M-Vac®-Collected Mixture Samples Deconvoluted by STRmix™

By

KATHERINE HAMRICK
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Approved:

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Kent Pinkerton, Chair

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Ruth Dickover

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Rhonda Roby

Committee in Charge

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**Abstract**

Traditionally, forensic evidence items have been sampled using methods such as the double swab technique and any resulting profiles have been deconvoluted by conventional methods. However, some evidence is difficult to sample using traditional forensic methods. For example, the double swab technique, a wet swabbing followed by a dry swabbing, may not effectively collect biological evidence from large surface areas such as a shirt or from grooves and crevices of rough surfaces. In addition, the ability of either the wet or dry swab to absorb liquid could potentially impact how much DNA is retrieved from either swab for later DNA testing. The M-Vac® Wet-Vacuum System is a sampling technique that can cover large, rough, and/or porous surface areas that could otherwise prove challenging from which to collect cells.

However, as sampling methods continue to be improved upon so that they collect more material, complex mixtures are more likely to be obtained. Complex mixtures can be difficult for an analyst to interpret manually. A tool that can aid an analyst in deconvoluting mixtures is STRmix™, a probabilistic genotyping software program. Probabilistic genotyping utilizes algorithms which apply statistical theory, biological modeling, and probability ratios to generate likelihood ratios for genotypes in a single-source or mixed DNA sample. Hence, STRmix™ probabilistic genotyping software was used to evaluate these complex mixtures.

Neat saliva from four donors was prepared in an equal mixture ratio by volume and diluted to 1:3, 1:10, 1:30, and 1:100. For each dilution, 1 mL was pipetted onto three 6” x 6” sterile cotton swatches, dried, and then sampled using the M-Vac® to evaluate the robustness of the M-Vac® collection system. Three additional 1:3 saliva sample swatches were prepared and sampled using the double swab technique. Control samples were used to estimate how much DNA is lost from sampling, and the amount of DNA recovered from the different sampling

techniques was compared. In addition, six denim swatches were spotted with 1 mL of the 1:3 saliva dilution. Three swatches were sampled with the double swab technique and three with the M-Vac®. Finally, 1 mL of the 1:3 saliva dilution was spotted on six bricks. Three bricks each were sampled with the double swab technique and with the M-Vac®.

This study evaluated the performance and utility of the M-Vac® Pre-Filter system and the M-Vac® system in the collection of DNA from different substrates. The amount of DNA recovered by using the M-Vac® was significantly more than that obtained by the double swab technique from cotton fabric and denim fabric. However, the amount of DNA recovered by using the M-Vac® was similar to that obtained by the double swab technique from brick. In addition, some DNA loss was detected when using the M-Vac® Pre-Filter system. Furthermore, this research determined that mixture samples collected with the M-Vac® from cotton fabric, denim fabric, and brick can be deconvoluted with greater efficiency using STRmix™ than manual interpretation of DNA profiles. More contributors were identified and more mixtures were able to be analyzed when combining the M-Vac® collection technique with STRmix™ probabilistic genotyping.

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## Introduction

Choosing sampling methods that are appropriate for DNA evidence is important to the field of forensic sciences. Traditional sampling methods may still perform well, but on certain substrates, their ability to collect DNA may be limited. One example of a traditional sampling method is the double swab technique. In the double swab technique, a wet swab is first rolled back and forth over an object's surface to hydrate the area and collect cellular material, and then a dry swab is rolled back and forth over the area to absorb and collect more cellular material (Sweet *et al.* 1997). The double swab technique can collect cells from various surfaces. However, the double swab technique has drawbacks. For example, the double swab technique is not an ideal technique for collecting cells from grooves or crevices in surfaces. In addition, the effectiveness of the wet and dry swabs in absorbing liquid during sampling can result in how much DNA is eventually extracted from them (Hanson and Ballantyne 2013). An alternative to the double swab technique is the M-Vac® System (M-Vac Systems, Inc., Sandy, UT). The M-Vac® functions by spraying solution from its sampling head onto a surface while simultaneously vacuuming up the solution and any cellular material. The M-Vac®, a vacuum system, can be used to sample from large and/or uneven surface areas, while the double swab technique is best suited for use on smaller surface areas (Garrett *et al.* 2014; Hedman *et al.* 2015). Even when cells are recovered using the double swab technique, the resulting DNA profiles may consist of complex, difficult-to-interpret mixtures resulting from several people handling the same object or touching the same surface (van Oorschot and Jones 1997).

If traditional methods such as the double swab technique are unable to collect cells from an object or surface, an alternative sampling method would be preferred. One such method involves using the M-Vac® Microbial Wet-Vacuum System (Figure 1). In brief, the M-Vac®

works by spraying a Surface Rinse Solution (M-Vac Systems, Inc.) (SRS) onto a surface to hydrate and loosen cells while simultaneously vacuuming the SRS along with cells. The M-Vac®-collected sample is then vacuum-filtered through either a 0.45 µm or 0.20 µm polyethersulfone (PES) vacuum filter, also called the M-Vac® Filter (M-Vac® Systems, Inc.). Because the M-Vac® uses a wet-vacuum, it can effectively collect cells from rough, porous surfaces that are difficult to sample from using the double swab technique (McLamb *et al.* 2020; Vickar *et al.* 2018).

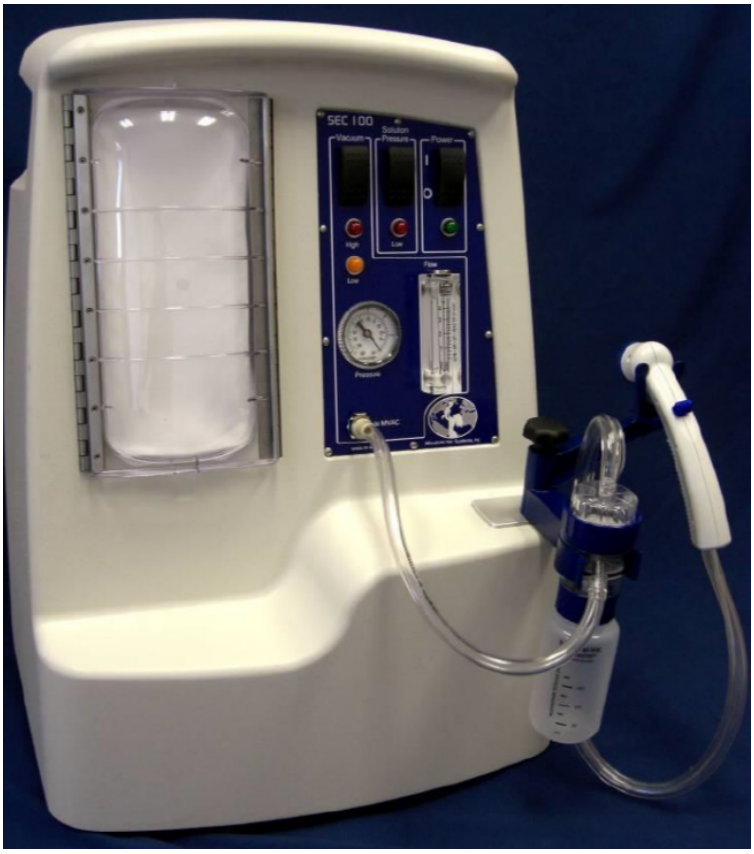


**Figure 1.** The M-Vac® System (M-Vac® Systems, Inc.).

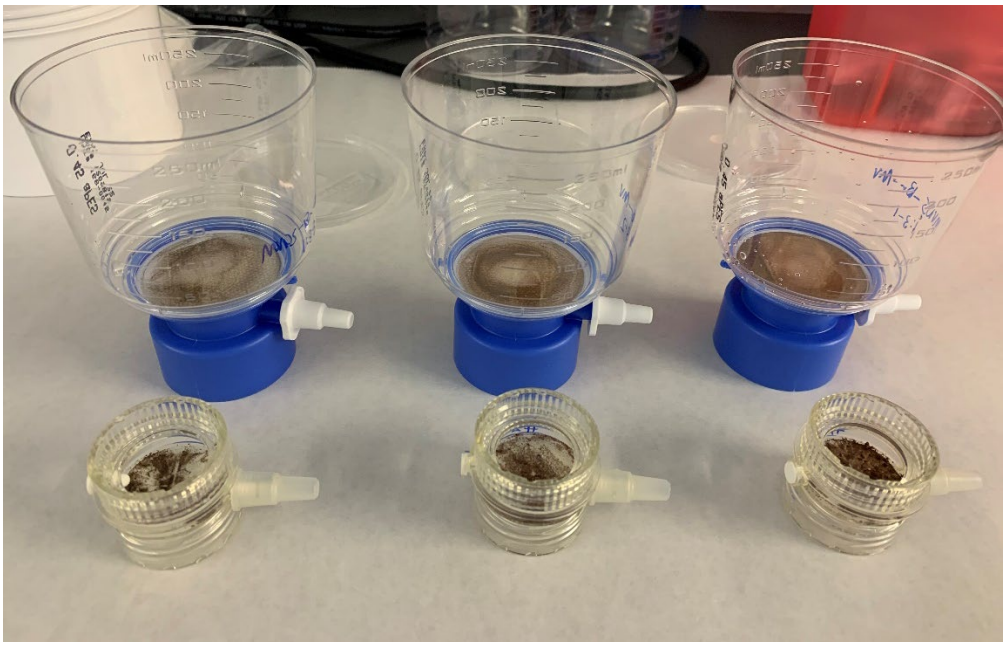

**Figure 2.** Used M-Vac® Pre-Filters (foreground) and used M-Vac® Filters (background).

An optional M-Vac® Pre-Filter can be used after a sample has been collected by the M-Vac®, but before it is vacuum-filtered through the M-Vac® Filter (Figure 2). The M-Vac® Pre-Filter is used by vacuum-filtering M-Vac®-collected sample first through the M-Vac® Pre-Filter, which is designed to trap particles larger than 40 µm while anything smaller flows through and is collected as filtrate in a 50 mL conical tube connected below the M-Vac® Pre-Filter. The resulting filtrate is then vacuum-filtered through the M-Vac® Filter (M-Vac® Systems, Inc.). While the purpose of the M-Vac® Pre-Filter is to remove large debris in the sample, there are concerns that cells could be trapped in the M-Vac® Pre-Filter along with the debris (M-Vac® Systems, Inc.). One component of this research project seeks to determine how much DNA can be extracted from the M-Vac® Pre-Filter in comparison to the M-Vac® Filter. The results of this study would better inform crime laboratory personnel or scientists as to how much DNA loss can be expected when using an M-Vac® Pre-Filter, so that a more informed decision on the use of an M-Vac® Pre-Filter on M-Vac®-collected samples can be made.

After sample collection is performed, the collected cellular material is extracted for DNA and then quantified. For DNA samples that are expected to have low amounts of DNA, the resulting extracts can undergo a concentration step conducted between extraction and quantification to increase the DNA concentration in the eluent. This step is useful for those samples suspected of having little cellular material at collection. DNA concentration can be performed by using a concentrator such as the SpeedVac™ Vacuum Concentrator (ThermoFisher Scientific, Waltham, MA). The SpeedVac™ works by using a combination of a vacuum, heat, and centrifugation, which, by decreasing the amount of liquid in the DNA extract, increases the total concentration of the DNA in the extract. M-Vac®-collected samples in particular are expected to need this additional step, due to the large volume of SRS initially collected along with the cells, and because the M-Vac® is most likely to be used on objects or surfaces from which little or no cells are recovered using traditional sampling methods. After quantification, short tandem repeats (STRs) in DNA are amplified. Next, the amplified DNA samples undergo capillary electrophoresis (CE), in which amplification products are separated by a CE instrument. The resulting information is output as an electropherogram, a pictorial representation of CE data that displays alleles amplified for STR loci. This electropherogram is then analyzed using genotyping software that calls alleles which correspond to the number of repeats for each STR locus so as to create a DNA profile for that sample.

