | 89161.4 | 246.3022 | 9120.676 |
| **AL 5** | 362 | 89152.5 | 246.2776 | 9119.444 |
| **AL 6** | 362 | 89152.62 | 246.278 | 9117.448 |
| **AL 7** | 362 | 89156.5 | 246.2887 | 9120.728 |
| **AL 8** | 362 | 89157.27 | 246.2908 | 9121.239 |
| **AL 9** | 362 | 89155.43 | 246.2857 | 9118.195 |
| **AL 10** | 362 | 89157.66 | 246.2919 | 9119.563 |
| **AL 11** | 362 | 89165.3 | 246.313 | 9124.789 |
| **AL 12** | 362 | 89130.22 | 246.2161 | 9124.238 |
| **AL 13** | 362 | 89135.45 | 246.2305 | 9122.788 |
| **AL 14** | 362 | 89084.85 | 246.0907 | 9127.425 |
| **AL 15** | 362 | 89135.44 | 246.2305 | 9126.821 |
| **AL 16** | 362 | 89140.93 | 246.2457 | 9126.125 |
| **AL 17** | 362 | 89084.97 | 246.0911 | 9130.639 |
| **AL 18** | 362 | 89165.8 | 246.3144 | 9124.826 |
| **AL 19** | 362 | 89134.89 | 246.229 | 9119.312 |
| **AL 20** | 362 | 89149.51 | 246.2694 | 9121.287 |
| **AL 21** | 362 | 89154.69 | 246.2837 | 9124.201 |
| **AL 22** | 362 | 89136.38 | 246.2331 | 9120.749 |
| **AL 23** | 362 | 89146.91 | 246.2622 | 9125.475 |
| **AL 24** | 362 | 89138.05 | 246.2377 | 9120.773 |
| **AL 25** | 362 | 89154.29 | 246.2826 | 9117.779 |
| **AL 26** | 362 | 89180.4 | 246.3547 | 9115.658 |
| **AL 27** | 362 | 89102.77 | 246.1402 | 9130.976 |
| **AL 28** | 362 | 89140.33 | 246.244 | 9118.91 |
| **AL 29** | 362 | 89174.27 | 246.3378 | 9118.941 |
| **AL 30** | 362 | 89185.58 | 246.369 | 9117.593 |
| **AL 31** | 362 | 89101.27 | 246.1361 | 9128.724 |
| **AL 32** | 362 | 89190.23 | 246.3819 | 9120.295 |
| **AL 33** | 362 | 89103.56 | 246.1424 | 9131.32 |
| **AL 34** | 362 | 89090.07 | 246.1052 | 9130.612 |

| **ANOVA** | | | | | | |
|---|---|---|---|---|---|---|
| **Source of Variation** | SS | df | MS | F | P-value | F crit |
| **Between Groups** | 69.41863 | 33 | 2.103595 | 0.000231 | 1 | 1.43732 |
| **Within Groups** | 1.12E+08 | 12274 | 9122.822 | | | |
| | | | | | | |
| **Total** | 1.12E+08 | 12307 | | | | |

### 3.5 DNA Mixture Study

Mixture study is conducted to define and mimic the range of detectable mixture ratios, including detection of major and minor components. To assess the ability to resolve minor contributor alleles with the assay, a study was performed with nine mixture sample of male: male at different ratios (1:1, 3:1, 7:1, 10:1, 15:1 and vice versa) (Appendix 1) using standard SRM 2391c (component B and C). The total amount of mixed DNA used in this study was 500 pg (Table 11); a 15:1 mixture thus contains 30 pg of the minor component DNA and 470 pg of the major component. Samples were run in replicates of 4. The limit of detection of the minor component was determined by analyzing non-overlapping alleles of both DNAs.

Table 11 illustrates the % of detected alleles in each mixture. Full STR profile was obtained until the 1:3 ratio (130 pg for the minor contributor). 98% of expected alleles were identified for minor components of 1:7, 7:1 and 10:1 mixture, and 96% for 1:10 mixture. For 15:1 91% of the minor component alleles were identified.

Table 11: Mixture study

| Mixture ratio | Total DNA input (pg) | Number of detected alleles in the mixture | % of detected alleles in the mixture |
|---|---|---|---|
| **1:15** | 30 / 470 | 55/59 | 93 |
| **1:10** | 43 /457 | 57/59 | 96.6 |
| **1:7** | 63 / 437 | 58/59 | 98 |
| **1:3** | 130 / 370 | 59/59 | 100 |
| **1:1** | 250 / 250 | 59/59 | 100 |
| **3:1** | 370 / 130 | 59/59 | 100 |
| **7:1** | 437/ 63 | 58/59 | 98 |
| **10:1** | 457 / 43 | 58/59 | 98 |
| **15:1** | 470 / 30 | 54/59 | 91.5 |

### 3.6 Stability Study

Two set of different PCR inhibitors was used to assess the tolerance of Verifiler™ Plus and 500 pg of control DNA 007 was used as a template. PCR was performed under standard conditions. Performance levels were assessed based on the number of alleles correctly called.

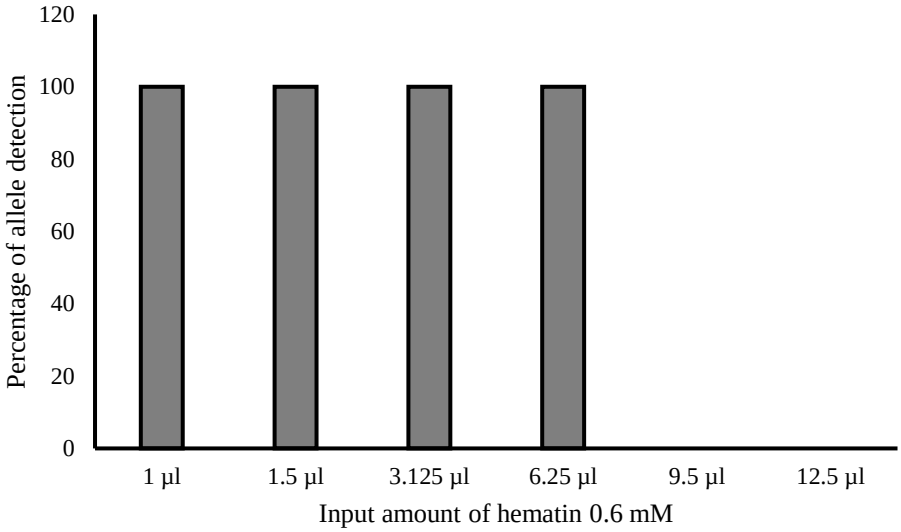


Figure 12: The effect of hematin porcine 0.6 mM on allele detection of DNA control 007

In the first set, hematin porcine (0.6mM) was added to the PCR reactions in the following amounts: 1, 1.5, 3.125, 6.25, 9.5 and 12.5 µl. Full profile were generated in the presence of 6.25 µl, with no alleles being detected in the presence of 9.5 µl and 12.5 µl (Figure 12).

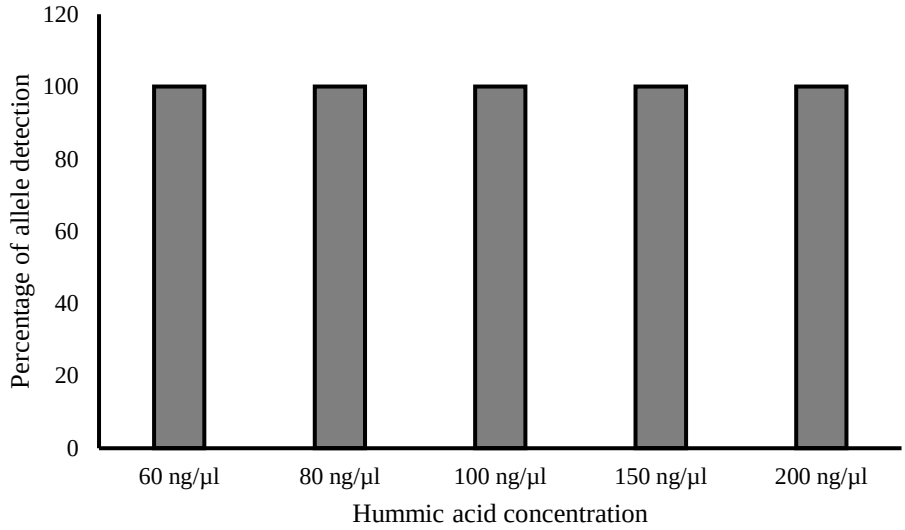


Figure 13: The effect of humic acid on allele detection of DNA control 007

In another set of experiment, humic acid was added in different concentrations ranging from 60 - 200 ng/µl. Full profiles were generated for all concentrations tested (up to 200 ng/µl) (Figure 13).

### 3.7 Success Rate and Concordance

Concordance study was conducted to assess the success rate of the assay. A total of 16 blood samples on FTA card, which were directly amplified, and another 16 buccal swabs along with 3 samples from the Standard Reference Materials 2391c (component A, B and C) were analyzed using this multiplex. The success rate was 100%. All genotyping was performed with GeneMapper *ID-X* v1.4 software. Data tables were exported into Microsoft Excel and compared to data generated previously with the Applied Biosystem GlobalFiler$^{TM}$ PCR kit. Allele call of Components A, B and C were analyzed against the certified STR allele values. All 35 samples were 100% concordance (Appendix 2).

### 3.8 Casework Samples

A study was performed to assess the ability to obtain reliable results from casework samples that represent the typical variation in DNA quantity and quality encountered with samples received by forensic laboratories. Different casework samples with different ranges of concentration which can impact the result and the interpretation of obtained data were selectively chose and tested to assess the performance of the assay. Eight Bn, 7 Bt, 5 CB, 1 P, 1 Bl along with Ts were analyzed and compared with data previously obtained using GlobalFiler$^{TM}$ PCR kit (Table 12). Almost all of the samples tested yielded more alleles compared to the results previously obtained with GlobalFiler$^{TM}$ PCR kit (Figure 16). While the DNA concentration in swab of bottle-4 (Bt 4) (4 ng/µl) is lower than DNA concentration in cigarette butt-1 (CB 1) (8 pg/ µl), The number of allele call in (Bt 4) is significantly higher than the number obtained in (CB 1). This could be due to the presence of potential PCR inhibitors in the cigarette butt such as: tars and phenolics from the smoke, paper additives and flavor additives [26].

Table 12: Different casework samples used to assess the multiplex assay

| Sample | Sample DNA | Allele called | | % of total alleles | |
|---|---|---|---|---|---|
| | pg/µl | GF | VFP | GF | VFP |
| **CB 1** | 8 | 8 | 18 | 17 | 36 |
| **CB 2** | 400 | 46 | 49 | 100 | 100 |
| **CB 3** | 1200 | 46 | 49 | 100 | 100 |
| **CB 4** | 200 | 46 | 49 | 100 | 100 |
| **CB 5** | 1000 | 46 | 49 | 100 | 100 |
| **Bt 1** | 30 | 43 | 49 | 93 | 100 |
| **Bt 2** | 100 | 46 | 49 | 100 | 100 |
| **Bt 3** | 30 | 46 | 49 | 100 | 100 |
| **Bt 4** | 4 | 30 | 49 | 65 | 100 |
| **Bt 5** | 90 | 46 | 49 | 100 | 100 |
| **Bt 6** | 80 | 46 | 49 | 100 | 100 |
| **Bt 7** | 400 | 46 | 49 | 100 | 100 |
| **Ts** | 400 | 39 | 49 | 84 | 100 |
| **P** | 600 | 35 | 48 | 76 | 98 |
| **Bl** | 1400 | 46 | 49 | 100 | 100 |
| **Bn 1** | 60 | 46 | 49 | 100 | 100 |
| **Bn 2** | 20 | 46 | 49 | 100 | 100 |
| **Bn 3** | 30 | 46 | 49 | 100 | 100 |
| **Bn 4** | 30 | 46 | 49 | 100 | 100 |
| **Bn 5** | 30 | 44 | 49 | 100 | 100 |
| **Bn 6** | 16 | 43 | 46 | 93 | 94 |
| **Bn 7** | 20 | 46 | 49 | 100 | 100 |
| **Bn 8** | 500 | 46 | 49 | 100 | 100 |

CB: cigarette butt; Bt: bottle; Ts: human tissue; P: plug; Bl: blood; Bn: bone

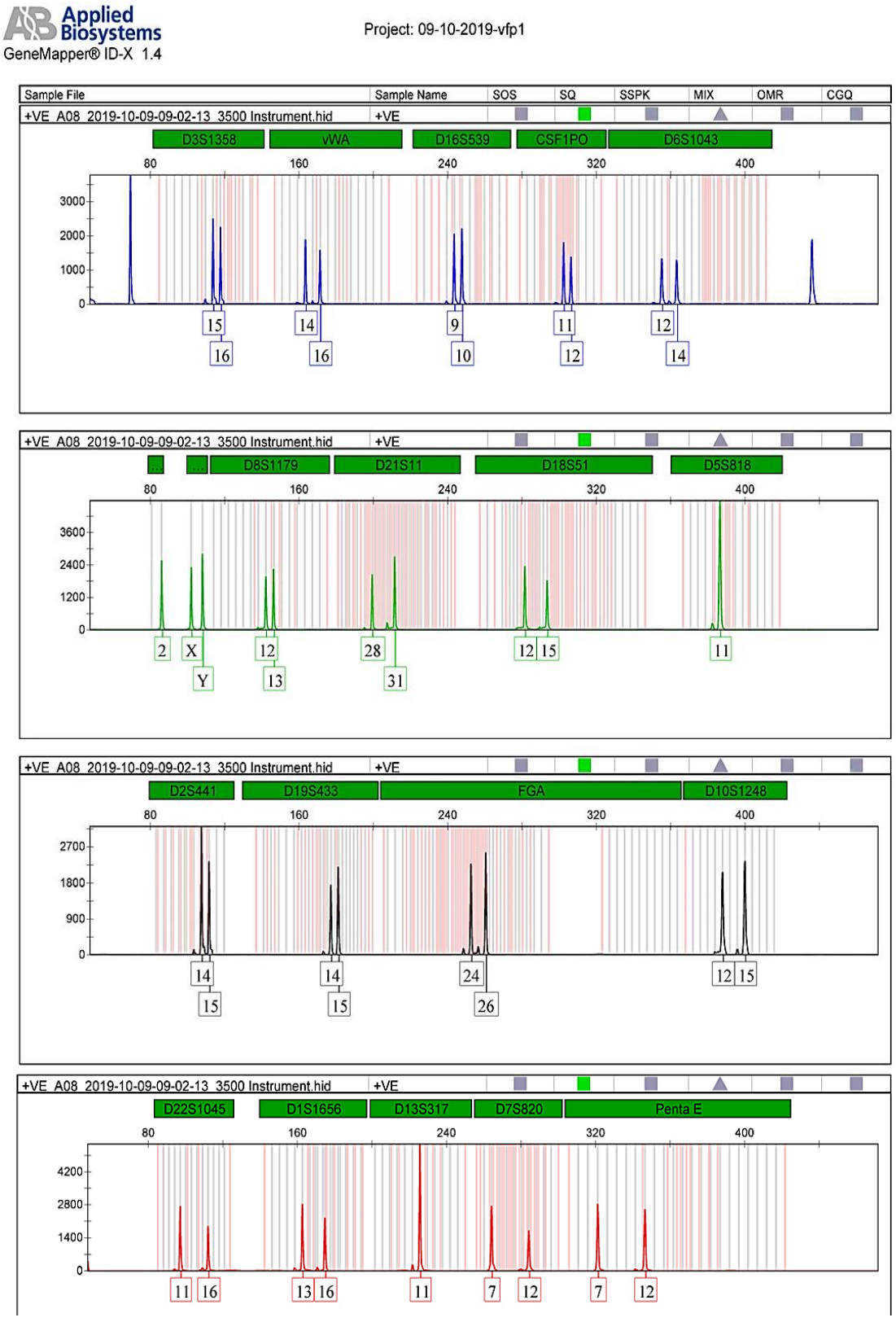


Figure 14: Electropherogram of DNA control 007 with 500 pg

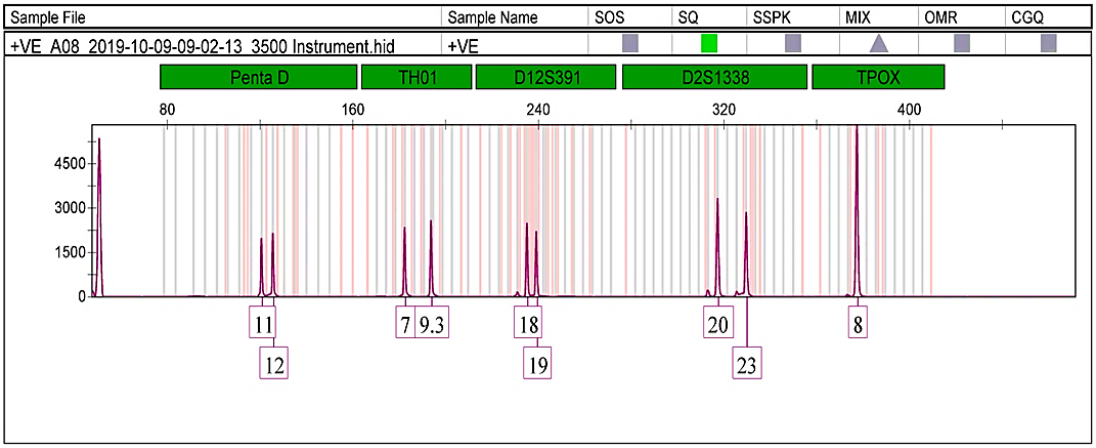


Figure 14: Electropherogram of DNA control 007 with 500 pg (Continued)

Figure 14 illustrates a full profile obtained from 500 pg of DNA control 007 which shows balanced peak height ratio.

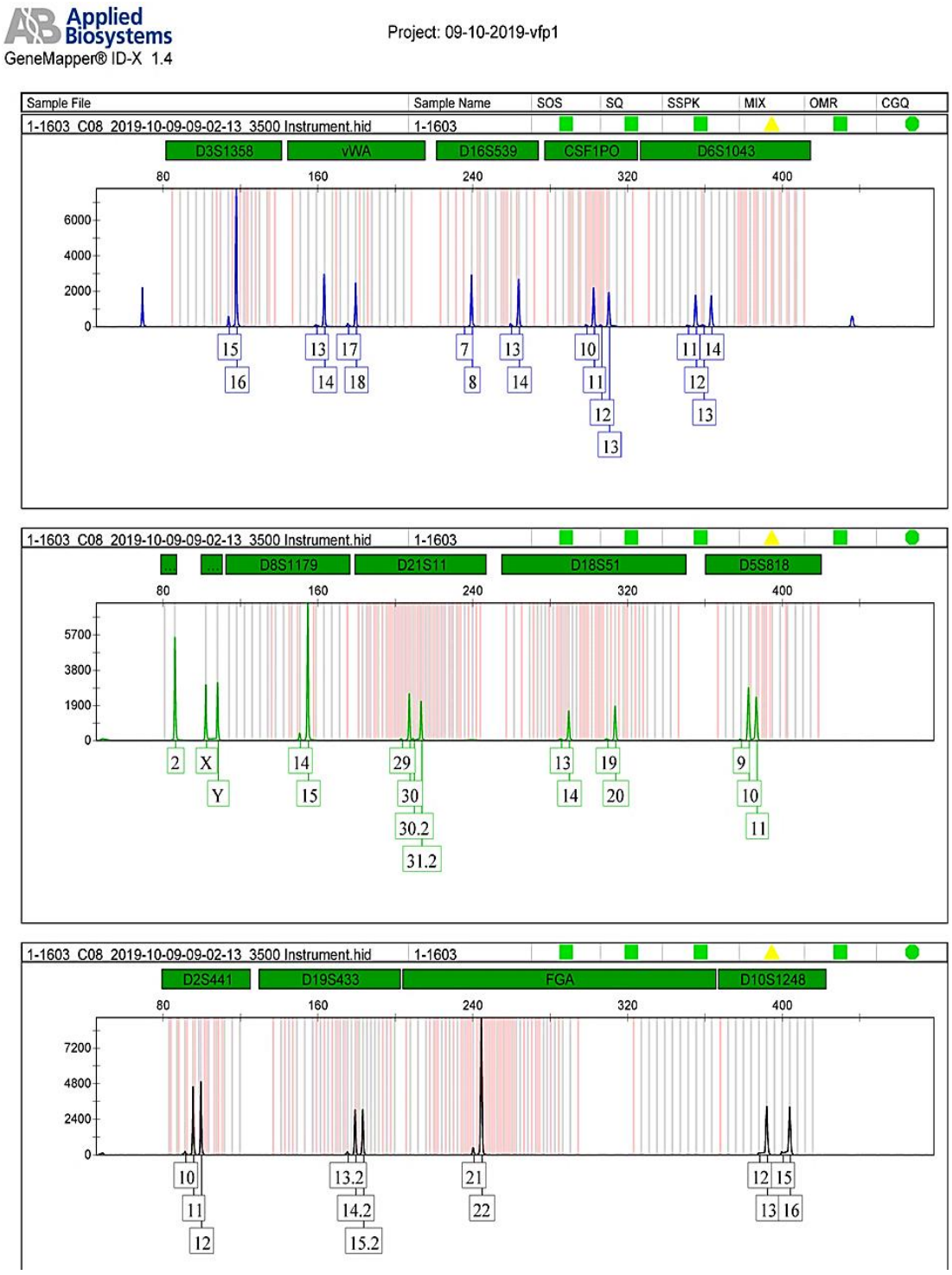


Figure 15: Electropherogram of a cigarette butt sample (1000 pg)

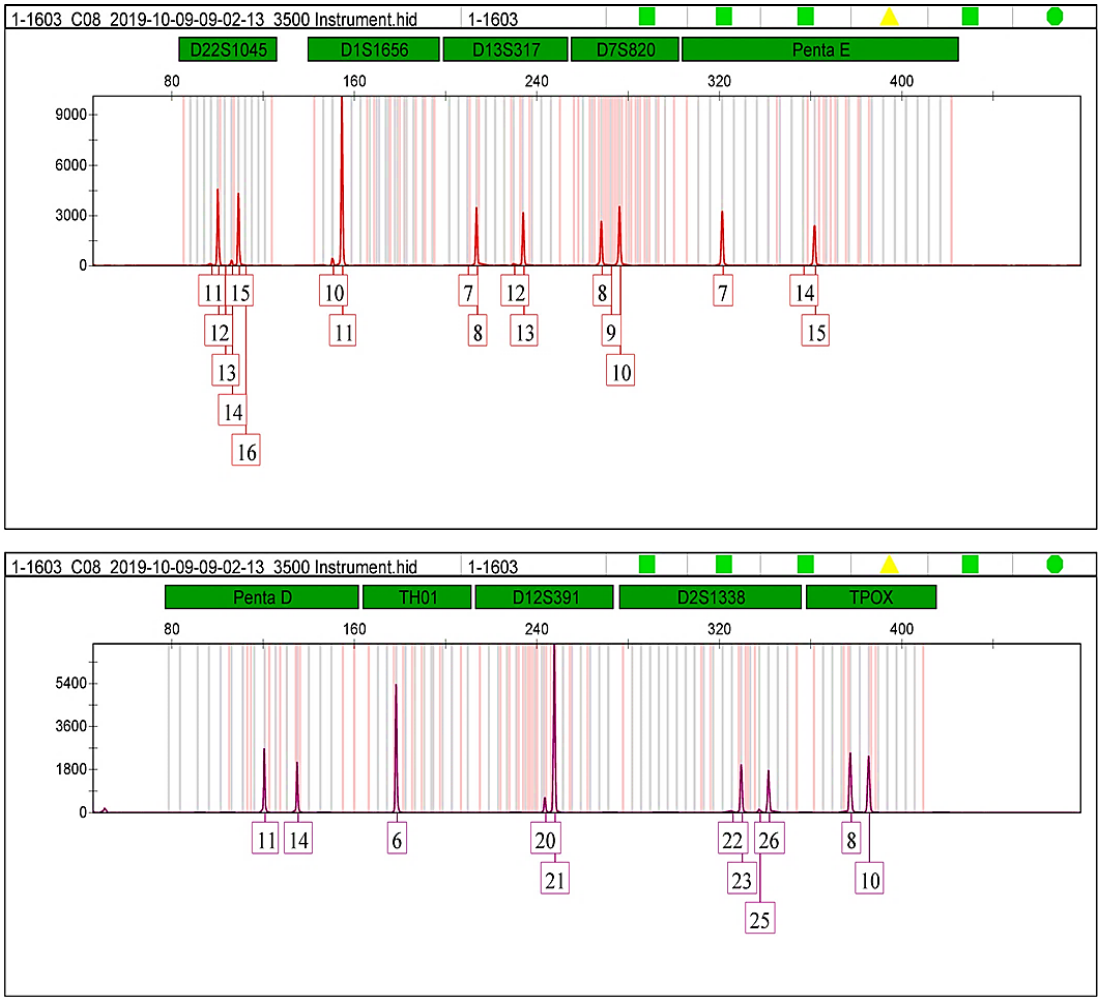


Figure 15: Electropherogram of a cigarette butt sample (1000 pg) (Continued)

Generally, profiles obtained from Verifiler™ Plus recorded higher peak heights compared to the GlobalFiler™ PCR kit (Figure 15).