The resulting DNA profile may exhibit a mixture; mixtures may need to be deconvoluted so that useful information can be obtained. The traditional method of deconvolution is manual deconvolution, in which an analyst uses their training and experience to determine the number of contributors to a DNA profile as well as which alleles correspond to which contributors. Manual deconvolution of DNA profiles can be useful to distinguish mixtures of two or three people.

However, there are limits to how much information can be obtained from manual deconvolutions, such as when there are three or more contributors to a DNA profile, when the DNA profile contains complex mixtures in which peaks have similar heights that make it difficult to assign specific peaks to specific contributors, or when the DNA results are low level and exhibit dropout (Moretti *et al.* 2017). In addition, the crime laboratory protocols have defined limits on which DNA profiles can be manually interpreted based on the number of contributors. For example, a crime laboratory's protocol for manual interpretation may only allow analysts to interpret single-source samples and mixtures of two people. Given that restriction, an analyst from that crime laboratory would not be able to manually deconvolute mixtures of three or more people, and if no other method of deconvolution is available, they may not be able to interpret a mixture of three or more people at all.

One alternative to manual interpretation is probabilistic genotyping. Probabilistic genotyping software uses biological modeling and computer algorithms to compare the likelihood that an individual has contributed to a mixture to the likelihood that a person unrelated to the individual but who shares their alleles at STR loci in their DNA profile contributed to the mixture (SWGDAM 2015). One example of probabilistic genotyping software is STRmix™ (ESR, Porirua, New Zealand). STRmix™ is a fully continuous probabilistic genotyping software program, which means that it takes all available information (including peak heights and stutter) into consideration in addition to allele calls when it deconvolutes a DNA profile (Bright *et al.* 2016). STRmix™ has been shown to be able to deconvolute mixtures of two to five profiles. These complex mixtures could prove to be difficult or impossible to interpret using manual interpretation (Bright *et al.* 2016). As a result of deconvoluting a DNA profile, STRmix™ produces likelihood ratios (LRs) that indicate how likely it is that a certain genotype at a certain

locus is an actual genotype from a contributor to a DNA profile. When comparing a deconvolution to one or more reference profiles, LRs indicate how likely a donor of a reference profile is to be included or excluded as a contributor to a DNA profile (Bright *et al.* 2016; Buckleton *et al.* 2019; Russell *et al.* 2019). A likelihood ratio is defined as the likelihood that the reference profile donor is a contributor to the DNA profile (the prosecution proposition, $H_p$) divided by the likelihood that the reference profile donor is not a contributor to the DNA profile (the defense proposition, $H_d$). In addition, SWGDAM has formulated a set of verbal qualifiers that correspond to the size of the LRs, which are displayed in the table below.

| **LR for $H_p$ Support (or 1/LR for $H_d$ support)** | **SWGDAM Verbal Qualifier** |
|---|---|
| 1 | Uninformative |
| 2-99 | Limited Support |
| 100-9,999 | Moderate Support |
| 10,000-999,999 | Strong Support |
| 1,000,000 or higher | Very Strong Support |

**Table 1.** SWGDAM verbal qualifiers and the LR values with which they are associated (SWGDAM 2018).

The purpose of this project is to investigate whether a combination of a more recent sampling method (the M-Vac®) and a more recent method of deconvolution (STRmix™ probabilistic genotyping software) can provide more information about mixture samples collected from cotton fabric, denim, and brick than the combination of a traditional sampling method (the double swab technique) and a traditional method of deconvolution (manual deconvolution). The results of this project could help crime laboratories understand the conditions traditional methods and more recent methods for sampling and deconvolution perform best. The results of this project will also demonstrate that DNA profiles obtained from M-Vac®-collected samples can be effectively extracted, concentrated, amplified, and deconvoluted using STRmix™ probabilistic genotyping software.

**Hypotheses:**

**$H_1$** Controlled mixed DNA samples collected by the M-Vac® System and subjected to interpretation with the use of STRmix™ probabilistic genotyping software will provide more information than the respective double swab and manual interpretation techniques.

**$H_0$** Controlled mixed DNA samples collected by the M-Vac® System and subjected to interpretation with the use of STRmix™ probabilistic genotyping software will not provide more information than the respective double swab and manual interpretation techniques.

Multiple statistical methods available in Microsoft Excel were used to test these hypotheses. First, a two-way ANOVA was conducted using two treatments (either the double swab technique or M-Vac® sample collection) using three sample types (cotton fabric, denim fabric, or brick) to evaluate whether the combination of specific sample types and specific treatments, rather than just the sampling method itself, could cause a significant difference in the amount of DNA that was obtained during sampling. Next, a one-way ANOVA was conducted on samples from all substrate types (cotton fabric, denim fabric, or brick) collected using the double swab technique from a 1:3 dilution of a four-person saliva mixture. This one-way ANOVA was conducted to compare DNA yield of the 1:3 diluted four-person saliva mixture samples collected with the double swab technique across the different sample types. A separate one-way ANOVA was conducted on samples from all substrate types (cotton fabric, denim fabric, or brick) collected using the M-Vac® on the 1:3 dilution of the four-person saliva mixture. This one-way ANOVA was conducted to compare DNA yield of the 1:3 diluted four-person saliva mixture samples collected with the M-Vac® across different substrates.

Two-sample t-tests assuming unequal variances were also used for comparison. One set of two-sample t-tests assuming unequal variances compared the double swab technique to the M-Vac® across different substrates upon which samples of the 1:3 dilution of the four-person saliva were deposited. These samples were compared to determine whether there were significant differences in how much DNA the double swab technique and the M-Vac® technique collected on different substrates. A second set of two-sample t-tests assuming unequal variances compared how much DNA the same sampling method (either the double swab technique or the M-Vac®) collected from the same samples of the 1:3 dilution of the four-person mixture across different substrates (cotton fabric, denim fabric, or brick). These samples were compared to determine whether there were significant differences in how much DNA the same sampling method collected across different substrates. A third set of two-sample t-tests assuming unequal variances compared how much DNA was collected by the M-Vac® from different sample dilutions (1:3, 1:10, 1:30, or 1:100) of the four-person saliva mixture on the same cotton fabric substrate. These samples were compared to determine whether there were significant differences in how much DNA the M-Vac® was able to collect from different dilutions of the four-person saliva mixture on the same substrate.

## Materials and Methods

### Methods Used Across Multiple Studies

#### DNA Collection Via the Double Swab Technique:

The double swab technique published by Sweet *et al.* was used in this study. First, one Puritan Sterile Cotton Tipped Applicator (Puritan Medical Products, Guilford, ME) was taken out of a package of two applicators, which will henceforth be referred to as "swabs." An AddiPak® Sterile Water Solution Unit (Teleflex Incorporated, Wayne, PA) was then used to wet the first swab. The wet swab was then rolled back and forth over the surface of the fabric square or brick. The second dry swab from the package was rolled back and forth over the area where the wet swab had been rolled. Both the wet and dry swabs were inserted into a swab rack to dry.

#### DNA Collection Via the M-Vac® System:

Before sample collection, the M-Vac® sample head was connected to the vacuum tube from the M-Vac® system and its SRS fluid tube was connected to the SRS Buffer held in the M-Vac® system's buffer chamber. Either a reusable plastic board or a single-use portion of Polyshield® Fluid Barrier (VWR, Radnor, PA) was cleaned by wiping it with 70% ethanol and then set on top of butcher paper. The fabric square or brick was then set on the cleaned surface.

The M-Vac® system's power switch was then powered on, and then the M-Vac® sampling head was positioned over the surface of the fabric square or brick. After the M-Vac®'s solution pressure switch and then its vacuum switch were powered on, the M-Vac® sampling head was used to sample the surface of the fabric square or brick using a unidirectional sampling method.

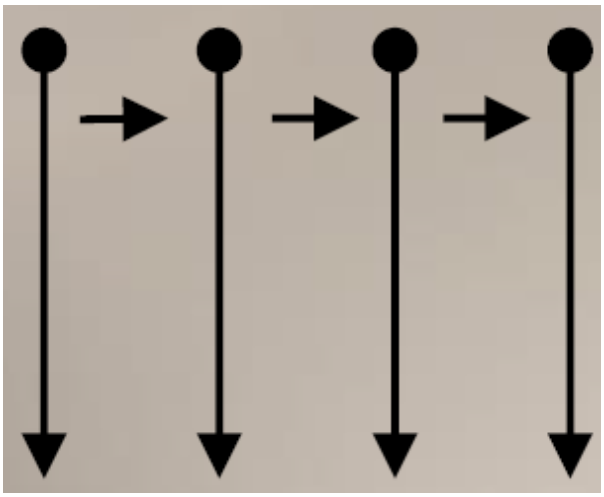

**Figure 3.** The unidirectional sampling method for the M-Vac® (M-Vac® Systems, Inc.).

In this unidirectional sampling method, the M-Vac® sampling head was rubbed in a line in one direction across the surface of the fabric square or brick. The M-Vac® sampling head was then rubbed in another line in one direction across the surface of the fabric square or brick, so that it was parallel to but partially overlapped the previous line. This sampling technique was repeated until the entire surface of the fabric square or brick was sampled. The surface of the fabric square or brick was then sampled again using this same sampling technique until 150 mL of SRS fluid was collected in the M-Vac® sample collection bottle.

**DNA Extraction:**

For samples extracted using Incubation Buffer (Promega Corporation, Madison, WI), the following extraction protocol was followed (Appendix C). Each solid sample was placed in a Nucleospin® Forensic Filter (Macherey-Nagel, Düren, Germany) (forensic filter) set inside a Costar® 2 mL Snap Cap Microcentrifuge Polypropylene Tube (dolphin nose). Digestion Master Mix was prepared so that there were 190 µL Incubation Buffer and 10 µL proteinase K for each sample. A total of 200 µL Digestion Master Mix was added to each sample, incubated at 56°C for 60-90 minutes, and then centrifuged at 15,000 RPM for 10 minutes. After removing the forensic filter, 400 µL Lysis Buffer was added to each sample tube and mixed. Each sample was

then pipetted into its own DNA IQ™ Casework Pro Kit for Maxwell® 16 cartridge (Promega Corporation), and then all samples were extracted using the Maxwell® 16 Instrument (Promega Corporation). All samples were then eluted in 50 µL Elution Buffer (Promega Corporation).

For samples extracted using Casework Extraction Buffer (Promega Corporation), the following extraction protocol was followed. All solid samples were placed in a Nucleospin® Forensic Filter set inside a Costar® 2 mL Snap Cap Microcentrifuge Polypropylene Tube (dolphin nose). Digestion Master Mix was prepared with 386 µL Casework Extraction Buffer (Promega Corporation), 10 µL 18 mg/mL proteinase K, and 4 µL 1-Thioglycerol for each sample. A total of 400 µL Digestion Master Mix was incubated at 56°C for 60 to 90 minutes, and then centrifuged at 15,000 RPM for 10 minutes. After removing the Nucleospin® Forensic Filter, 200 µL Lysis Buffer (Promega Corporation) was added to each sample tube and mixed. Each sample was then pipetted into its own Maxwell® 16 cartridge, and then all samples were extracted using the Maxwell® 16. All samples were then eluted in either 50 µL Elution Buffer (Promega Corporation) or 50 µL UltraPure™ Water (Thermo Fisher, Waltham, MA), depending on the sample type.

**DNA Quantification:**

QIAgility Robotics (Qiagen, Germantown, MD) were used to aliquot small portions of samples onto a MicroAmp® Optical 96-Well Reaction Plate (Thermo Fisher Scientific, Carlsbad, CA) and prepare these aliquots for quantification. A set of dilutions from 50 ng/µL PowerQuant® Male gDNA Standard from the PowerQuant® System kit (Promega Corporation) was also prepared using the QIAgility and pipetted onto the same 96-well plate, to serve as standards for comparison with the samples. The 96-well plate with prepared samples, standards,

and controls was run in an Applied Biosystems® 7500 Real-Time PCR System (Applied Biosystems, South San Francisco, CA).