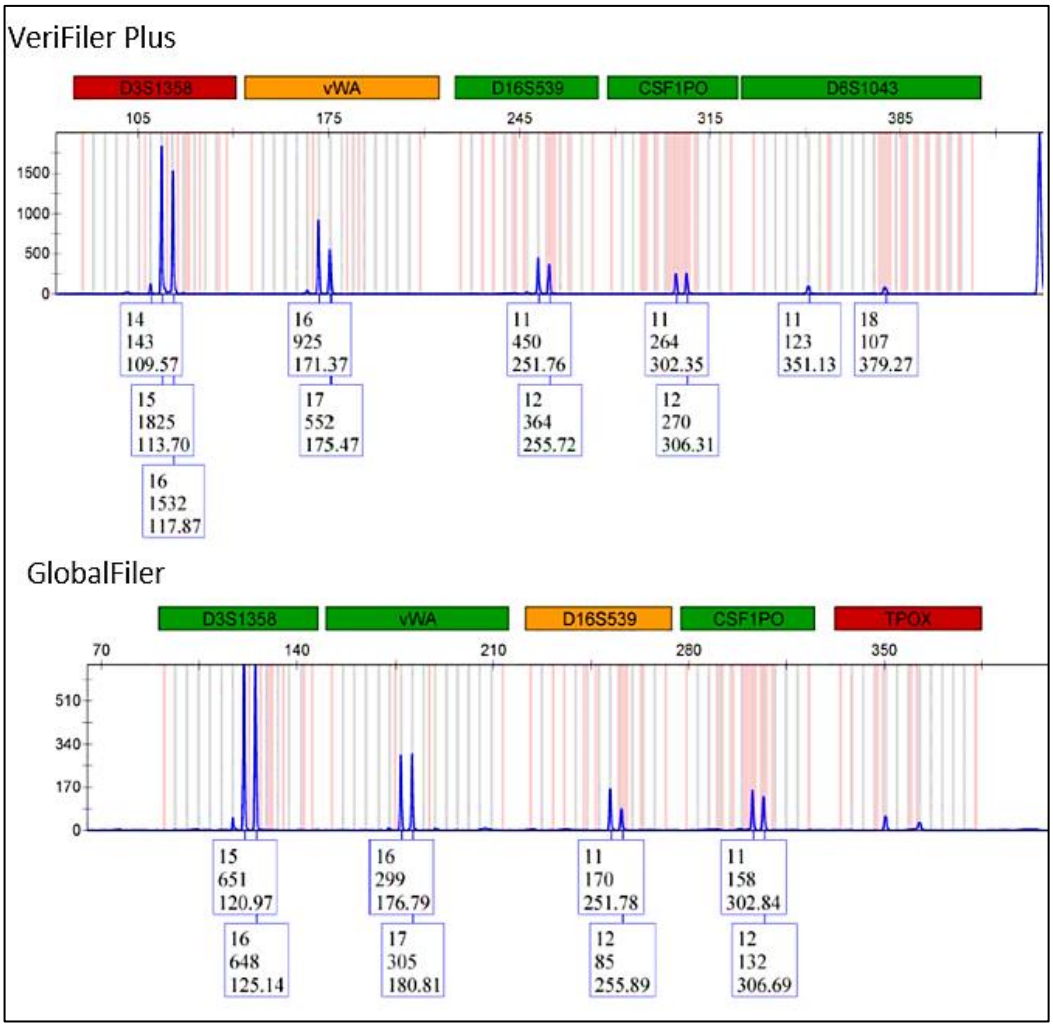


Figure 16: Electropherogram of a swab of bottle (30 pg) using two PCR assays

Figure 16 illustrates a comparison between the number of alleles generated from the same swab of bottle using Verifiler$^{TM}$ Plus and GlobalFiler$^{TM}$ PCR kit. Results shows obtaining greater number of alleles using Verifiler$^{TM}$ Plus.

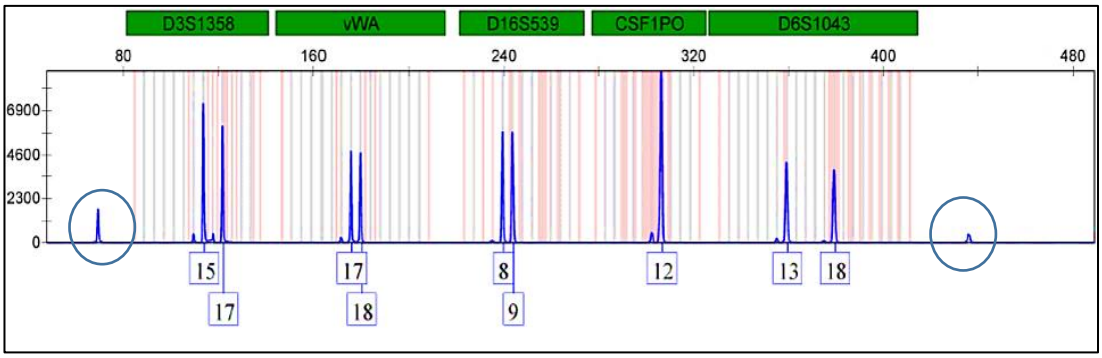


Figure 17: Illustration of two internal quality control sensors

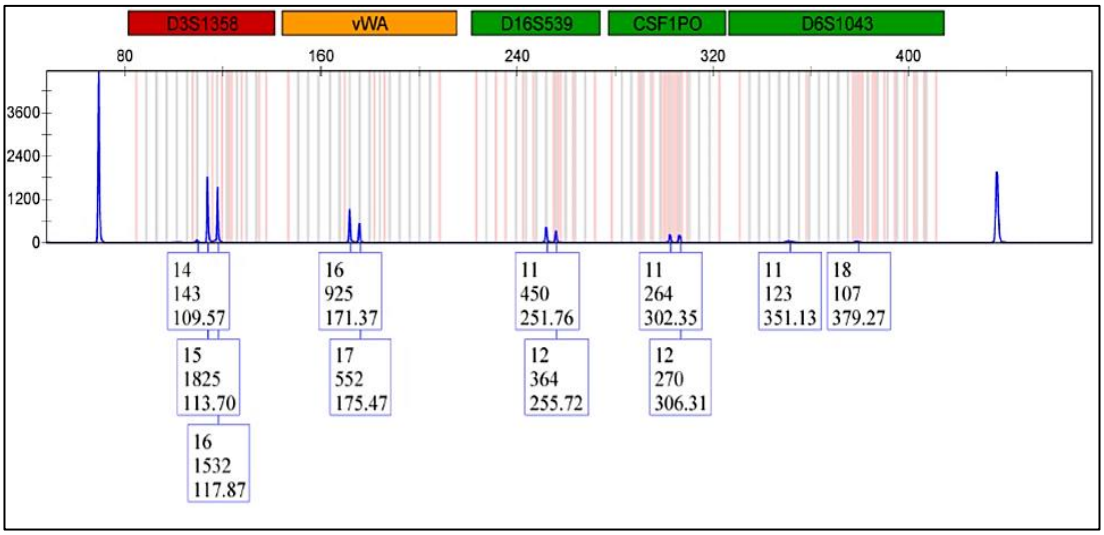


Figure 18: Interpretation of the sample in the presence of IQC

Two internal quality control sensors are included in this assay which provides extra information on the quality and the integrity of DNA sample (Figure 17). It can evaluate the PCR reaction, infer possible sample degradation or inhibition.

In Figure 18, both IQC sensors are > 2000 rfu which indicates successful PCR reaction was performed [27], while a ski slope pattern observed in the DNA profile which indicates a degradation pattern.

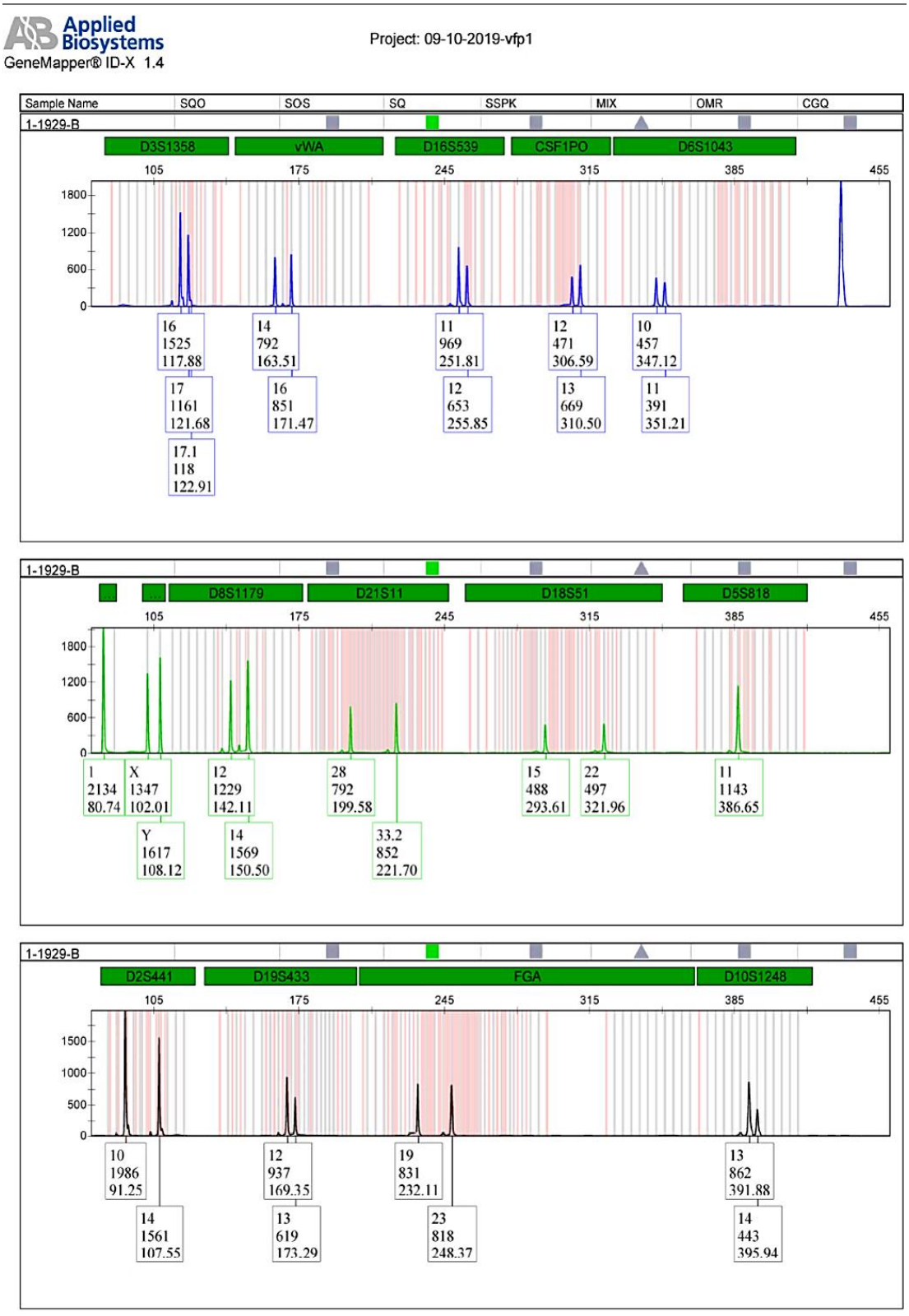


Figure 19: Electropherogram of bone sample (500 pg)

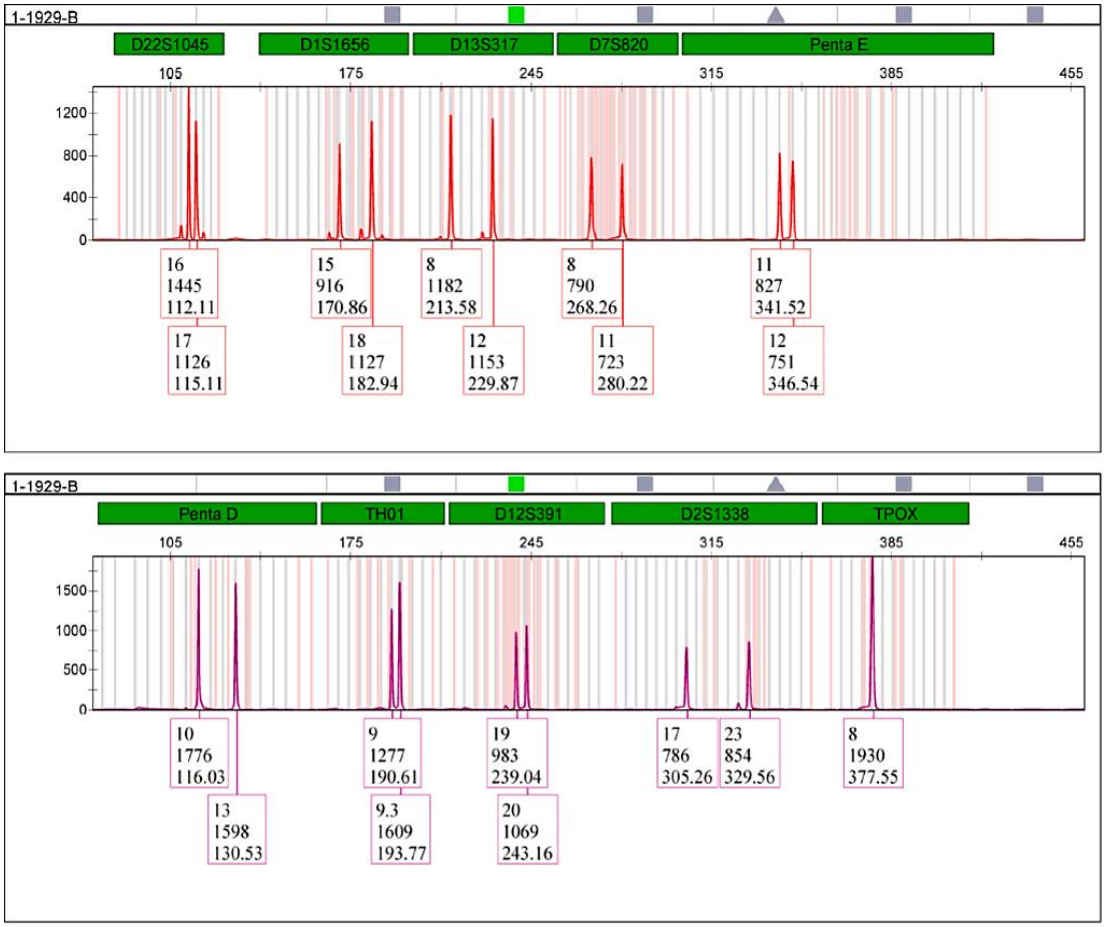


Figure 19: Electropherogram of bone sample (500 pg) (Continued)

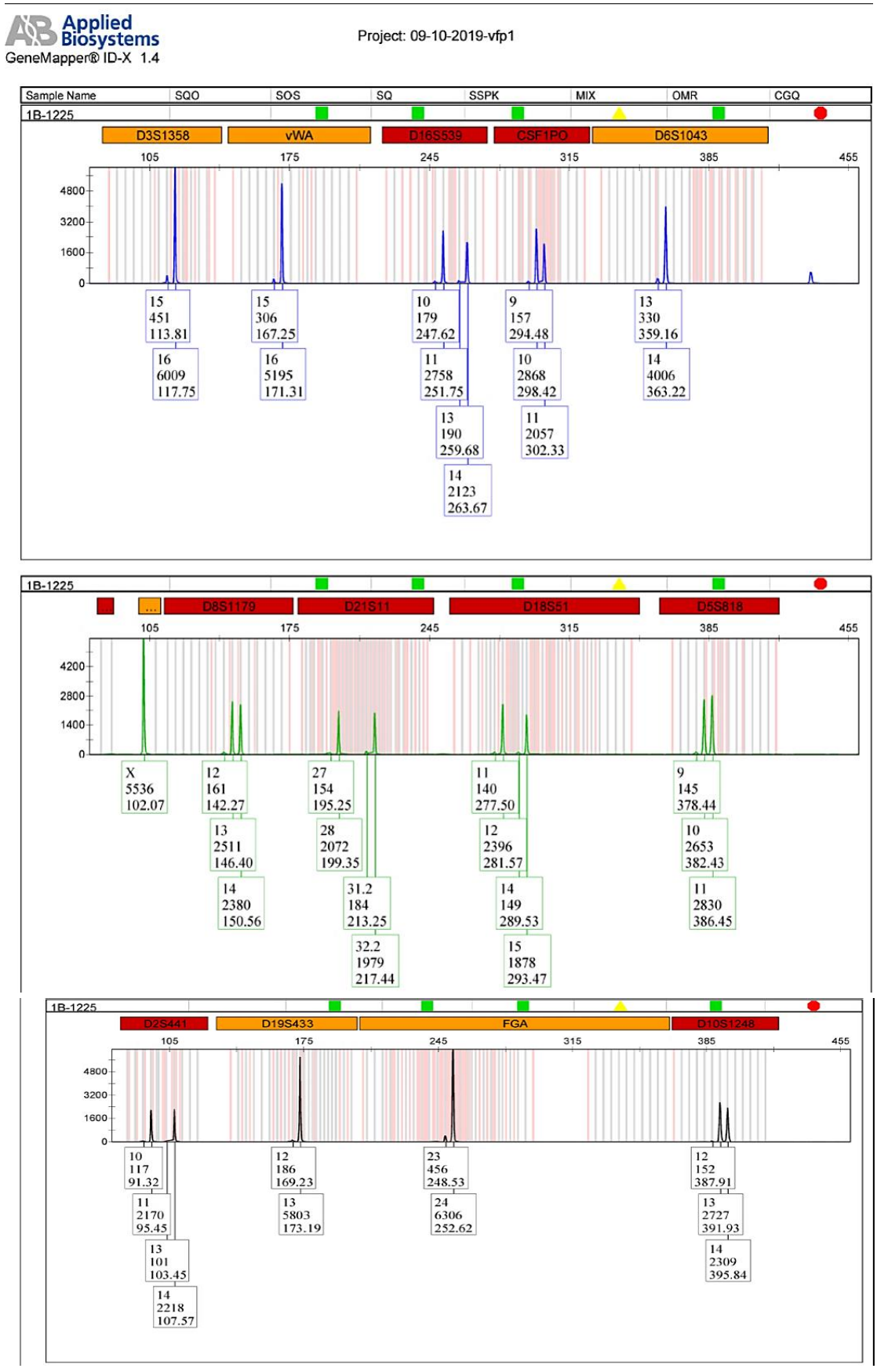


Figure 20: Electropherogram of blood sample (1400 pg)

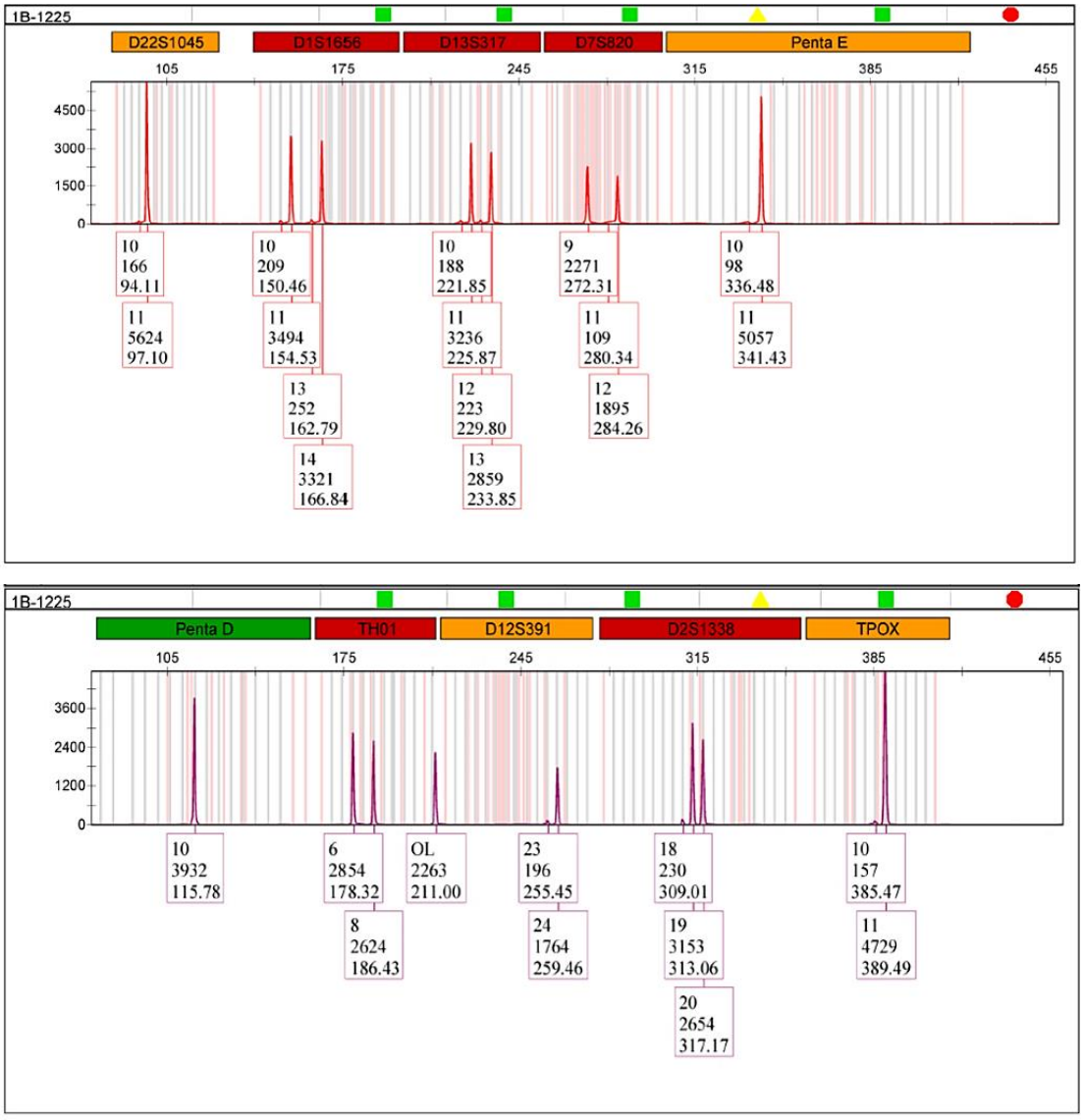


Figure 20: Electropherogram of blood sample (1400 pg) (Continued)

The main results in this study highlight the minimum DNA input required to generate a full profile is 63 pg (Figure 19). The AT was set to 80 RFU while the SD was set to 150 RFU. Size precision of Verifiler$^{TM}$ Plus PCR kit varied between 0.02 bp and 0.08 bp. Samples were 100% concordance and the success rate of generated profiles was 100%. The assay showed high tolerance toward humic acid (60-200 ng/µl) and hematin porcine (0.6 mM) (Figure 20).

# Chapter 4: Discussion

This study is the first to evaluate the performance of (VeriFiler™ Plus) a six-dye STR multiplex assay with casework samples in forensic applications. Sensitivity of the assay toward DNA template input was tested and full STR profiles (100% of allele call) were obtained from all the samples ranging from 1000 pg down to 63 pg showing consistent result with a study conducted using AmpFlSTR® NGM SElect™ PCR Amplification Kit [20].

However, it shows higher sensitivity compared to two other six-dye multiplexes, GlobalFiler® PCR Amplification and Investigator® 24plex QS kits [4,17, 21]. 95% of expected alleles were recoverd from 32 pg DNA, which is significantly higher than the percentage of recovered allele at 32 pg in GlobalFiler® PCR Amplification [16]. 70% and 52% of all expected alleles were recovered at 16 pg and 8 pg respectively. A study reported that the Investigator® kit produced 50% of expected alleles with 8 pg of DNA [17] which is more consistent with result obtained from 8 pg DNA.