**DNA Amplification:**

QIAgility Robotics were used to pipette samples onto a 96-well plate and prepare them for amplification. Using PowerPlex® Fusion 6C Systems (Promega Corporation), 1 ng of DNA per amplification was targeted. The 9700 Thermal Cycler (Applied Biosystems) was used to amplify the samples.

**STR Fragment Analysis Setup:**

QIAgility Robotics were used to pipette amplified samples onto a 96-well plate and prepare them for capillary electrophoresis. The amplified samples were then electrophoresed on the Applied Biosystems® 3500 Genetic Analyzer (Applied Biosystems).

**Initial Concentration Studies**

Previously collected DNA samples from the same donor (X19-19) were pooled in one tube. A total of 100 µL of this pooled sample was then pipetted into each of two tubes. In addition, previously collected reagent blanks corresponding to the previously collected DNA samples were also pooled in one tube, and 100 µL of pooled reagent blank was then pipetted into each of two tubes. A 1:10 dilution of the pooled sample as well as a 1:10 dilution of the pooled reagent blank was made. Next, a 1:1 dilution of this 1:10 dilution was made to make a 1:20 dilution for both the pooled sample and the pooled reagent blank. A total of 100 µL of the 1:10 sample dilution was pipetted into one tube, 100 µL of the 1:10 reagent blank dilution was pipetted into a second tube, 100 µL of the 1:20 sample dilution was pipetted into a third tube, and 100 µL of the 1:20 reagent blank was pipetted into a fourth tube.

Before any samples or reagent blanks were put in, the SpeedVac™ Vacuum Concentrator was pre-heated at medium heat for ten minutes. Next, the pooled sample and its corresponding reagent blank were put into the SpeedVac™ for 22 minutes at medium heat. After 22 minutes, the two tubes were removed and the volume of liquid left in each tube was measured. UltraPure™ water was then added to the sample and its reagent blank to bring both to a volume of 50 µL. Next, the other pooled sample and its corresponding reagent blank were put into the SpeedVac™ for 35 minutes at medium heat. After 35 minutes, the two tubes were removed and the volume of liquid left in each tube was measured. UltraPure™ Water was then added to the sample and its reagent blank to bring both to a volume of 25 µL.

The 1:10 sample dilution and the 1:10 reagent blank dilution were put into the SpeedVac™ for 22 minutes at medium heat. After 22 minutes, the two tubes were removed and the volume of liquid left in each tube was measured. UltraPure™ Water was then added to the 1:10 sample dilution and its 1:10 reagent blank dilution to bring both to a volume of 50 µL. The 1:20 sample dilution and the 1:20 reagent blank dilution were put into the SpeedVac™ for 35 minutes at medium heat. After 35 minutes, the two tubes were removed and the volume of liquid left in each tube was measured. UltraPure™ Water was then added to the 1:20 sample dilution and its 1:20 reagent blank dilution to bring both to a volume of 25 µL. The samples and a reagent blank were extracted on Maxwell® 16 instruments according to the Alameda County Sheriff's Office Crime Laboratory FBU protocol, and all samples were eluted in 50 µL Elution Buffer. The samples and a reagent blank were then quantified and amplified according to the Alameda County Sheriff's Office Crime Laboratory FBU protocol, and DNA profiles for each sample were obtained using capillary electrophoresis.

**Investigating Potential DNA Loss Caused by the M-Vac® Pre-Filter System**

Saliva was obtained from a donor (X19-63). After collecting two aliquots each of 20 µL neat saliva and 100 µL neat saliva, the remaining saliva was diluted 1:10 by volume with PBS. Positive controls were prepared by pipetting 3 mL of the 1:10 diluted saliva onto each of three M-Vac® filters. Each filter was then vacuum-filtered and dried in a laminar flow hood. A negative control was prepared by collecting 150 mL of SRS solution using the M-Vac® (Microbial-Vac Systems Inc., Sandy, UT), vacuum-filtering it through an M-Vac® Pre-Filter, then vacuum-filtering it through an M-Vac® filter, and finally drying the filter in a laminar flow hood.

Samples were prepared by pipetting 3 mL of the 1:10 diluted saliva onto each of three fabric squares, cut from a woven green cotton sweater. Three days later, a sample was collected from each fabric square using ~125 mL SRS and the M-Vac®. Each sample was vacuum-filtered through an M-Vac® Pre-Filter and then vacuum-filtered through an M-Vac® filter. Finally, each sample's M-Vac® Pre-Filter and M-Vac® filter were dried in a laminar flow hood.

After drying overnight, each sample's M-Vac® filter was cut out, each filter was divided in half, and each half of a filter was put in a Nucleospin® Forensic Filter in its own extraction tube. For each sample, fibers sitting on top of the M-Vac® Pre-Filter were put into a Nucleospin® Forensic Filter in their own extraction tube. Then, for each sample, the membrane of the M-Vac® Pre-Filter was cut out and put into a Nucleospin® Forensic Filter in its own extraction tube.

The negative control, positive controls, and samples were extracted on Maxwell® 16 instruments according to the Alameda County Sheriff's Office Crime Laboratory FBU protocol, and all samples were eluted in 50 µL Elution Buffer. For each sample, extracts from each half of the M-Vac® filter were combined after extraction. The negative control, positive controls, and

samples were then quantified according to the Alameda County Sheriff's Office Crime Laboratory FBU protocol. The volumes of the negative control, positive controls, and samples were measured after quantification.

After quantification, for each sample, the extracts of the pre-filter fibers, pre-filter membrane, and filter were combined into the same tube. 100 µL of combined sample was aliquoted into a new tube for each sample. Next, the 100 µL sample aliquots were concentrated. First, the SpeedVac™ was pre-heated at medium heat for 10 minutes, and then the samples were concentrated in the SpeedVac™ for 32 minutes at medium heat. After concentration, the sample volumes were measured, and any samples with volumes below 25 µL were brought up to 25 µL using UltraPure™ Water. The concentrated samples were then quantified according to the Alameda County Sheriff's Office Crime Laboratory FBU protocol. One concentrated sample was amplified according to the Alameda County Sheriff's Office Crime Laboratory FBU protocol.

After the first quantification, one positive control, half of one sample's M-Vac® filter, and one reagent blank were all re-extracted using Casework Extraction Buffer instead of the Incubation Buffer used in the Alameda County Sheriff's Office Crime Laboratory FBU protocol. They were also extracted with 1-thiogylcerol and 200 µL Lysis Buffer, instead of only 400 µL Lysis Buffer as in the FBU protocol. The positive control, sample, and reagent blank were then quantified according to the Alameda County Sheriff's Office Crime Laboratory FBU protocol. The sample was amplified according to the Alameda County Sheriff's Office Crime Laboratory FBU protocol, and DNA profiles for each sample were obtained using capillary electrophoresis. Due to low peak heights and issues with peak shape, samples PF-Fil-1A-CWE, PF-Mvac-2, and PF-Mvac-1 100 to 25 were reinjected.

In a separate experiment, the M-Vac® was tested to see whether DNA could go through the M-Vac® filter into the flow-through in the flask below. First, 2 mL of SRS was pipetted onto an M-Vac® filter and vacuum-filtered through. Approximately 1 mL of the resulting flow-through was collected as a reagent blank. Next, an approximate 1:1 dilution by volume of neat blood was made using SRS. 50 µL of this diluted blood was saved as a positive control, and 2 mL was pipetted onto the M-Vac® filter and vacuum-filtered through the filter. Approximately 1.2 mL of the resulting flow-through was collected as a sample and divided up into four 2-mL tubes containing ~300 µL flow-through each. All sample tubes and the tubes containing the reagent blank were put in the SpeedVac™ concentrator at low heat for approximately 105 minutes. Afterwards, all tubes were taken out of the SpeedVac™. All sample tubes were combined into one tube, and all reagent blank tubes were combined into one tube. The reagent blank, positive control, unconcentrated sample, and concentrated sample were extracted according to the Alameda County Sheriff's Office Crime Laboratory FBU protocol. The negative control, positive control, and samples were then quantified according to the Alameda County Sheriff's Office Crime Laboratory FBU protocol.

**Investigation of M-Vac®'s Performance to Collect Cellular Material from Various Substrates Compared to the Double Swab Technique**

Country Classics CC3 White Solid 100% Cotton Fabric was purchased. This white, thin, loose-weave 100% cotton fabric was then washed in a washing machine in preparation for this experiment. The fabric was first washed on express wash with a Tide detergent pod and hot water. The fabric was then washed for a second time on express wash with a Tide detergent pod and hot water, with three extra rinses. The fabric was washed for a third time on normal/casual wash without a Tide detergent pod but with extra hot water. The cotton fabric was then dried in a

drying machine, before being cut into squares. Denim fabric squares were cut from a pair of dark-colored, tightly-woven men's jeans.

Neat saliva was collected from four donors, two of which were female (X19-15 and X19-49) and two of which were male (Y19-26 and Y19-36). All donors' neat saliva samples were rocked back and forth in an incubator at 37°C for approximately 30 minutes. An approximate 1:1:1:1 mixture by volume of the four donors' saliva was prepared, some of which was saved as a mixture of neat saliva. Next, approximate 1:3, 1:10, 1:30, and 1:100 dilutions by volume of this mixture were prepared.

Positive controls were prepared by pipetting 1 mL of the neat saliva mixture onto an M-Vac® filter and 1 mL of each dilution of that mixture onto its own separate M-Vac® filter. Each filter was then vacuum-filtered and dried in a laminar flow hood. A negative control was prepared by collecting 150 mL of SRS solution using the M-Vac®, vacuum-filtering it through an M-Vac® filter, and drying the filter in a laminar flow hood. For each dilution, samples were prepared by pipetting 1 mL of diluted saliva onto each of three cotton fabric squares. 1 mL of the 1:3 saliva mixture dilution was also pipetted onto each of three extra cotton fabric squares, six denim fabric squares, and six bricks.

After at least several days of drying, the following samples were collected using ~150 mL SRS and the M-Vac®: three cotton fabric squares each for each dilution (1:3, 1:10, 1:30, 1:100 diluted saliva mixtures), three denim fabric squares with 1:3 diluted saliva mixture, and three bricks with 1:3 diluted saliva mixture. Each M-Vac®-collected sample taken from cotton or denim fabric squares was vacuum-filtered through an M-Vac® filter, which was then dried in a laminar flow hood. Each M-Vac®-collected sample taken from a brick was vacuum-filtered through an M-Vac® Pre-Filter, then vacuum-filtered through an M-Vac® filter, and finally, the

filter was dried in a laminar flow hood. For all positive controls and samples, some flow-through that went through the M-Vac® filter was used to wash the sample collection bottle before being re-filtered through the M-Vac® filter.

The following samples were collected using the double swab technique: three cotton fabric squares with 1:3 diluted saliva mixture, three denim fabric squares with 1:3 diluted saliva mixture, and three bricks with 1:3 diluted saliva mixture. The double swab technique that was used for sample collection is as follows. For each sample, water from an AddiPak® Sterile Water Solution Unit was dropped on a new sterile swab to make a wet swab. The wet swab was then rolled back and forth over the entire face of the fabric square or brick that previously had saliva deposited onto it. A new sterile, dry swab was then rolled back and forth over the same area previously swabbed by the wet swab. Both wet and dry swabs for each sample were put in a swab stand and dried at least overnight.