Sizing Precision is critical for determination of correct genotyping. Therefore, migration of each fragment and sizing precision must be consistent and within the bin window to ensure proper allele designation. The degree of variation generated in this study was between 0.02 bp and 0.08 bp which is within the recommended bin window for genotyping as it is mentioned by ENFSI (2010) [23] showing similar results to other studies conducted in this field [17, 25].

Mixture study can be used to assist forensic laboratories in establishing interpretation guidelines. Full STR profile was obtained until the 1:3 ratio comparing

to the results obtained from GlobalFiler Amplification kit, there is no significant difference [4].

Due to the wide range of forensic sample types and the different sampling models in the crime scene, substances known to be inhibitory toward PCR reactions are often encountered in DNA extracted from casework samples resulting either in loss of peaks or lowering of peak heights. Inhibition study was conducted to assess the tolerance and the robustness of the assay in the presence of common type of inhibitors that could bear in casework samples. Hematin porcine (0.6 mM), formed by the oxidation of heme which is the main component of blood and humic acid (60-200 ng/µl), a principle component of humic substances that may present in forensic samples collected from soil [17]. Full profiles were generated for all humic acid concentrations tested (up to 200 ng/µl). This is in agreement with findings in other studies [17]. These data demonstrate that this essay can generate a high number of alleles in the presence of the mentioned inhibitory substances.

# Chapter 5: Conclusion

This study was conducted to assess and evaluate the performance of the 6-dye multiplex assay containing 23 autosomal loci with casework samples. The result of each quality control test was analyzed, sensitivity test results showed the minimum amount of DNA input resulting in a full DNA profile was 63 pg. However, the optimum range determined was 125 - 500 pg. Minimal limit of detection was calculated for every dye set, and the result obtained was used to estimate the overall analytical threshold at 80 RFU. Stochastic threshold calculation resulted in highest value to be set at 180 RFU. Heterozygous alleles were balanced for total DNA input ranging from 1000 - 500 pg while it was less than 60% for samples with total DNA input below 250 pg. All expected alleles were detected for minor contributor of 1:1, 1:3, mixture ratios, however 98% of alleles were detected for the minor contributor of 1:7, 10:1 mixture ratio. For 15:1 91% of the alleles were detected. Stability study demonstrated that this assay can generate a high number of alleles in the presence of of Hematin porcine (0.6 mM) and humic acid (200 ng/µl). DNA profile results are concordant to those obtained from Applied Biosystem GlobalFiler$^{TM}$ PCR kit.

Overall, this study demonstrate that the assay can produce reliable and reproducible results, and it enhances the success rate of obtaining positive results from challenging forensic samples. This assay is fit to be used with casework samples in forensic DNA identification. Further studies are recommended to assess the resistance of this assay toward other PCR inhibitors such as inhibitors encountered in cigarette butt samples. Species specificity tests are also recommended to be done.

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# Appendix

(1)

- Sample setup for Mixture study using Component B and C (1.6 ng/µl)
    - Samples were diluted to reach a final concentration of 0.5 ng/ µl in 100 µl. To prepare a stock solution for each component (B & C), 31.25 µl was added to 68.75 µl of the diluent.
    - Mixtures were prepared using the following amounts:

Table 13: Volume of each diluted DNA sample required to prepare mixture set 1

| Ratio | Volume of B (µl) | Volume of C (µl) |
|---|---|---|
| **1:1** | 8.75 | 8.75 |
| **3:1** | 13.1 | 4.4 |
| **7:1** | 15.3 | 2.2 |
| **10:1** | 16 | 1.5 |
| **15:1** | 16.5 | 1 |

Table 14: Volume of each diluted DNA sample required to prepare mixture set 2

| Ratio | Volume of C (µl) | Volume of B (µl) |
|---|---|---|
| **1:3** | 13.1 | 4.4 |
| **1:7** | 15.3 | 2.2 |
| **1:10** | 16 | 1.5 |
| **1:15** | 16.5 | 1 |

(2)

Table 15: Concordance study with SRM 2391c samples (component A, B and C)

| sample | markers | Verifiler Plus | | Certificate of Ananlysis- Standard Reference Material 2391c | |
|---|---|---|---|---|---|
| **A** | D3S1358 | 15 | 16 | 15 | 16 |
| | vWA | 18 | 19 | 18 | 19 |
| | D16S539 | 10 | 11 | 10 | 11 |
| | CSF1PO | 10 | 10 | 10 | 10 |
| | D6S1043 | 11 | 18 | 11 | 18 |
| | Yindel | | | | |
| | AMEL | X | X | X | X |
| | D8S1179 | 13 | 14 | 13 | 14 |
| | D21S11 | 28 | 32.2 | 28 | 32.2 |
| | D18S51 | 12 | 15 | 12 | 15 |
| | D5S818 | 11 | 12 | 11 | 12 |
| | D2S441 | 10 | 10 | 10 | 10 |
| | D19S433 | 13 | 14 | 13 | 14 |
| | FGA | 21 | 23 | 21 | 23 |
| | D10S1248 | 15 | 16 | 15 | 16 |
| | D22S1045 | 15 | 15 | 15 | 15 |
| | D1S1656 | 17.3 | 17.3 | 17.3 | 17.3 |
| | D13S317 | 8 | 8 | 8 | 8 |
| | D7S820 | 11 | 11 | 11 | 11 |
| | Penta E | 5 | 10 | 5 | 10 |
| | Penta D | 9 | 13 | 9 | 13 |
| | TH01 | 8 | 9.3 | 8 | 9.3 |
| | D12S391 | 18.3 | 22 | 18.3 | 22 |
| | D2S1338 | 18 | 23 | 18 | 23 |
| | TPOX | 8 | 8 | 8 | 8 |
| **B** | D3S1358 | 15 | 19 | 15 | 19 |
| | vWA | 17 | 18 | 17 | 18 |
| | D16S539 | 10 | 13 | 10 | 13 |
| | CSF1PO | 10 | 11 | 10 | 11 |
| | D6S1043 | 14 | 19 | 14 | 19 |
| | Yindel | 2 | | | |
| | AMEL | X | Y | X | Y |
| | D8S1179 | 10 | 13 | 10 | 13 |
| | D21S11 | 32 | 32.2 | 32 | 32.2 |
| | D18S51 | 13 | 16 | 13 | 16 |
| | D5S818 | 12 | 13 | 12 | 13 |
| | D2S441 | 10 | 14 | 10 | 14 |
| | D19S433 | 16 | 16.2 | 16 | 16.2 |
| | FGA | 20 | 23 | 20 | 23 |

Table 15: Concordance study with SRM 2391c sample (component A, B and C) (continued)

| **sample** | markers | Verifiler Plus | | Certificate of Ananlysis- Standard Reference Material 2391c | |
|---|---|---|---|---|---|
| **B** | D10S1248 | 13 | 13 | 13 | 13 |
| | D22S1045 | 15 | 17 | 15 | 17 |
| | D1S1656 | 11 | 14 | 11 | 14 |
| | D13S317 | 9 | 12 | 9 | 12 |
| | D7S820 | 10 | 10 | 10 | 10 |
| | Penta E | 7 | 15 | 7 | 15 |
| | Penta D | 8 | 12 | 8 | 12 |
| | TH01 | 6 | 9.3 | 6 | 9.3 |
| | D12S391 | 19 | 24 | 19 | 24 |
| | D2S1338 | 17 | 17 | 17 | 17 |
| | TPOX | 8 | 11 | 8 | 11 |
| **C** | D3S1358 | 16 | 18 | 16 | 18 |
| | vWA | 16 | 18 | 16 | 18 |
| | D16S539 | 10 | 10 | 10 | 10 |
| | CSF1PO | 10 | 12 | 10 | 12 |
| | D6S1043 | 11 | 14 | 11 | 14 |
| | Yindel | 2 | | | |
| | AMEL | X | Y | X | Y |
| | D8S1179 | 10 | 17 | 10 | 17 |
| | D21S11 | 29 | 30 | 29 | 30 |
| | D18S51 | 16 | 19 | 16 | 19 |
| | D5S818 | 10 | 11 | 10 | 11 |
| | D2S441 | 10 | 10 | 10 | 10 |
| | D19S433 | 13.2 | 15.2 | 13.2 | 15.2 |
| | FGA | 24 | 26 | 24 | 26 |
| | D10S1248 | 12 | 16 | 12 | 16 |
| | D22S1045 | 16 | 16 | 16 | 16 |
| | D1S1656 | 11 | 15 | 11 | 15 |
| | D13S317 | 11 | 11 | 11 | 11 |
| | D7S820 | 10 | 12 | 10 | 12 |
| | Penta E | 12 | 13 | 12 | 13 |
| | Penta D | 10 | 11 | 10 | 11 |
| | TH01 | 6 | 8 | 6 | 8 |
| | D12S391 | 19 | 23 | 19 | 23 |
| | D2S1338 | 19 | 19 | 19 | 19 |
| | TPOX | 11 | 11 | 11 | 11 |

Table 16: Concordance study with blood and buccal swab samples using GlobalFiler (GF) and Verifiler Plus (VFP) PCR kits

| Sample | markers | GF | | markers | VFP- Blood | | VFP- Buccal swab | |
|---|---|---|---|---|---|---|---|---|
| **1** | D3S1358 | 15 | 18 | D3S1358 | 15 | 18 | 15 | 18 |
| | vWA | 15 | 17 | vWA | 15 | 17 | 15 | 17 |
| | D16S539 | 10 | 12 | D16S539 | 10 | 12 | 10 | 12 |
| | CSF1PO | 11 | 12 | CSF1PO | 11 | 12 | 11 | 12 |
| | TPOX | 8 | 8 | D6S1043 | 12 | 17 | 12 | 17 |
| | Yindel | 2 | | Yindel | 2 | | 2 | |
| | AMEL | X | Y | AMEL | X | Y | X | Y |
| | D8S1179 | 14 | 14 | D8S1179 | 14 | 14 | 14 | 14 |
| | D21S11 | 28 | 29 | D21S11 | 28 | 29 | 28 | 29 |
| | D18S51 | 14 | 14 | D18S51 | 14 | 14 | 14 | 14 |
| | DYS391 | 10 | 10 | D5S818 | 11 | 12 | 11 | 12 |
| | D2S441 | 10 | 14 | D2S441 | 10 | 14 | 10 | 14 |
| | D19S433 | 14 | 15 | D19S433 | 14 | 15 | 14 | 15 |
| | TH01 | 7 | 8 | FGA | 21 | 24 | 21 | 24 |
| | FGA | 21 | 24 | D10S1248 | 15 | 16 | 15 | 16 |
| | D22S1045 | 11 | 15 | D22S1045 | 11 | 15 | 11 | 15 |
| | D5S818 | 11 | 12 | D1S1656 | 17 | 17.3 | 17 | 17.3 |
| | D13S317 | 11 | 13 | D13S317 | 11 | 13 | 11 | 13 |
| | D7S820 | 10 | 11 | D7S820 | 10 | 11 | 10 | 11 |
| | SE33 | 19 | 27.2 | Penta E | 7 | 10 | 7 | 10 |
| | D10S1248 | 15 | 16 | Penta D | 11 | 12 | 11 | 12 |
| | D1S1656 | 17 | 17.3 | TH01 | 7 | 8 | 7 | 8 |
| | D12S391 | 22 | 24 | D12S391 | 22 | 24 | 22 | 24 |
| | D2S1338 | 18 | 23 | D2S1338 | 18 | 23 | 18 | 23 |
| | | | | TPOX | 8 | 8 | 8 | 8 |
| **2** | D3S1358 | 15 | 15 | D3S1358 | 15 | 15 | 15 | 15 |
| | vWA | 17 | 18 | vWA | 17 | 18 | 17 | 18 |
| | D16S539 | 9 | 13 | D16S539 | 9 | 13 | 9 | 13 |
| | CSF1PO | 12 | 12 | CSF1PO | 12 | 12 | 12 | 12 |
| | TPOX | 8 | 11 | D6S1043 | 11 | 14 | 11 | 14 |
| | Yindel | | | Yindel | | | | |
| | AMEL | X | X | AMEL | X | X | X | X |
| | D8S1179 | 12 | 13 | D8S1179 | 12 | 13 | 12 | 13 |
| | D21S11 | 28 | 30 | D21S11 | 28 | 30 | 28 | 30 |
| | D18S51 | 12 | 14 | D18S51 | 12 | 14 | 12 | 14 |
| | DYS391 | | | D5S818 | 12 | 13 | 12 | 13 |
| | D2S441 | 11 | 11 | D2S441 | 11 | 11 | 11 | 11 |
| | D19S433 | 13 | 14 | D19S433 | 13 | 14 | 13 | 14 |
| | TH01 | 6 | 7 | FGA | 19 | 25 | 19 | 25 |
| | FGA | 19 | 25 | D10S1248 | 13 | 14 | 13 | 14 |
| | D22S1045 | 11 | 15 | D22S1045 | 11 | 15 | 11 | 15 |

Table 16: Concordance study with blood and buccal swab samples using GlobalFiler (GF) and Verifiler Plus (VFP) PCR kits (continued)

| Sample | markers | GF | | markers | VFP- Blood | | VFP- Buccal swab | |
|---|---|---|---|---|---|---|---|---|
| | D5S818 | 12 | 13 | D1S1656 | 12 | 16 | 12 | 16 |
| | D13S317 | 12 | 12 | D13S317 | 12 | 12 | 12 | 12 |
| | D7S820 | 8 | 11 | D7S820 | 8 | 11 | 8 | 11 |
| | SE33 | 28.2 | 29.2 | Penta E | 5 | 19 | 5 | 19 |
| | D10S1248 | 13 | 14 | Penta D | 12 | 14 | 12 | 14 |
| | D1S1656 | 12 | 16 | TH01 | 6 | 7 | 6 | 7 |
| | D12S391 | 18 | 18 | D12S391 | 18 | 18 | 18 | 18 |
| | D2S1338 | 17 | 19 | D2S1338 | 17 | 19 | 17 | 19 |
| | | | | TPOX | 8 | 11 | 8 | 11 |
| **3** | D3S1358 | 15 | 18 | D3S1358 | 15 | 18 | 15 | 18 |
| | vWA | 17 | 18 | vWA | 17 | 18 | 17 | 18 |
| | D16S539 | 12 | 13 | D16S539 | 12 | 13 | 12 | 13 |
| | CSF1PO | 11 | 12 | CSF1PO | 11 | 12 | 11 | 12 |
| | TPOX | 8 | 8 | D6S1043 | 11 | 13 | 11 | 13 |
| | Yindel | | | Yindel | | | | |
| | AMEL | X | X | AMEL | X | X | X | X |
| | D8S1179 | 15 | 16 | D8S1179 | 15 | 16 | 15 | 16 |
| | D21S11 | 29 | 31 | D21S11 | 29 | 31 | 29 | 31 |
| | D18S51 | 16 | 17 | D18S51 | 16 | 17 | 16 | 17 |
| | DYS391 | | | D5S818 | 12 | 13 | 12 | 13 |
| | D2S441 | 11 | 14 | D2S441 | 11 | 14 | 11 | 14 |
| | D19S433 | 15 | 15.2 | D19S433 | 15 | 15.2 | 15 | 15.2 |
| | TH01 | 6 | 6 | FGA | 22 | 22 | 22 | 22 |
| | FGA | 22 | 22 | D10S1248 | 14 | 15 | 14 | 15 |
| | D22S1045 | 15 | 16 | D22S1045 | 15 | 16 | 15 | 16 |
| | D5S818 | 12 | 13 | D1S1656 | 11 | 17 | 11 | 17 |
| | D13S317 | 8 | 13 | D13S317 | 8 | 13 | 8 | 13 |
| | D7S820 | 10 | 11 | D7S820 | 10 | 11 | 10 | 11 |
| | SE33 | 23.2 | 36 | Penta E | 12 | 13 | 12 | 13 |
| | D10S1248 | 14 | 15 | Penta D | 11 | 14 | 11 | 14 |
| | D1S1656 | 11 | 17 | TH01 | 6 | 6 | 6 | 6 |
| | D12S391 | 17 | 18 | D12S391 | 17 | 18 | 17 | 18 |
| | D2S1338 | 19 | 19 | D2S1338 | 19 | 19 | 19 | 19 |
| | | | | TPOX | 8 | 8 | 8 | 8 |
| **4** | D3S1358 | 15 | 18 | D3S1358 | 15 | 18 | 15 | 18 |
| | vWA | 17 | 18 | vWA | 17 | 18 | 17 | 18 |
| | D16S539 | 12 | 12 | D16S539 | 12 | 12 | 12 | 12 |
| | CSF1PO | 12 | 12 | CSF1PO | 12 | 12 | 12 | 12 |
| | TPOX | 8 | 11 | D6S1043 | 11 | 19 | 11 | 19 |
| | Yindel | | | Yindel | | | | |
| | AMEL | X | X | AMEL | X | X | X | X |

Table 16: Concordance study with blood and buccal swab samples using GlobalFiler (GF) and Verifiler Plus (VFP) PCR kits (continued)

| Sample | markers | GF | | markers | VFP- Blood | | VFP- Buccal swab | |
|---|---|---|---|---|---|---|---|---|
| | D8S1179 | 12 | 13 | D8S1179 | 12 | 13 | 12 | 13 |
| | D21S11 | 29 | 29 | D21S11 | 29 | 29 | 29 | 29 |
| | D18S51 | 14 | 15 | D18S51 | 14 | 15 | 14 | 15 |
| | DYS391 | | | D5S818 | 13 | 13 | 13 | 13 |
| | D2S441 | 11 | 14 | D2S441 | 11 | 14 | 11 | 14 |
| | D19S433 | 13 | 14 | D19S433 | 13 | 14 | 13 | 14 |
| | TH01 | 6 | 9 | FGA | 21 | 23 | 21 | 23 |
| | FGA | 21 | 23 | D10S1248 | 14 | 15 | 14 | 15 |
| | D22S1045 | 15 | 15 | D22S1045 | 15 | 15 | 15 | 15 |
| | D5S818 | 13 | 13 | D1S1656 | 15 | 17.3 | 15 | 17.3 |
| | D13S317 | 11 | 12 | D13S317 | 11 | 12 | 11 | 12 |
| | D7S820 | 9 | 11 | D7S820 | 9 | 11 | 9 | 11 |
| | SE33 | 18 | 21.1 | Penta E | 5 | 13 | 5 | 13 |
| | D10S1248 | 14 | 15 | Penta D | 9 | 9 | 9 | 9 |
| | D1S1656 | 15 | 17.3 | TH01 | 6 | 9 | 6 | 9 |
| | D12S391 | 20 | 23 | D12S391 | 20 | 23 | 20 | 23 |
| | D2S1338 | 17 | 21 | D2S1338 | 17 | 21 | 17 | 21 |
| | | | | TPOX | 8 | 11 | 8 | 11 |
| **5** | D3S1358 | 16 | 17 | D3S1358 | 16 | 17 | 16 | 17 |
| | vWA | 17 | 17 | vWA | 17 | 17 | 17 | 17 |
| | D16S539 | 10 | 14 | D16S539 | 10 | 14 | 10 | 14 |
| | CSF1PO | 11 | 12 | CSF1PO | 11 | 12 | 11 | 12 |
| | TPOX | 8 | 8 | D6S1043 | 11 | 11 | 11 | 11 |
| | Yindel | | | Yindel | | | | |
| | AMEL | X | X | AMEL | X | X | X | X |
| | D8S1179 | 11 | 13 | D8S1179 | 11 | 13 | 11 | 13 |
| | D21S11 | 29 | 32.2 | D21S11 | 29 | 32.2 | 29 | 32.2 |
| | D18S51 | 16 | 18 | D18S51 | 16 | 18 | 16 | 18 |
| | DYS391 | | | D5S818 | 10 | 11 | 10 | 11 |
| | D2S441 | 10 | 12 | D2S441 | 10 | 12 | 10 | 12 |
| | D19S433 | 13 | 14 | D19S433 | 13 | 14 | 13 | 14 |
| | TH01 | 6 | 9 | FGA | 20 | 23 | 20 | 23 |
| | FGA | 20 | 23 | D10S1248 | 15 | 15 | 15 | 15 |
| | D22S1045 | 11 | 15 | D22S1045 | 11 | 15 | 11 | 15 |
| | D5S818 | 10 | 11 | D1S1656 | 11 | 17.3 | 11 | 17.3 |
| | D13S317 | 14 | 14 | D13S317 | 14 | 14 | 14 | 14 |
| | D7S820 | 9 | 10 | D7S820 | 9 | 10 | 9 | 10 |
| | SE33 | 18 | 20.2 | Penta E | 7 | OL | 7 | OL |
| | D10S1248 | 15 | 15 | Penta D | 11 | 12 | 11 | 12 |
| | D1S1656 | 11 | 17.3 | TH01 | 6 | 9 | 6 | 9 |
| | D12S391 | 18.3 | 21 | D12S391 | 18.3 | 21 | 18.3 | 21 |

Table 16: Concordance study with blood and buccal swab samples using GlobalFiler (GF) and Verifiler Plus (VFP) PCR kits (continued)

| Sample | markers | GF | | markers | VFP- Blood | | VFP- Buccal swab | |
|---|---|---|---|---|---|---|---|---|
| | D2S1338 | 19 | 19 | D2S1338 | 19 | 19 | 19 | 19 |
| | | | | TPOX | 8 | 8 | 8 | 8 |
| **6** | D3S1358 | 17 | 18 | D3S1358 | 17 | 18 | 17 | 18 |
| | vWA | 14 | 14 | vWA | 14 | 14 | 14 | 14 |
| | D16S539 | 9 | 9 | D16S539 | 9 | 9 | 9 | 9 |
| | CSF1PO | 10 | 12 | CSF1PO | 10 | 12 | 10 | 12 |
| | TPOX | 8 | 8 | D6S1043 | 12 | 18 | 12 | 18 |
| | Yindel | | | Yindel | | | | |
| | AMEL | X | X | AMEL | X | X | X | X |
| | D8S1179 | 13 | 13 | D8S1179 | 13 | 13 | 13 | 13 |
| | D21S11 | 31 | 32 | D21S11 | 31 | 32 | 31 | 32 |
| | D18S51 | 12 | 16 | D18S51 | 12 | 16 | 12 | 16 |
| | DYS391 | | | D5S818 | 10 | 12 | 10 | 12 |
| | D2S441 | 10 | 12 | D2S441 | 10 | 12 | 10 | 12 |
| | D19S433 | 13.2 | 14 | D19S433 | 13.2 | 14 | 13.2 | 14 |
| | TH01 | 9 | 9 | FGA | 21 | 24 | 21 | 24 |
| | FGA | 21 | 24 | D10S1248 | 13 | 14 | 13 | 14 |
| | D22S1045 | 15 | 15 | D22S1045 | 15 | 15 | 15 | 15 |
| | D5S818 | 10 | 12 | D1S1656 | 16 | 16 | 16 | 16 |
| | D13S317 | 9 | 12 | D13S317 | 9 | 12 | 9 | 12 |
| | D7S820 | 11 | 13 | D7S820 | 11 | 13 | 11 | 13 |
| | SE33 | 25.2 | 27.2 | Penta E | 11 | 17 | 11 | 17 |
| | D10S1248 | 13 | 14 | Penta D | 10 | 11 | 10 | 11 |
| | D1S1656 | 16 | 16 | TH01 | 9 | 9 | 9 | 9 |
| | D12S391 | 18 | 25 | D12S391 | 18 | 25 | 18 | 25 |
| | D2S1338 | 19 | 21 | D2S1338 | 19 | 21 | 19 | 21 |
| | | | | TPOX | 8 | 8 | 8 | 8 |
| **7** | D3S1358 | 15 | 16 | D3S1358 | 15 | 16 | 15 | 16 |
| | vWA | 17 | 18 | vWA | 17 | 18 | 17 | 18 |
| | D16S539 | 11 | 12 | D16S539 | 11 | 12 | 11 | 12 |
| | CSF1PO | 12 | 12 | CSF1PO | 12 | 12 | 12 | 12 |
| | TPOX | 8 | 8 | D6S1043 | 12 | 12 | 12 | 12 |
| | Yindel | 2 | | Yindel | 2 | | 2 | |
| | AMEL | X | | AMEL | X | | X | |
| | D8S1179 | 12 | 12 | D8S1179 | 12 | 12 | 12 | 12 |
| | D21S11 | 28 | 29 | D21S11 | 28 | 29 | 28 | 29 |
| | D18S51 | 12 | 14 | D18S51 | 12 | 14 | 12 | 14 |
| | DYS391 | 10 | 10 | D5S818 | 12 | 12 | 12 | 12 |
| | D2S441 | 12 | 12 | D2S441 | 12 | 12 | 12 | 12 |
| | D19S433 | 13 | 15.2 | D19S433 | 13 | 15.2 | 13 | 15.2 |
| | TH01 | 6 | 8 | FGA | 21 | 21 | 21 | 21 |