Swabs were prepared for extraction. Wet and dry swabs of the same sample were cut out and put into a Nucleospin® Forensic Filter in the same tube. M-Vac® filters were also prepared for extraction. After drying the filters overnight, the M-Vac® filters were cut out, each filter was divided in half, and each half of a filter was put in a Nucleospin® Forensic Filter in its own extraction tube. For each sample that was collected using an M-Vac® Pre-Filter, debris sitting on top of the M-Vac® Pre-Filter membrane were put into the same extraction tube as the membrane.

Each donor's neat saliva sample, the negative control, the positive controls, and samples were extracted on Maxwell® 16 instruments with Casework Extraction Buffer, according to Promega's Casework Extraction Buffer Extraction Protocol. Extracts of each donor's neat saliva sample, the negative control, the positive controls, and all samples (except the ones noted below)

were eluted in 50 µL Elution Buffer. Extracts of M-Vac®-collected samples of 1:30 diluted saliva mixture on cotton fabric, 1:100 diluted saliva mixture on cotton fabric, and 1:3 diluted saliva mixture on brick (along with corresponding M-Vac® Pre-Filters) were eluted in 50 µL UltraPure™ Water. Extracts of each half of an M-Vac® filter were then combined for each M-Vac® filter. All extracts were quantified according to the Alameda County Sheriff's Office Crime Laboratory FBU protocol. The volumes of the negative control, positive controls, and samples were measured after quantification.

Extracts of M-Vac®-collected samples of the 1:3 diluted saliva mixture deposited on brick, 1:30 diluted saliva mixture on cotton fabric, and 1:100 diluted saliva mixture on cotton fabric were concentrated alongside an UltraPure™ Water reagent blank. These extracts and the reagent blank were concentrated in the SpeedVac™ at medium heat for ~52-54 minutes to dry them down to approximately only a few microliters of solution. Next, 25 µL of $TE^{-4}$ was pipetted into each sample tube and the reagent blank, and all tubes were incubated at 56°C for 10 minutes to aid in resuspension of DNA. The concentrated samples were then quantified according to the Alameda County Sheriff's Office Crime Laboratory FBU protocol.

All positive controls, the negative control, and a selection of samples were chosen to be amplified. If necessary, controls or samples were manually diluted prior to amplification. The positive controls, the negative control, and a selection of samples were then amplified according to the Alameda County Sheriff's Office Crime Laboratory FBU protocol. DNA profiles for each control and sample were obtained using capillary electrophoresis.

DNA profiles for controls and samples were analyzed with GeneMapper *ID-X*. Artifacts were manually removed from each profile before the profiles were exported to STRmix™. Using an analytical threshold of 70 RFU, STRmix™ was then used to deconvolute each control and

sample. Next, STRmix™ was used to calculate LRs for each contributor in each sample using the mixture donors' DNA profiles for comparison. Finally, STRmix™'s ability to exclude noncontributors was tested. Using a different set of donor profiles that were not contributors to the four-person mixture, STRmix™ was used to calculate LRs for each contributor in each sample using the non-contributor donors' DNA profiles for comparison.

Touch DNA samples on bricks were prepared using a procedure adapted from Vickar *et al.*'s study (Vickar *et al.* 2018). Donors were instructed not to wash their hands at least an hour before sample collection and were allowed to work at their desks in the hour before sample collection. Just before sample collection, donors were instructed to "groom" themselves for ~30 seconds by rubbing their hands on their neck and head, before rubbing their hands together for ~5 seconds. Donors then placed one hand on a brick, put pressure on it for ~30 seconds, and then their hand was scraped across the brick. This process was repeated with their other hand. Each donor only touched one brick, in an attempt to create a different single-source touch DNA sample on each of the three bricks.

Each touch DNA on brick sample was sampled first using the double swab technique, then with the M-Vac®. Each M-Vac®-collected sample was vacuum-filtered through an M-Vac® Pre-Filter and then vacuum-filtered through an M-Vac® filter. Swabs were dried overnight in a swab rack, while M-Vac® Pre-Filter membranes and M-Vac® filters were dried overnight in a laminar flow hood.

Swabs, M-Vac® Pre-Filter membranes and associated debris, and M-Vac® filters were then prepared for extraction. Wet and dry swabs of the same sample were cut out and put into a Nucleospin® Forensic Filter in the same tube. For each sample that was collected using an M-Vac® Pre-Filter, debris sitting on top of the M-Vac® Pre-Filter membrane were put into the

same extraction tube as the membrane. The M-Vac® filters were cut out, each filter was divided in half, and each half of a filter was put in a Nucleospin® Forensic Filter in its own extraction tube.

Samples were extracted on Maxwell® 16 instruments with Casework Extraction Buffer, according to Promega's Casework Extraction Buffer Extraction Protocol. Extracts from swabs were eluted in 50 µL Elution Buffer, and extracts from M-Vac®-collected Pre-Filter membrane and filter samples were eluted in 50 µL UltraPure™ Water. Extracts from M-Vac® Pre-Filter membranes and filters were then combined for membranes and filters from the same sample. These extracts and the reagent blank were then concentrated in the SpeedVac™. Before any samples were added, the SpeedVac™ was pre-heated at medium heat for 10 minutes. The samples and the reagent blank were then concentrated at medium heat for 1 hour to dry them down to approximately only a few microliters of solution. Next, 25 µL of $TE^{-4}$ was pipetted into each sample tube and the reagent blank, and all tubes were incubated at 56°C to aid in resuspension of DNA. The concentrated samples were then quantified according to the Alameda County Sheriff's Office Crime Laboratory FBU protocol.

All samples were amplified. If necessary, controls or samples were manually diluted prior to amplification. The positive controls, the negative control, and a selection of samples were then amplified according to the Alameda County Sheriff's Office Crime Laboratory FBU protocol. DNA profiles for each control and sample were obtained using capillary electrophoresis. These DNA profiles were then analyzed with GeneMapper *ID-X* using the same analysis method used to analyze the four-person saliva mixture samples. Using an analytical threshold of 70 RFU, STRmix™ was then used to deconvolute each control and sample. Next, STRmix™ was used to calculate LRs for the contributor to each sample using the donors' DNA profiles for comparison.

Finally, STRmix™'s ability to exclude noncontributors was tested. Using donor profiles that were not expected to be contributors to each touch DNA on brick sample, STRmix™ was used to calculate LRs for the contributor in each sample using the non-contributor donors' DNA profiles for comparison.

## Results

### Initial Concentration Studies

**Quantification Results**

| Sample Name | [Autosomal] (ng/ µL) after concentrating | Expected concentration (ng/ µL) |
|---|---|---|
| A | 0.2008 | - |
| A-1 100 to 50 | 0.3168 | 0.4016 |
| A-2 100 to 25 | 0.6816 | 0.8032 |
| B 1:10 of A | 0.0111 | - |
| B-1 1:10_100 to 50 | 0.0076 | 0.0222 |
| C 1:20 of A | 0.0041 | - |
| C-1 1:20_100 to 25 | 0.0072 | 0.0222 |
| A-1 RB 100 to 50 | 0 | 0 |
| A-2 RB 100 to 25 | 0 | 0 |

**Table 2.** Comparison of autosomal DNA concentrations after concentrating DNA samples to the expected DNA concentrations for those samples.

In general, samples increased in concentration after being concentrated in the SpeedVac™, but they did not reach their expected concentrations. Expected concentrations for samples A-1 and A-2 were calculated using the concentration for sample A, and expected concentrations for samples B-1 and C-1 were calculated using the concentration for samples B and C, respectively. For samples that started at 100 µL and concentrated to 50 µL, the concentration was expected to double since the solution volume was halved. For samples that started at 100 µL and were concentrated to 25 µL, the concentration was expected to quadruple since the solution volume was dried to a quarter of what it originally was. No DNA was quantified for either reagent blank.

Compared to the original pooled sample A, both concentrated aliquots of sample A (samples A-1 and A-2) had increased concentrations. However, these increased concentrations were still less than the expected increased concentration. Sample A-1's concentration was expected to be double sample A's concentration, and Sample A-2's concentration was expected to be quadruple sample A's concentration.

Sample B-1's and sample C-1's expected concentrations were calculated using the observed concentration for sample C. Sample B was a 1:10 dilution of sample A. Compared to sample B, the concentrated aliquot sample B-1 did not have an increased concentration and was about half its expected increased concentration. Sample C was a 1:20 dilution of sample A. Compared to sample C, the concentration sample C-1 had an increased concentration. However, this increased concentration was about half its expected concentration. Since sample C-1 was aliquoted from a 1:20 dilution of sample A and was expected to quadruple after concentration, and sample B-1 was aliquoted from a 1:10 dilution of sample A and was expected to double after concentration, sample C-1 and sample B-1 had the same expected concentrations. Because sample B-1 and sample C-1's observed concentrations were similar to each other, this expectation was met.

**Amplification Results**

| Sample Name | Average Peak Height (RFU) | Average Peak Height Ratio for Heterozygous Peaks | Amount of template DNA amplified (ng) |
|---|---|---|---|
| A | 6,362 | .88 | 1.000 |
| A-1 100 to 50* | 1,776 | .85 | 1.000 |
| A-2 100 to 25* | 3,062 | .80 | 1.000 |
| B 1:10 | 1,316 | .80 | 0.166 |
| B-1 1:10_100 to 50 | 489 | .71 | 0.114 |
| C-1 1:20_100 to 25 | 907 | .71 | 0.108 |

**Table 3.** Comparison of average peak heights, average peak height ratio for heterozygous peaks, and amount of template DNA amplified across all DNA samples.

All samples yielded full, concordant profiles and acceptable average peak height ratios for heterozygous peaks. All samples other than sample B-1 and sample C-1 had high peak heights and an acceptable average peak height ratio for heterozygous peaks. Sample B-1 and sample C-1 had lower peak heights, but this was expected as the amounts of template DNA amplified were significantly lower than 1.000 ng (0.114 ng and 0.108 ng, respectively). The

samples' states of degradation and inhibition did not change with concentration. No alleles were called for reagent blank A-2 RB 100 to 25, as expected.

**Investigating Potential DNA Loss Caused by the M-Vac® Pre-Filter System**

**Quantification Results (Extraction with Incubation Buffer)**

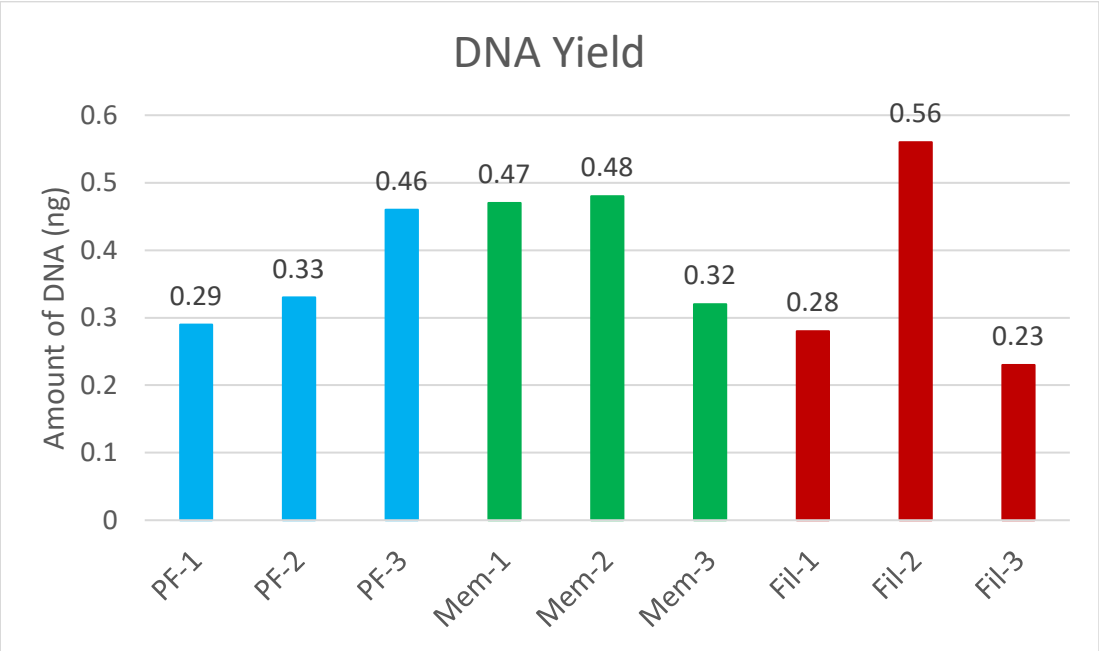


**Figure 4.** Comparison of total DNA yields from fibers recovered from the M-Vac® Pre-Filter membrane (PF), the M-Vac® Pre-Filter membrane (Mem), and the M-Vac® filter (Fil). The average DNA yield from the controls was 8.07 ng.