Table 16: Concordance study with blood and buccal swab samples using GlobalFiler (GF) and Verifiler Plus (VFP) PCR kits (continued)

| Sample | markers | GF | | markers | VFP- Blood | | VFP- Buccal swab | |
|---|---|---|---|---|---|---|---|---|
| | FGA | 21 | 21 | D10S1248 | 14 | 14 | 14 | 14 |
| | D22S1045 | 11 | 17 | D22S1045 | 11 | 17 | 11 | 17 |
| | D5S818 | 12 | 12 | D1S1656 | 12 | 16 | 12 | 16 |
| | D13S317 | 11 | 13 | D13S317 | 11 | 13 | 11 | 13 |
| | D7S820 | 10 | 11 | D7S820 | 10 | 11 | 10 | 11 |
| | SE33 | 27.2 | 28.2 | Penta E | 7 | 7 | 7 | 7 |
| | D10S1248 | 14 | 14 | Penta D | 8 | 12 | 8 | 12 |
| | D1S1656 | 12 | 16 | TH01 | 6 | 8 | 6 | 8 |
| | D12S391 | 19 | 21 | D12S391 | 19 | 21 | 19 | 21 |
| | D2S1338 | 21 | 22 | D2S1338 | 21 | 22 | 21 | 22 |
| | | | | TPOX | 8 | 8 | 8 | 8 |
| | D3S1358 | 15 | 16 | D3S1358 | 15 | 16 | 15 | 16 |
| | vWA | 16 | 18 | vWA | 16 | 18 | 16 | 18 |
| | D16S539 | 9 | 11 | D16S539 | 9 | 11 | 9 | 11 |
| | CSF1PO | 11 | 12 | CSF1PO | 11 | 12 | 11 | 12 |
| | TPOX | 8 | 11 | D6S1043 | 11 | 11 | 11 | 11 |
| **8** | Yindel | | | Yindel | | | | |
| | AMEL | X | X | AMEL | x | x | X | x |
| | D8S1179 | 16 | 17 | D8S1179 | 16 | 17 | 16 | 17 |
| | D21S11 | 32.2 | 32.2 | D21S11 | 32.2 | 32.2 | 32.2 | 32.2 |
| | D18S51 | 14 | 15 | D18S51 | 14 | 15 | 14 | 15 |
| | DYS391 | | | D5S818 | 11 | 12 | 11 | 12 |
| | D2S441 | 14 | 14 | D2S441 | 14 | 14 | 14 | 14 |
| | D19S433 | 13 | 14 | D19S433 | 13 | 14 | 13 | 14 |
| | TH01 | 6 | 7 | FGA | 20 | 21 | 20 | 21 |
| | FGA | 20 | 21 | D10S1248 | 14 | 15 | 14 | 15 |
| | D22S1045 | 15 | 15 | D22S1045 | 15 | 15 | 15 | 15 |
| | D5S818 | 11 | 12 | D1S1656 | 15 | 16 | 15 | 16 |
| | D13S317 | 11 | 11 | D13S317 | 11 | 11 | 11 | 11 |
| | D7S820 | 10 | 10 | D7S820 | 10 | 10 | 10 | 10 |
| | SE33 | 14 | 29.2 | Penta E | 13 | 13 | 13 | 13 |
| | D10S1248 | 14 | 15 | Penta D | 11 | 11 | 11 | 11 |
| | D1S1656 | 15 | 16 | TH01 | 6 | 7 | 6 | 7 |
| | D12S391 | 20 | 20 | D12S391 | 20 | 20 | 20 | 20 |
| | D2S1338 | 19 | 23 | D2S1338 | 19 | 23 | 19 | 23 |
| | | | | TPOX | 8 | 11 | 8 | 11 |
| **9** | D3S1358 | 15 | 17 | D3S1358 | 15 | 17 | 15 | 17 |
| | vWA | 18 | 19 | vWA | 18 | 19 | 18 | 19 |
| | D16S539 | 11 | 13 | D16S539 | 11 | 13 | 11 | 13 |
| | CSF1PO | 11 | 12 | CSF1PO | 11 | 12 | 11 | 12 |
| | TPOX | 8 | 9 | D6S1043 | 11 | 19 | 11 | 19 |

Table 16: Concordance study with blood and buccal swab samples using GlobalFiler (GF) and Verifiler Plus (VFP) PCR kits (continued)

| Sample | markers | GF | | markers | VFP- Blood | | VFP- Buccal swab | |
|---|---|---|---|---|---|---|---|---|
| | Yindel | 2 | | Yindel | 2 | | 2 | |
| | AMEL | X | Y | AMEL | X | Y | X | Y |
| | D8S1179 | 13 | 14 | D8S1179 | 13 | 14 | 13 | 14 |
| | D21S11 | 30 | 34.2 | D21S11 | 30 | 34.2 | 30 | 34.2 |
| | D18S51 | 12 | 14 | D18S51 | 12 | 14 | 12 | 14 |
| | DYS391 | 11 | | D5S818 | 11 | 13 | 11 | 13 |
| | D2S441 | 11 | 14 | D2S441 | 11 | 14 | 11 | 14 |
| | D19S433 | 12 | 16.2 | D19S433 | 12 | 16.2 | 12 | 16.2 |
| | TH01 | 6 | 7 | FGA | 24 | 24 | 24 | 24 |
| | FGA | 24 | 24 | D10S1248 | 15 | 15 | 15 | 15 |
| | D22S1045 | 15 | 15 | D22S1045 | 15 | 15 | 15 | 15 |
| | D5S818 | 11 | 13 | D1S1656 | 15 | 17 | 15 | 17 |
| | D13S317 | 11 | 12 | D13S317 | 11 | 12 | 11 | 12 |
| | D7S820 | 8 | 10 | D7S820 | 8 | 10 | 8 | 10 |
| | SE33 | 27.2 | 33 | Penta E | 7 | 14 | 7 | 14 |
| | D10S1248 | 15 | 15 | Penta D | 12 | 13 | 12 | 13 |
| | D1S1656 | 15 | 17 | TH01 | 6 | 7 | 6 | 7 |
| | D12S391 | 15 | 22 | D12S391 | 15 | 22 | 15 | 22 |
| | D2S1338 | 18 | 24 | D2S1338 | 18 | 24 | 18 | 24 |
| | | | | TPOX | 8 | 9 | 8 | 9 |
| **10** | D3S1358 | 16 | 17 | D3S1358 | 16 | 17 | 16 | 17 |
| | vWA | 13 | 14 | vWA | 13 | 14 | 13 | 14 |
| | D16S539 | 9 | 11 | D16S539 | 9 | 11 | 9 | 11 |
| | CSF1PO | 11 | 12 | CSF1PO | 11 | 12 | 11 | 12 |
| | TPOX | 8 | 11 | D6S1043 | 11 | 13 | 11 | 13 |
| | Yindel | 2 | | Yindel | 2 | | 2 | |
| | AMEL | X | Y | AMEL | X | Y | X | Y |
| | D8S1179 | 13 | 14 | D8S1179 | 13 | 14 | 13 | 14 |
| | D21S11 | 27 | 30 | D21S11 | 27 | 30 | 27 | 30 |
| | D18S51 | 15 | 19 | D18S51 | 15 | 19 | 15 | 19 |
| | DYS391 | 10 | | D5S818 | 12 | 12 | 12 | 12 |
| | D2S441 | 11 | 14 | D2S441 | 11 | 14 | 11 | 14 |
| | D19S433 | 15 | 15.2 | D19S433 | 15 | 15.2 | 15 | 15.2 |
| | TH01 | 7 | 9 | FGA | 21 | 23 | 21 | 23 |
| | FGA | 21 | 23 | D10S1248 | 14 | 14 | 14 | 14 |
| | D22S1045 | 14 | 15 | D22S1045 | 14 | 15 | 14 | 15 |
| | D5S818 | 12 | 12 | D1S1656 | 11 | 11 | 11 | 11 |
| | D13S317 | 12 | 12 | D13S317 | 12 | 12 | 12 | 12 |
| | D7S820 | 10 | 11 | D7S820 | 10 | 11 | 10 | 11 |
| | SE33 | 17 | 35.2 | Penta E | 17 | 18 | 17 | 18 |
| | D10S1248 | 14 | 14 | Penta D | 9 | 14 | 9 | 14 |

Table 16: Concordance study with blood and buccal swab samples using GlobalFiler (GF) and Verifiler Plus (VFP) PCR kits (continued)

| Sample | markers | GF | | markers | VFP- Blood | | VFP- Buccal swab | |
|---|---|---|---|---|---|---|---|---|
| | D1S1656 | 11 | 11 | TH01 | 7 | 9 | 7 | 9 |
| | D12S391 | 22 | 24 | D12S391 | 22 | 24 | 22 | 24 |
| | D2S1338 | 17 | 17 | D2S1338 | 17 | 17 | 17 | 17 |
| | | | | TPOX | 8 | 11 | 8 | 11 |
| **11** | D3S1358 | 15 | 17 | D3S1358 | 15 | 17 | 15 | 17 |
| | vWA | 17 | 18 | vWA | 17 | 18 | 17 | 18 |
| | D16S539 | 11 | 12 | D16S539 | 11 | 12 | 11 | 12 |
| | CSF1PO | 10 | 12 | CSF1PO | 10 | 12 | 10 | 12 |
| | TPOX | 8 | 9 | D6S1043 | 12 | 17 | 12 | 17 |
| | Yindel | 2 | | Yindel | 2 | | 2 | |
| | AMEL | X | Y | AMEL | X | Y | X | Y |
| | D8S1179 | 13 | 14 | D8S1179 | 13 | 14 | 13 | 14 |
| | D21S11 | 29 | 31 | D21S11 | 29 | 31 | 29 | 31 |
| | D18S51 | 13 | 18 | D18S51 | 13 | 18 | 13 | 18 |
| | DYS391 | 10 | | D5S818 | 12 | 13 | 12 | 13 |
| | D2S441 | 11 | 14 | D2S441 | 11 | 14 | 11 | 14 |
| | D19S433 | 14 | 14.2 | D19S433 | 14 | 14.2 | 14 | 14.2 |
| | TH01 | 9 | 9 | FGA | 21 | 26 | 21 | 26 |
| | FGA | 21 | 26 | D10S1248 | 13 | 15 | 13 | 15 |
| | D22S1045 | 16 | 16 | D22S1045 | 16 | 16 | 16 | 16 |
| | D5S818 | 12 | 13 | D1S1656 | 16 | 16.3 | 16 | 16.3 |
| | D13S317 | 13 | 13 | D13S317 | 13 | 13 | 13 | 13 |
| | D7S820 | 10 | 10 | D7S820 | 10 | 10 | 10 | 10 |
| | SE33 | 17 | 20 | Penta E | 17 | 18 | 17 | 18 |
| | D10S1248 | 13 | 15 | Penta D | 9 | 14 | 9 | 14 |
| | D1S1656 | 16 | 16.3 | TH01 | 9 | 9 | 9 | 9 |
| | D12S391 | 22 | 22 | D12S391 | 22 | 22 | 22 | 22 |
| | D2S1338 | 20 | 21 | D2S1338 | 20 | 22 | 20 | 22 |
| | | | | TPOX | 8 | 9 | 8 | 9 |
| **12** | D3S1358 | 16 | 18 | D3S1358 | 16 | 18 | 16 | 18 |
| | vWA | 15 | 16 | vWA | 15 | 16 | 15 | 16 |
| | D16S539 | 12 | 12 | D16S539 | 12 | 12 | 12 | 12 |
| | CSF1PO | 5 | 11 | CSF1PO | 5 | 11 | 5 | 11 |
| | TPOX | 8 | 9 | D6S1043 | 12 | 19 | 12 | 19 |
| | Yindel | 2 | | Yindel | 2 | | 2 | |
| | AMEL | X | Y | AMEL | X | Y | X | Y |
| | D8S1179 | 14 | 14 | D8S1179 | 14 | 14 | 14 | 14 |
| | D21S11 | 29 | 31 | D21S11 | 29 | 31 | 29 | 31 |
| | D18S51 | 13 | 15 | D18S51 | 13 | 15 | 13 | 15 |
| | DYS391 | 11 | | D5S818 | 9 | 12 | 9 | 12 |
| | D2S441 | 11 | 14 | D2S441 | 11 | 14 | 11 | 14 |

Table 16: Concordance study with blood and buccal swab samples using GlobalFiler (GF) and Verifiler Plus (VFP) PCR kits (continued)

| Sample | markers | GF | | markers | VFP- Blood | | VFP- Buccal swab | |
|---|---|---|---|---|---|---|---|---|
| | D19S433 | 13 | 14 | D19S433 | 13 | 14 | 13 | 14 |
| | TH01 | 6 | 9 | FGA | 23 | 24 | 23 | 24 |
| | FGA | 23 | 24 | D10S1248 | 15 | 16 | 15 | 16 |
| | D22S1045 | 15 | 15 | D22S1045 | 15 | 15 | 15 | 15 |
| | D5S818 | 9 | 12 | D1S1656 | 15.3 | 16 | 15.3 | 16 |
| | D13S317 | 8 | 11 | D13S317 | 8 | 11 | 8 | 11 |
| | D7S820 | 9 | 11 | D7S820 | 9 | 11 | 9 | 11 |
| | SE33 | 14 | 17 | Penta E | 11 | 13 | 11 | 13 |
| | D10S1248 | 15 | 16 | Penta D | 9 | 15 | 9 | 15 |
| | D1S1656 | 15.3 | 16 | TH01 | 6 | 9 | 6 | 9 |
| | D12S391 | 18 | 24 | D12S391 | 18 | 24 | 18 | 24 |
| | D2S1338 | 16 | 20 | D2S1338 | 16 | 20 | 16 | 20 |
| | | | | TPOX | 8 | 9 | 8 | 9 |
| | D1S1656 | 15.3 | 16 | TH01 | 6 | 9 | 6 | 9 |
| | D12S391 | 18 | 24 | D12S391 | 18 | 24 | 18 | 24 |
| | D2S1338 | 16 | 20 | D2S1338 | 16 | 20 | 16 | 20 |
| | | | | TPOX | 8 | 9 | 8 | 9 |
| **13** | D3S1358 | 15 | 18 | D3S1358 | 15 | 18 | 15 | 18 |
| | vWA | 16 | 16 | vWA | 16 | 16 | 16 | 16 |
| | D16S539 | 10 | 12 | D16S539 | 10 | 12 | 10 | 12 |
| | CSF1PO | 11 | 11 | CSF1PO | 11 | 11 | 11 | 11 |
| | TPOX | 9 | 10 | D6S1043 | 11 | 12 | 11 | 12 |
| | Yindel | 2 | | Yindel | 2 | | 2 | |
| | AMEL | X | Y | AMEL | X | Y | X | Y |
| | D8S1179 | 14 | 16 | D8S1179 | 14 | 16 | 14 | 16 |
| | D21S11 | 28 | 30 | D21S11 | 28 | 30 | 28 | 30 |
| | D18S51 | 14 | 16 | D18S51 | 14 | 16 | 14 | 16 |
| | DYS391 | 10 | | D5S818 | 10 | 12 | 10 | 12 |
| | D2S441 | 14 | 14 | D2S441 | 14 | 14 | 14 | 14 |
| | D19S433 | 12 | 13.2 | D19S433 | 12 | 13.2 | 12 | 13.2 |
| | TH01 | 9 | 9 | FGA | 21 | 24 | 21 | 24 |
| | FGA | 21 | 24 | D10S1248 | 14 | 15 | 14 | 15 |
| | D22S1045 | 15 | 16 | D22S1045 | 15 | 16 | 15 | 16 |
| | D5S818 | 10 | 12 | D1S1656 | 11 | 11 | 11 | 11 |
| | D13S317 | 11 | 11 | D13S317 | 11 | 11 | 11 | 11 |
| | D7S820 | 11 | 13 | D7S820 | 11 | 13 | 11 | 13 |
| | SE33 | 30.2 | 31.2 | Penta E | 9 | 12 | 9 | 12 |
| | D10S1248 | 14 | 15 | Penta D | 12 | 12 | 12 | 12 |
| | D1S1656 | 11 | 11 | TH01 | 9 | 9 | 9 | 9 |
| | D12S391 | 24 | 26 | D12S391 | 24 | 26 | 24 | 26 |
| | D2S1338 | 17 | 20 | D2S1338 | 17 | 20 | 17 | 20 |

Table 16: Concordance study with blood and buccal swab samples using GlobalFiler (GF) and Verifiler Plus (VFP) PCR kits (continued)

| Sample | markers | GF | | markers | VFP- Blood | | VFP- Buccal swab | |
|---|---|---|---|---|---|---|---|---|
| | | | | TPOX | 9 | 10 | 9 | 10 |
| **14** | D3S1358 | 16 | 17 | D3S1358 | 16 | 17 | 16 | 17 |
| | vWA | 15 | 16 | vWA | 15 | 16 | 15 | 16 |
| | D16S539 | 11 | 14 | D16S539 | 11 | 14 | 11 | 14 |
| | CSF1PO | 11 | 13 | CSF1PO | 11 | 13 | 11 | 13 |
| | TPOX | 9 | 9 | D6S1043 | 11 | 14 | 11 | 14 |
| | Yindel | | | Yindel | | | | |
| | AMEL | X | X | AMEL | X | X | X | X |
| | D8S1179 | 10 | 13 | D8S1179 | 10 | 13 | 10 | 13 |
| | D21S11 | 30 | 32.2 | D21S11 | 30 | 32.2 | 30 | 32.2 |
| | D18S51 | 13 | 18 | D18S51 | 13 | 18 | 13 | 18 |
| | DYS391 | | | D5S818 | 11 | 11 | 11 | 11 |
| | D2S441 | 11 | 14 | D2S441 | 11 | 14 | 11 | 14 |
| | D19S433 | 13 | 15 | D19S433 | 13 | 15 | 13 | 15 |
| | TH01 | 6 | 9 | FGA | 22 | 24 | 22 | 24 |
| | FGA | 22 | 24 | D10S1248 | 14 | 14 | 14 | 14 |
| | D22S1045 | 11 | 12 | D22S1045 | 11 | 12 | 11 | 12 |
| | D5S818 | 11 | 11 | D1S1656 | 12 | 16 | 12 | 16 |
| | D13S317 | 10 | 12 | D13S317 | 10 | 12 | 10 | 12 |
| | D7S820 | 9 | 11 | D7S820 | 9 | 11 | 9 | 11 |
| | SE33 | 27.2 | 32.2 | Penta E | 11 | 12 | 11 | 12 |
| | D10S1248 | 14 | 14 | Penta D | 13 | 14 | 13 | 14 |
| | D1S1656 | 12 | 16 | TH01 | 6 | 9 | 6 | 9 |
| | D12S391 | 20 | 21 | D12S391 | 20 | 21 | 20 | 21 |
| | D2S1338 | 20 | 21 | D2S1338 | 20 | 21 | 20 | 21 |
| | | | | TPOX | 9 | 9 | 9 | 9 |
| **15** | D3S1358 | 15 | 16 | D3S1358 | 15 | 16 | 15 | 16 |
| | vWA | 16 | 16 | vWA | 16 | 16 | 16 | 16 |
| | D16S539 | 11 | 11 | D16S539 | 11 | 11 | 11 | 11 |
| | CSF1PO | 12 | 12 | CSF1PO | 12 | 12 | 12 | 12 |
| | TPOX | 8 | 8 | D6S1043 | 19 | 19 | 19 | 19 |
| | Yindel | | | Yindel | | | | |
| | AMEL | X | X | AMEL | X | X | X | X |
| | D8S1179 | 10 | 12 | D8S1179 | 10 | 12 | 10 | 12 |
| | D21S11 | 31 | 31.2 | D21S11 | 31 | 31.2 | 31 | 31.2 |
| | D18S51 | 16 | 16 | D18S51 | 16 | 16 | 16 | 16 |
| | DYS391 | | | D5S818 | 11 | 12 | 11 | 12 |
| | D2S441 | 11 | 15 | D2S441 | 11 | 15 | 11 | 15 |
| | D19S433 | 13 | 13 | D19S433 | 13 | 13 | 13 | 13 |
| | TH01 | 6 | 9 | FGA | 22 | 25 | 22 | 25 |
| | FGA | 22 | 25 | D10S1248 | 13 | 16 | 13 | 16 |

Table 16: Concordance study with blood and buccal swab samples using GlobalFiler (GF) and Verifiler Plus (VFP) PCR kits (continued)

| Sample | markers | GF | | markers | VFP- Blood | | VFP- Buccal swab | |
|---|---|---|---|---|---|---|---|---|
| | D22S1045 | 11 | 16 | D22S1045 | 11 | 16 | 11 | 16 |
| | D5S818 | 11 | 12 | D1S1656 | 13 | 14.2 | 13 | 14.2 |
| | D13S317 | 11 | 12 | D13S317 | 11 | 12 | 11 | 12 |
| | D7S820 | 8 | 12 | D7S820 | 8 | 12 | 8 | 12 |
| | SE33 | 17 | 22 | Penta E | 11 | 20 | 11 | 20 |
| | D10S1248 | 13 | 16 | Penta D | 10 | 12 | 10 | 12 |
| | D1S1656 | 13 | OL | TH01 | 6 | 9 | 6 | 9 |
| | D12S391 | 17 | 20 | D12S391 | 17 | 20 | 17 | 20 |
| | D2S1338 | 17 | 23 | D2S1338 | 17 | 23 | 17 | 23 |
| | | | | TPOX | 8 | 8 | 8 | 8 |
| **16** | D3S1358 | 15 | 18 | D3S1358 | 15 | 18 | 15 | 18 |
| | vWA | 14 | 16 | vWA | 14 | 16 | 14 | 16 |
| | D16S539 | 12 | 12 | D16S539 | 12 | 12 | 12 | 12 |
| | CSF1PO | 10 | 12 | CSF1PO | 10 | 12 | 10 | 12 |
| | TPOX | 9 | 10 | D6S1043 | 13 | 14 | 13 | 14 |
| | Yindel | 2 | | Yindel | 2 | | 2 | |
| | AMEL | X | Y | AMEL | X | Y | X | Y |
| | D8S1179 | 12 | 13 | D8S1179 | 12 | 13 | 12 | 13 |
| | D21S11 | 28 | 29 | D21S11 | 28 | 29 | 28 | 29 |
| | D18S51 | 12 | 14 | D18S51 | 12 | 14 | 12 | 14 |
| | DYS391 | 10 | | D5S818 | 11 | 12 | 11 | 12 |
| | D2S441 | 11 | 14 | D2S441 | 11 | 14 | 11 | 14 |
| | D19S433 | 13 | 15 | D19S433 | 13 | 15 | 13 | 15 |
| | TH01 | 7 | 9 | FGA | 20 | 25 | 20 | 25 |
| | FGA | 20 | 25 | D10S1248 | 14 | 15 | 14 | 15 |
| | D22S1045 | 15 | 15 | D22S1045 | 15 | 15 | 15 | 15 |
| | D5S818 | 11 | 12 | D1S1656 | 13 | 16.3 | 13 | 16.3 |
| | D13S317 | 8 | 10 | D13S317 | 8 | 10 | 8 | 10 |
| | D7S820 | 11 | 11 | D7S820 | 11 | 11 | 11 | 11 |
| | SE33 | 15 | 18 | Penta E | 12 | 20 | 12 | 20 |
| | D10S1248 | 14 | 15 | Penta D | 2.2 | 9 | 2.2 | 9 |
| | D1S1656 | 13 | 16.3 | TH01 | 7 | 9 | 7 | 9 |
| | D12S391 | 21 | 24 | D12S391 | 21 | 24 | 21 | 24 |
| | D2S1338 | 17 | 19 | D2S1338 | 17 | 19 | 17 | 19 |
| | | | | TPOX | 9 | 10 | 9 | 10 |

Note: Markers in red represent the difference between the two PCR kits.