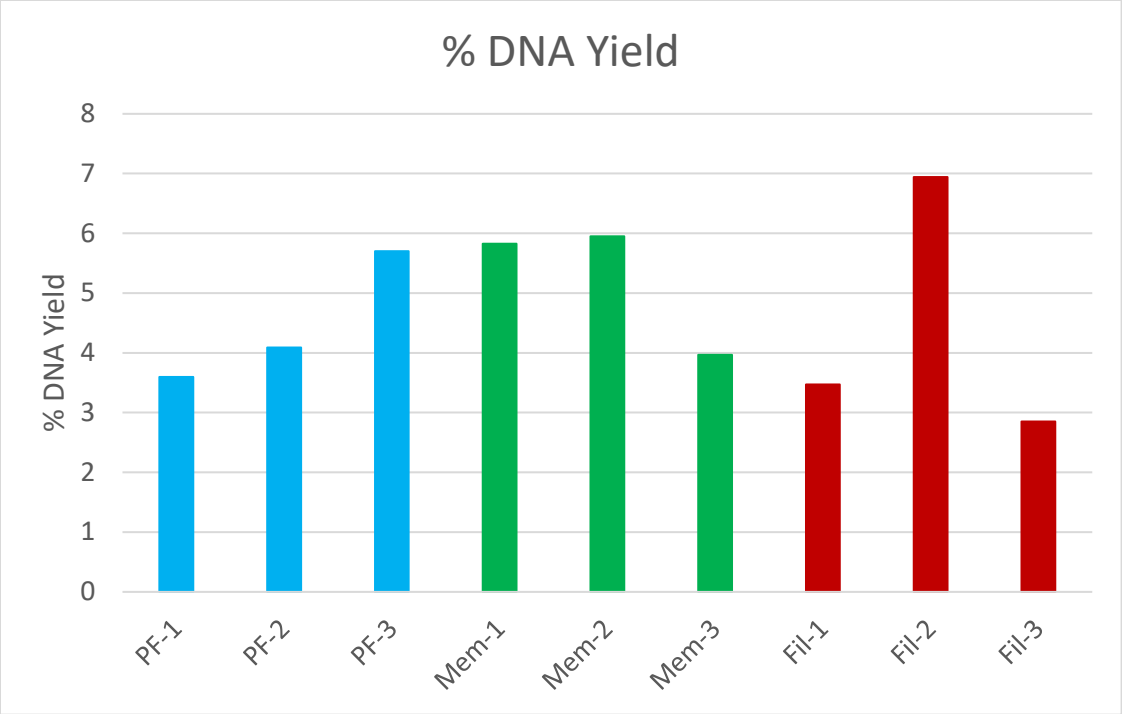


**Figure 5.** Percent DNA yields from samples from Figure 4. Comparison of total percent DNA yields from fibers recovered from the M-Vac® Pre-Filter membrane (PF), the M-Vac® Pre-Filter membrane (Mem), and the M-Vac® filter (Fil) for each sample.

DNA was recovered from both the positive controls and the samples. The average DNA yield from the controls was 8.07 ng. A similar amount of DNA for each sample was collected from fibers collected on the M-Vac® Pre-Filter membrane, the M-Vac® Pre-Filter membrane, and the M-Vac® filter for each sample, both within a sample and across samples. However, the concentrations of DNA recovered using the M-Vac® were much lower than expected, so a selection of samples was re-extracted using Casework Extraction Buffer instead of Incubation Buffer.

**Quantification Results (Extraction with Incubation Buffer vs. Re-extraction with Casework Extraction Buffer)**

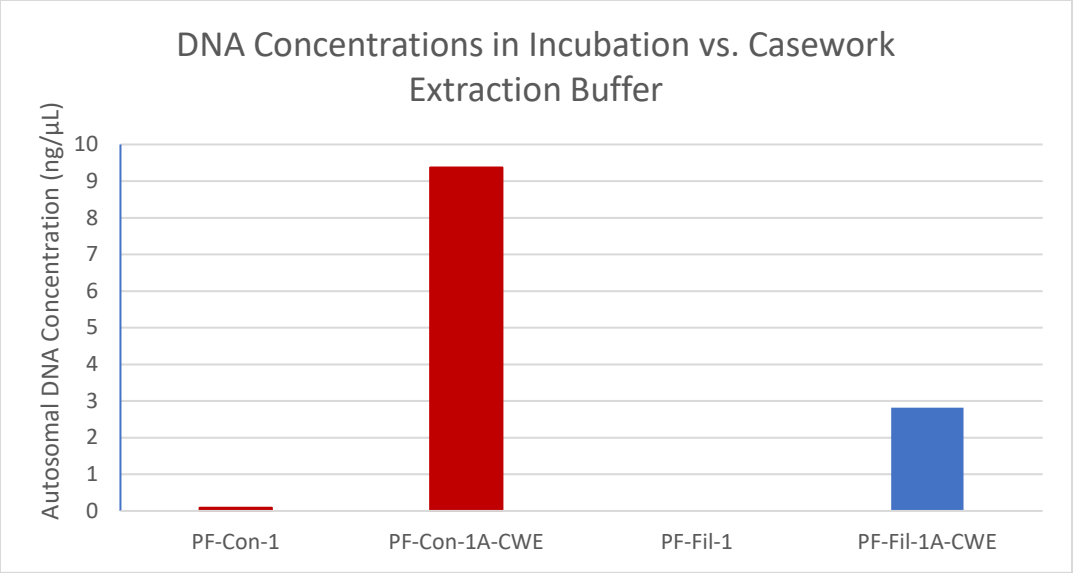


**Figure 6.** Autosomal DNA concentrations from PF-Con-1 and PF-Fil-1 when first extracted with Incubation Buffer, compared to when one half of the same control and sample were re-extracted with Casework Extraction Buffer.

The concentrations of the re-extracted control and sample were significantly higher than the concentrations of the same control and sample after their initial extractions with Incubation Buffer. It should be noted that PF-Con-1 and PF-Fil-1 are both a combination of two halves of the same filter extracted with Incubation Buffer, while PF-Fil-1A-CWE and PF-Con-1A-CWE are both re-extractions of one half of a filter. These results indicate that re-extracting even just one half of an M-Vac® filter with Casework Extraction Buffer yielded a far higher DNA concentration than the initial extraction of both halves of the filter with Incubation Buffer. (Note: The FBU adopted Casework Extraction Buffer in casework on January 29, 2021, due to the initial studies performed here.)

**Quantification Results (Unconcentrated vs. Concentrated Samples)**

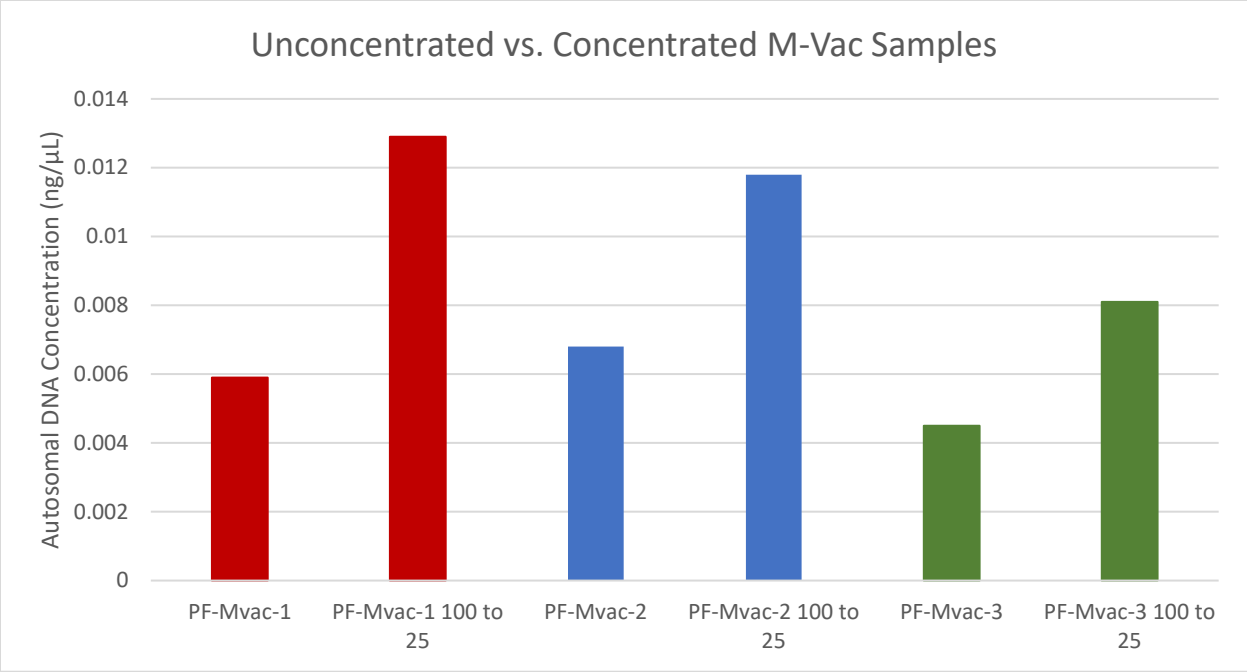


**Figure 7.** Comparison of autosomal DNA concentrations of the same samples before and after concentrating.

The autosomal DNA concentrations of the concentrated samples increased compared to the concentrations of those same samples before being concentrated. Since all the samples were concentrated from 100 µL to 25 µL, their autosomal DNA concentrations were expected to quadruple. However, their concentrations only approximately doubled. The samples' state of degradation did not change with concentration. (Note: M-Vac samples were later eluted in UltraPure™ Water due to negative effects observed with concentration of DNA extracts in Elution Buffer.)

**Quantification Results (Blood Flow-Through Study)**

| Sample Name | Autosomal DNA Concentration (ng/µL) |
|---|---|
| FT-Blood | 0.2579 |
| FT-NT-unfil | 1.7341 |
| FT-Blood-NC | 0.0714 |
| FT-RB | - |

**Table 4.** Comparison of autosomal DNA concentrations in concentrated flow-through, unfiltered blood, and unconcentrated flow-through.

Autosomal DNA concentrations were obtained from quantification of both concentrated and unconcentrated flow-through diluent obtained from vacuum-filtering diluted blood through the M-Vac® filter. The autosomal DNA concentration of the concentrated flow-through was higher than that of the unconcentrated flow-through. However, both the unconcentrated and concentrated portions of flow-through had lower autosomal DNA concentrations than a portion of diluted blood. No DNA was quantified for the reagent blank.

**Amplification Results**

One sample from the first extraction, one re-extracted sample, and one concentrated sample were all amplified. DNA profiles were successfully obtained from all three samples. However, while both the sample from the first extraction and the re-extracted sample had full DNA profiles that showed high peak heights, the DNA profile from the concentrated sample showed low, wide peaks. As a precaution, all three samples were re-injected. The second DNA profiles from the sample from the first extraction and the re-extracted sample were mostly the same compared to the first DNA profiles. The second DNA profile from the concentrated sample still had low peak heights in general, but the peak shapes were improved and the peak heights were more consistent across the profile.

**Investigation of M-Vac®'s Performance to Collect Cellular Material from Various Substrates Compared to the Double Swab Technique**

**Quantification Results**

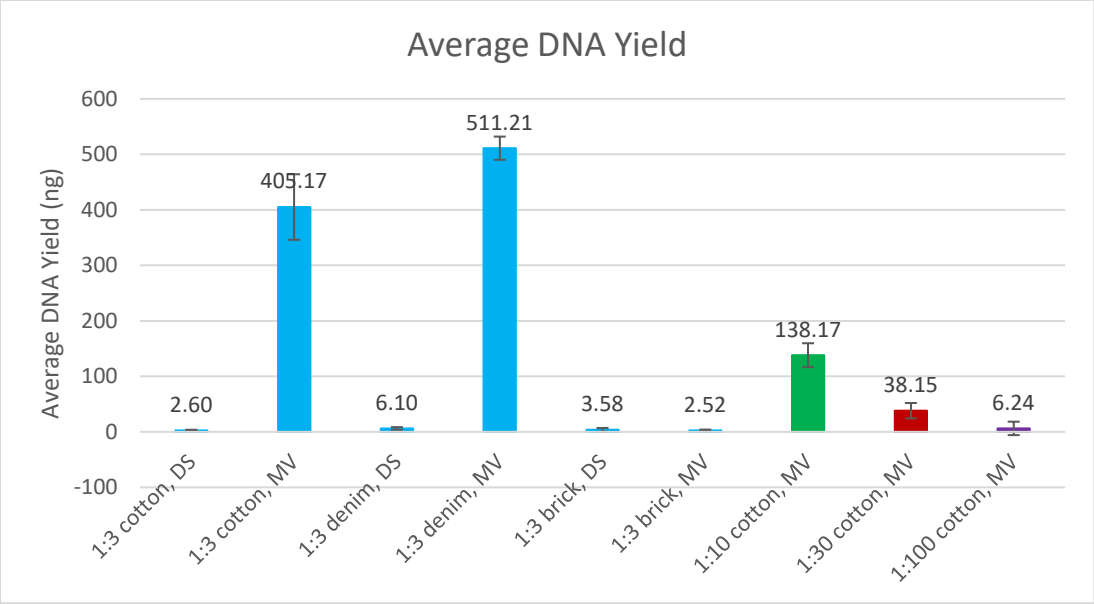


**Figure 8.** Comparison of average DNA yields across different sample types and dilutions of the four-person saliva mixture. DS = double swab technique, MV = M-Vac®.

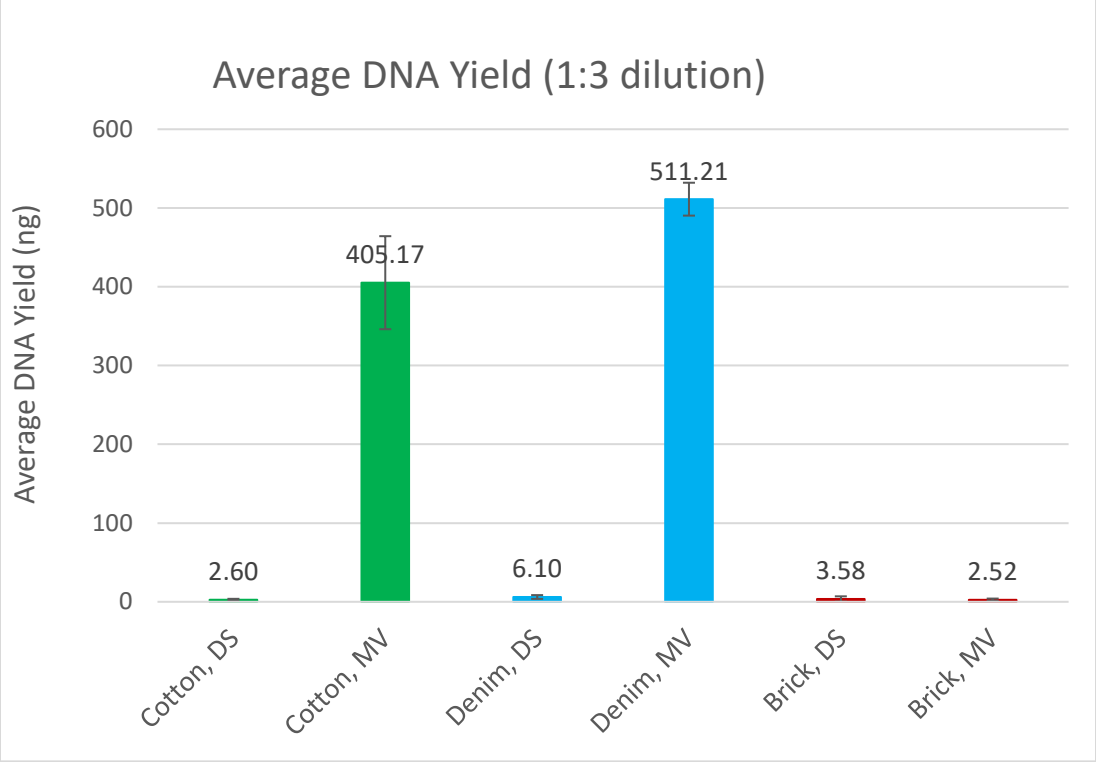


**Figure 9.** Average DNA yields of the 1:3 diluted saliva mixture samples from Figure 8, for comparison across different substrates and sampling methods. DS = double swab technique, MV = M-Vac®.

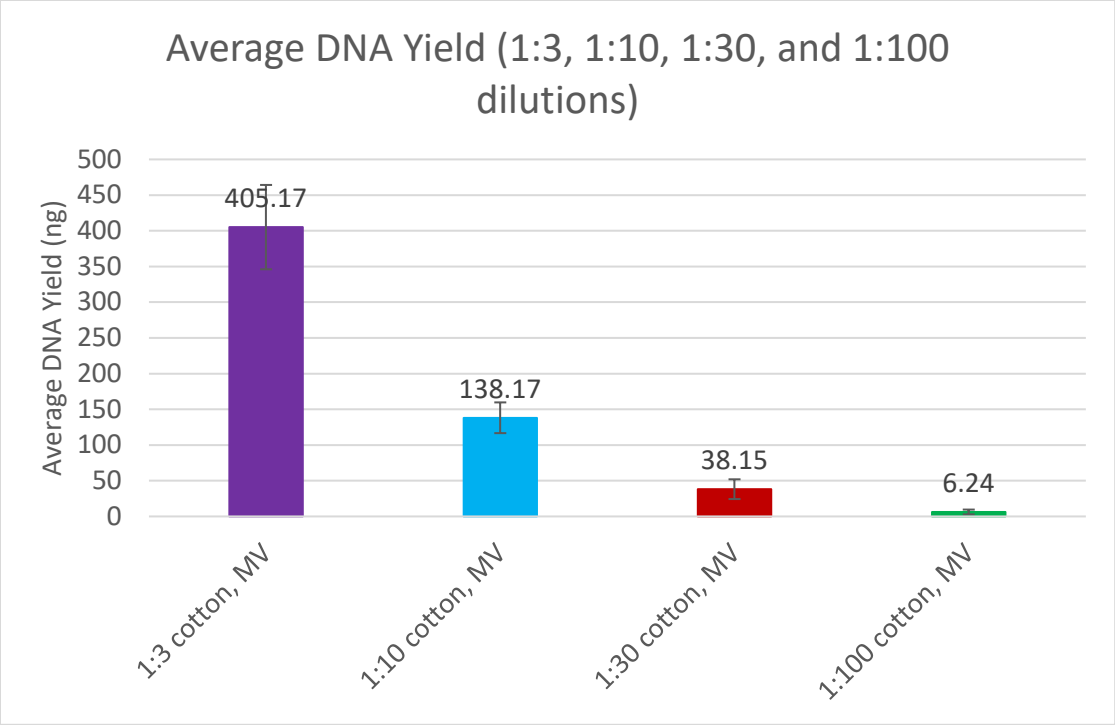


**Figure 10.** Average DNA yields of the 1:3, 1:10, 1:30, and 1:100 diluted saliva mixture samples from Figure 8 collected from cotton using the M-Vac®. MV = M-Vac®.

Figures 8, 9, and 10 illustrate that the M-Vac® was capable of collecting DNA from all dilutions of the four-person saliva mixture. In particular, with the 1:3 dilution samples collected from cotton and denim fabric, the M-Vac® was able to collect significantly more DNA than the double swab technique. When comparing samples of 1:3, 1:10, 1:30, and 1:100 dilutions of the four-person saliva mixture on cotton fabric, a trend emerges. Generally, the more diluted the saliva mixture was, the less DNA was collected by the M-Vac®. This trend was expected since more dilute samples have less DNA. However, both the M-Vac® and the double swab technique collected little DNA from the bricks, as evidenced by the low average DNA yields. Since average DNA yields from the M-Vac® and the double swab technique were similar in amount, neither method outperformed the other when sampling the four-person saliva mixture from a brick substrate. In addition, most samples from all sample types (cotton fabric, denim fabric, and brick) showed some degradation.

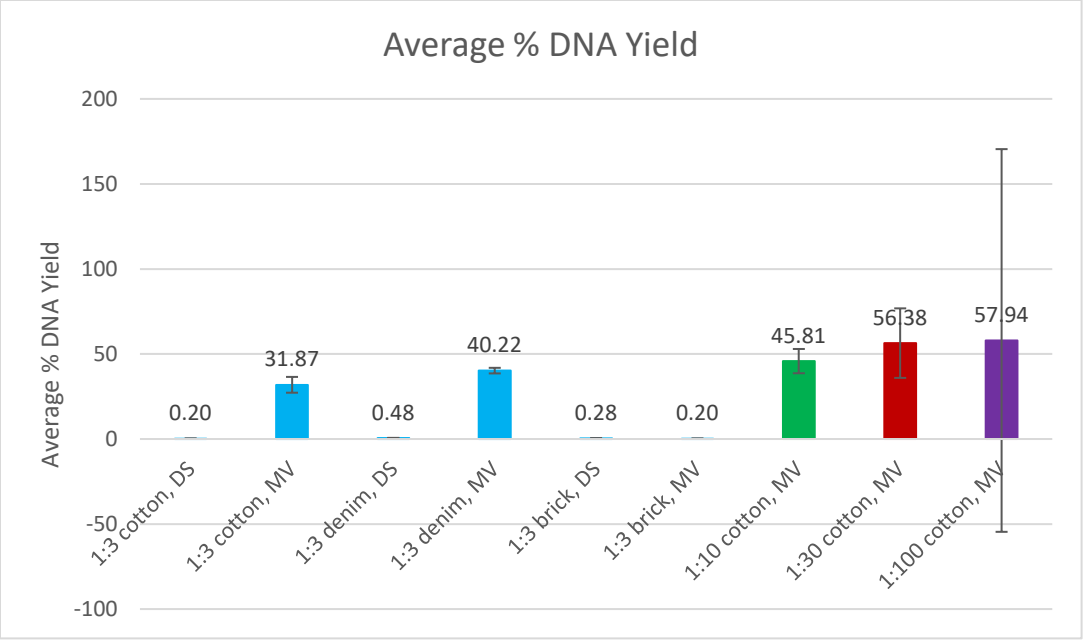


**Figure 11.** Comparison of average percent DNA yields across different sample types and dilutions of the four-person saliva mixture. For the 1:100 dilution, a large standard deviation is observed since there were only two measurements used in this set of data. DS = double swab technique, MV = M-Vac®. Blue bars = 1:3 dilution, green bar = 1:10 dilution, red bar = 1:30 dilution, purple bar = 1:100 dilution.

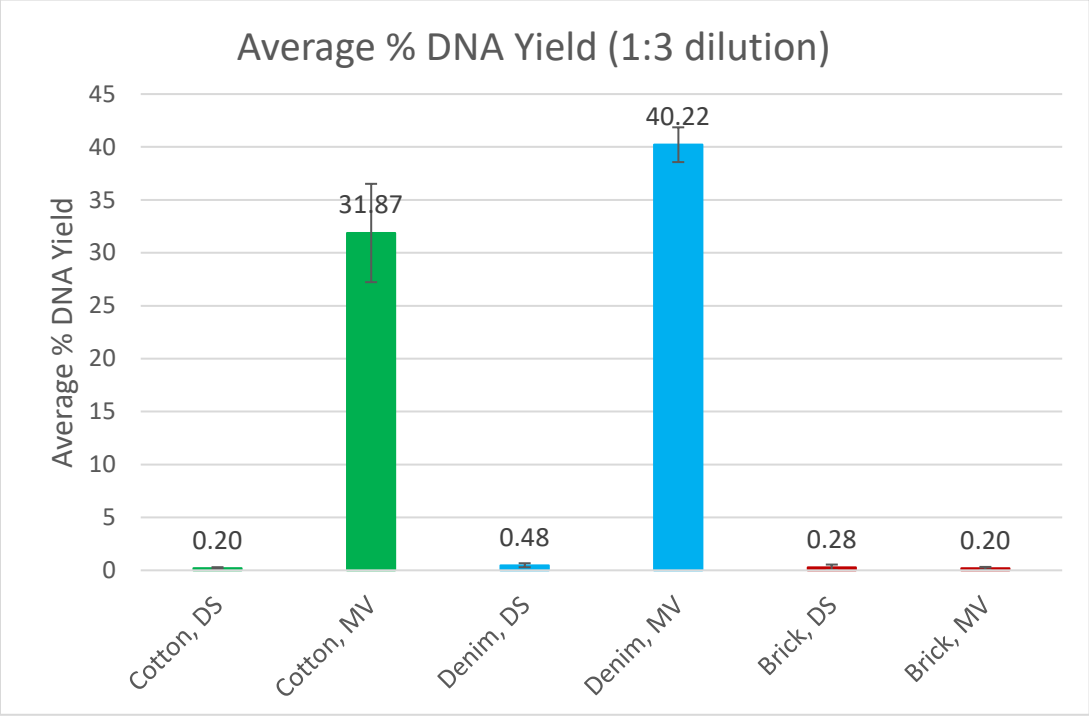

**Figure 12.** Average percent DNA yields of the 1:3 diluted saliva mixture samples from Figure 11, for comparison across different substrates and sampling methods. DS = double swab technique, MV = M-Vac®.

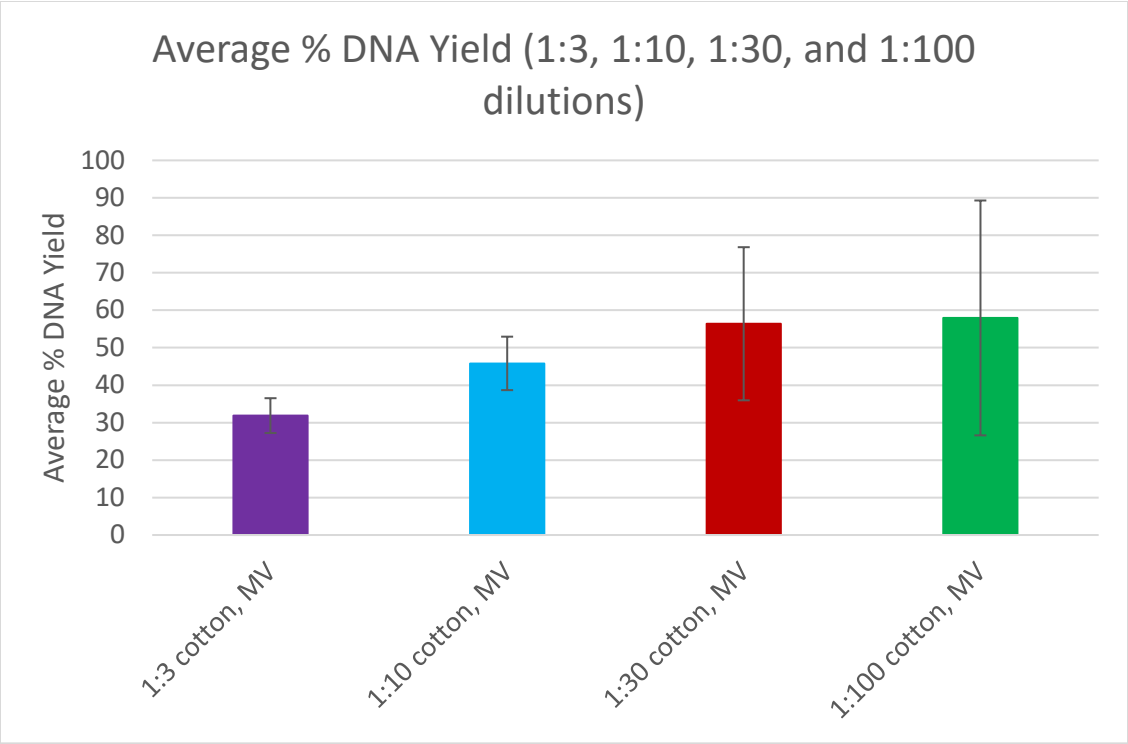


**Figure 13.** Average percent DNA yields of the 1:3, 1:10, 1:30, and 1:100 diluted saliva mixture samples from Figure 11 collected from cotton using the M-Vac®. MV = M-Vac®.

Figures 11, 12, and 13 show the average percent DNA yields for all dilutions of the four-person saliva mixture, across different substrates and sampling methods. Average percent DNA yield was calculated by dividing the amount of DNA in a dilution sample by the amount of DNA in its corresponding control (not pictured here), and then multiplying that by 100. Generally, the average percent DNA yield for M-Vac®-collected samples was 30-50%, compared to the controls. Within the M-Vac®-collected 1:100 diluted saliva mixture sample set, there was one sample that yielded more DNA than even the 1:100 diluted saliva mixture control. By including this replicate, the average percent DNA yield for this set was calculated to be higher than 100%, so this replicate was deemed an outlier and removed from the data set. Because only two

replicates were then considered for the 1:100 diluted saliva mixture sample, the standard deviation for that sample set was very large.

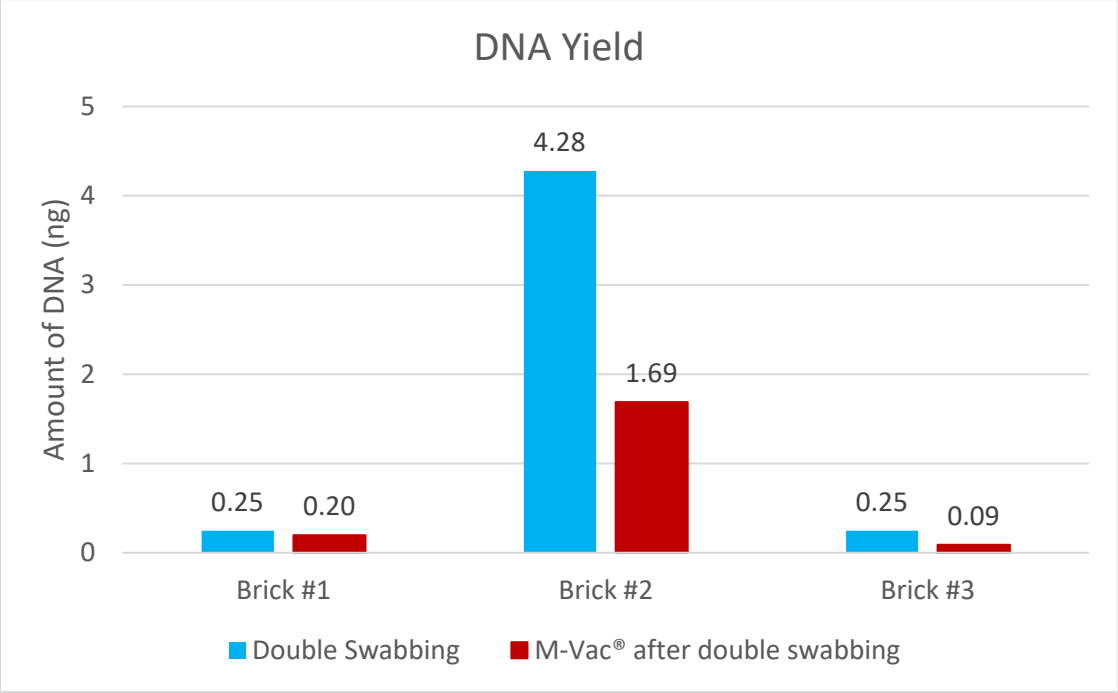


**Figure 14.** DNA yields for collecting touch DNA on each brick sample, first with the double swab technique, then with the M-Vac®.

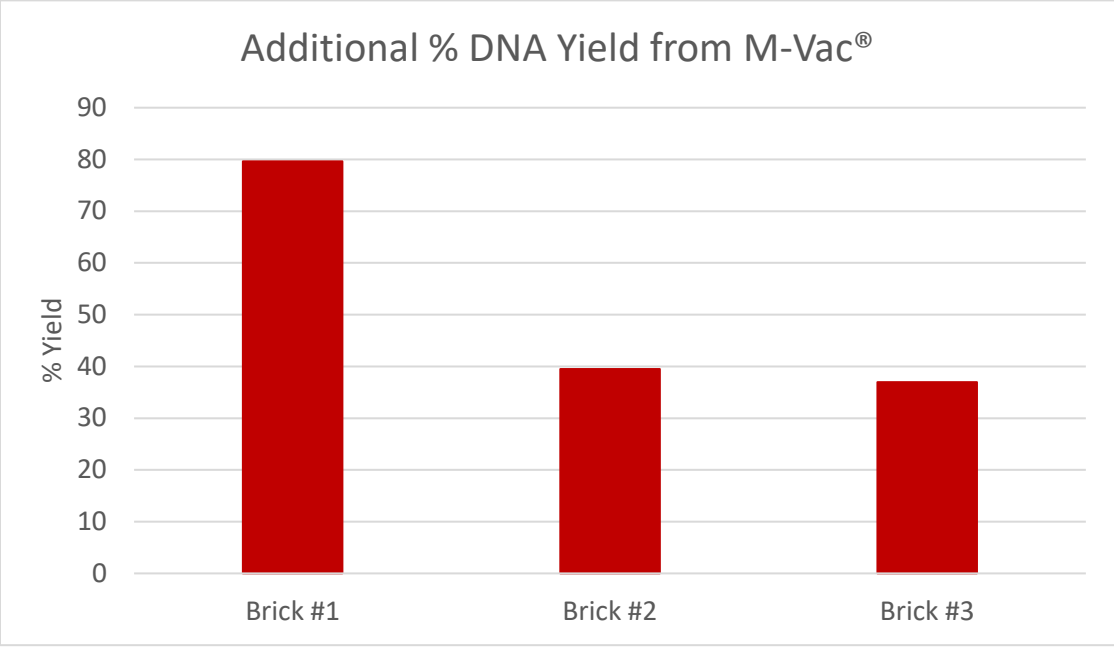

**Figure 15.** Additional percent DNA yield from the M-Vac® after using the double swab technique on each brick's surface.

The graph displayed in Figure 14 demonstrates that both the double swab technique and the M-Vac® were able to collect touch DNA from a brick substrate. Similar to the brick samples depicted in Figure 9, little DNA was able to be collected from the bricks using either method. In general, the amount of DNA collected first with the double swab technique was similar to or greater than the amount of DNA that was then collected off of the same brick using the M-Vac®. In addition, most samples showed some degradation.

The graph displayed in Figure 15 illustrates that using the M-Vac® even after first using the double swab technique on each brick's surface recovers DNA. The additional percent DNA yield was calculated for each brick by dividing the amount of DNA obtained using the M-Vac® by the amount of DNA obtained using the double swab technique, and then multiplying by 100.

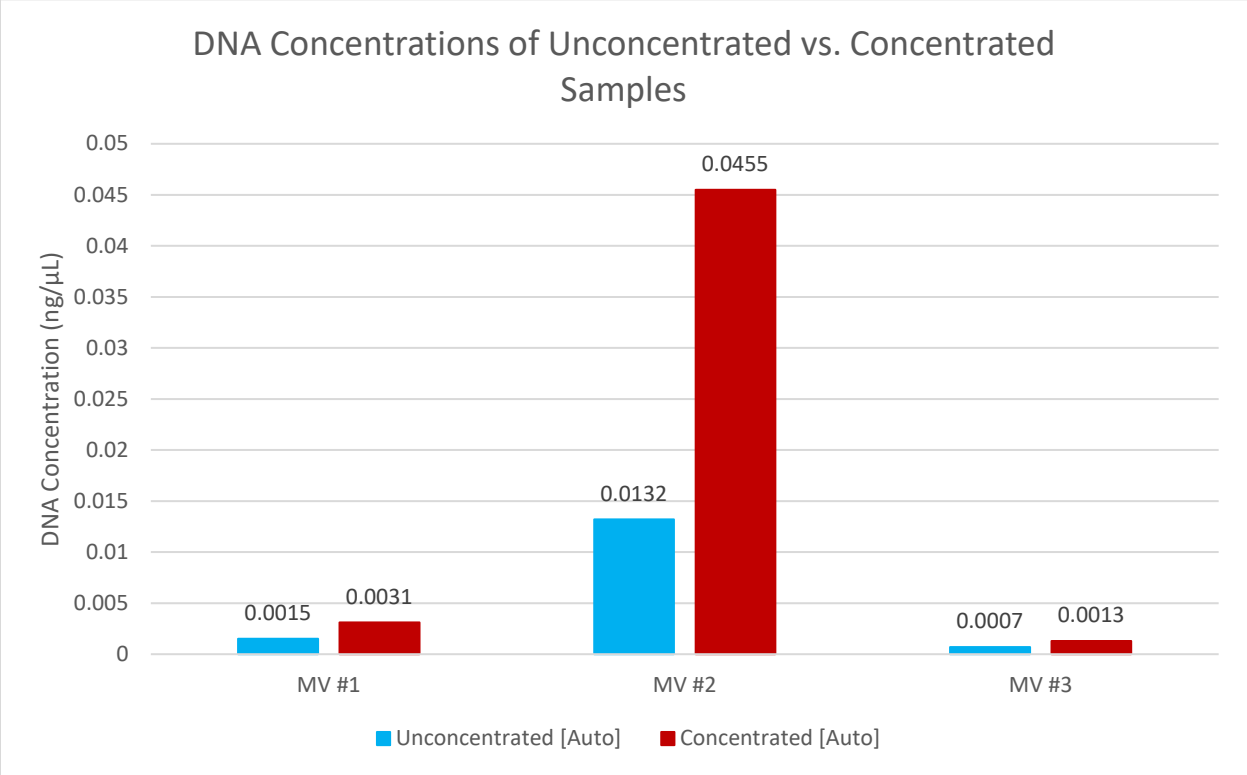

**Figure 16.** A comparison of unconcentrated and concentrated DNA concentrations for the same M-Vac®-collected touch DNA samples on brick. MV = M-Vac®.

Figure 16 demonstrates that the concentrations of M-Vac®-collected touch DNA on brick samples can increase after concentration in the SpeedVac™. After concentrating the M-Vac®-collected touch DNA on brick samples, the concentration of each sample increased by 2- to 3-fold. The samples' state of degradation did not change with concentration.

**Amplification Results**

The positive controls, the negative controls, and all samples were amplified. Full DNA profiles were successfully obtained from most samples, and partial DNA profiles were obtained for a few samples. A few samples, mostly those with partial DNA profiles, showed degradation in the form of "ski-slope" peak heights.

All DNA profiles of the four-person saliva mixture samples except one were identified as mixtures of at least four contributors, as expected. The one DNA profile of a four-person saliva mixture sample that was not identified as a mixture of at least four contributors had significant dropout and instead was identified as a mixture of at least three contributors.

Most DNA profiles of the single-person touch DNA samples were identified as mixtures of two contributors, with the donor as a major contributor and another person as a very minor contributor. Minor contributors in these profiles usually only contributed one allele total for all loci throughout the whole DNA profile. The remaining DNA profiles were either unable to be interpreted due to most alleles at most loci dropping out, or they were identified as single-source samples.

**STRmix™ Results**

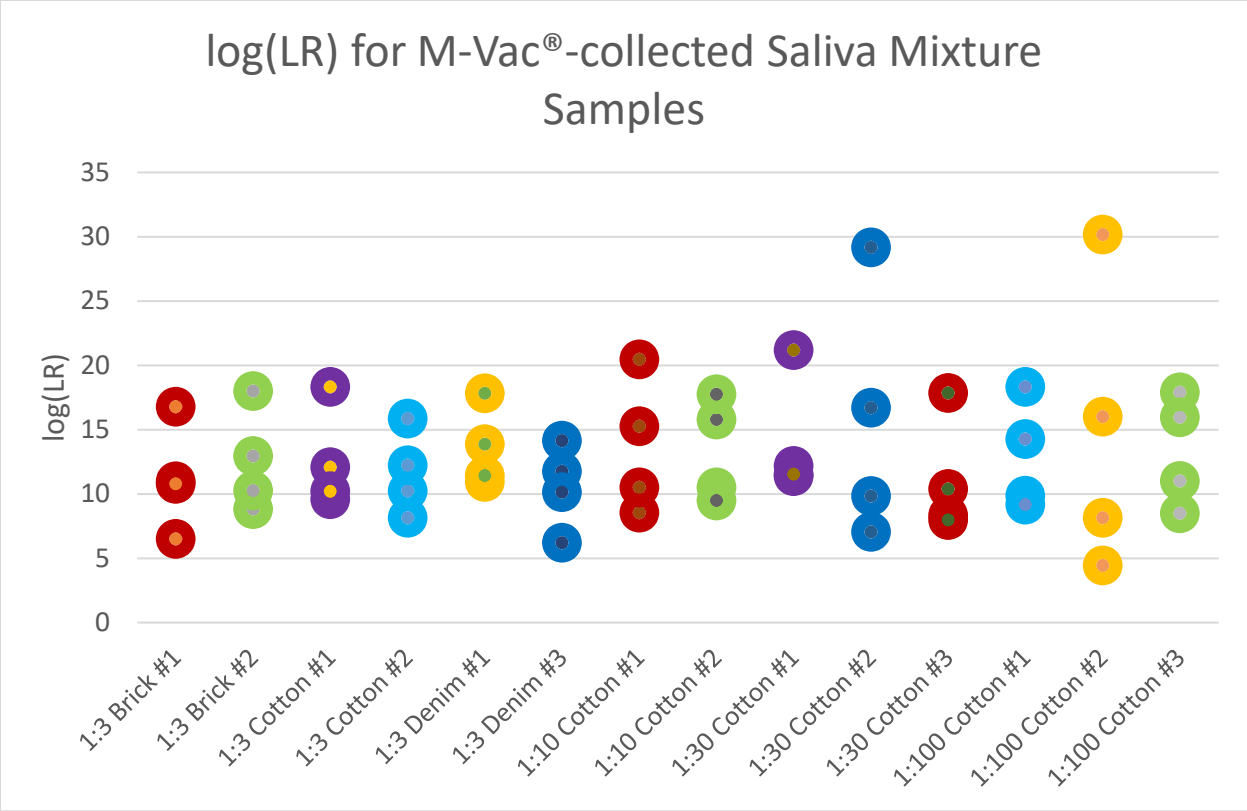


**Figure 17.** Dot plot for the logs of likelihood ratios calculated by STRmix™ for each contributor to the four-person saliva mixture, for comparison across different substrates and different dilutions of the mixture. These samples were collected with the M-Vac®.

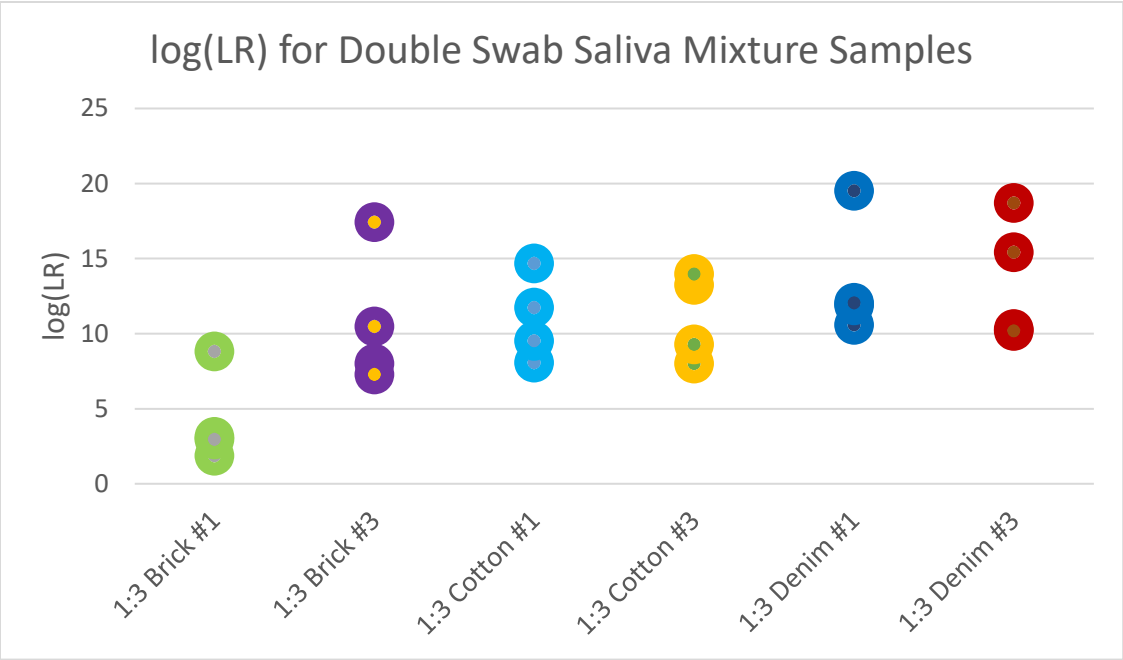

**Figure 18.** Dot plot for the logs of likelihood ratios calculated by STRmix™ for each contributor to the 1:3 dilution of the four-person saliva mixture, for comparison across different substrates. These samples were collected using the double swab technique.

All DNA profiles of samples of the four-person saliva mixture as well as those of touch DNA samples were able to be deconvoluted by STRmix™. Gelman Rubin scores for all samples ranged between 1 and 1.3, indicating that all the deconvolutions were successful. When using STRmix™ to calculate LRs by comparing sample profiles to donor reference profiles, LRs were able to be obtained for each comparison.

For all deconvolutions of all DNA profiles obtained from samples of the four-person saliva mixture, all donors were included in each mixture. In most deconvolutions, each donor was included with very strong support (represented in the dot plots in Figure 17 and Figure 18 by a log(LR) of 6 or greater) according to the SWGDAM verbal scale (SWGDAM 2018). A few deconvolutions of sample DNA profiles that showed signs of dropout had reduced support for some donors' LRs. In addition, STRmix™ calculated high stutter variance for a couple samples. However, this was expected because one of the donors had a 14 allele at the vWA locus, and it has been shown that STRmix™ can have trouble modeling stutter for the 14 allele at the vWA locus (Russell *et al.* 2019). When these two samples were deconvoluted in STRmix™ again with user instructions to ignore the vWA locus, their stutter variances decreased significantly.

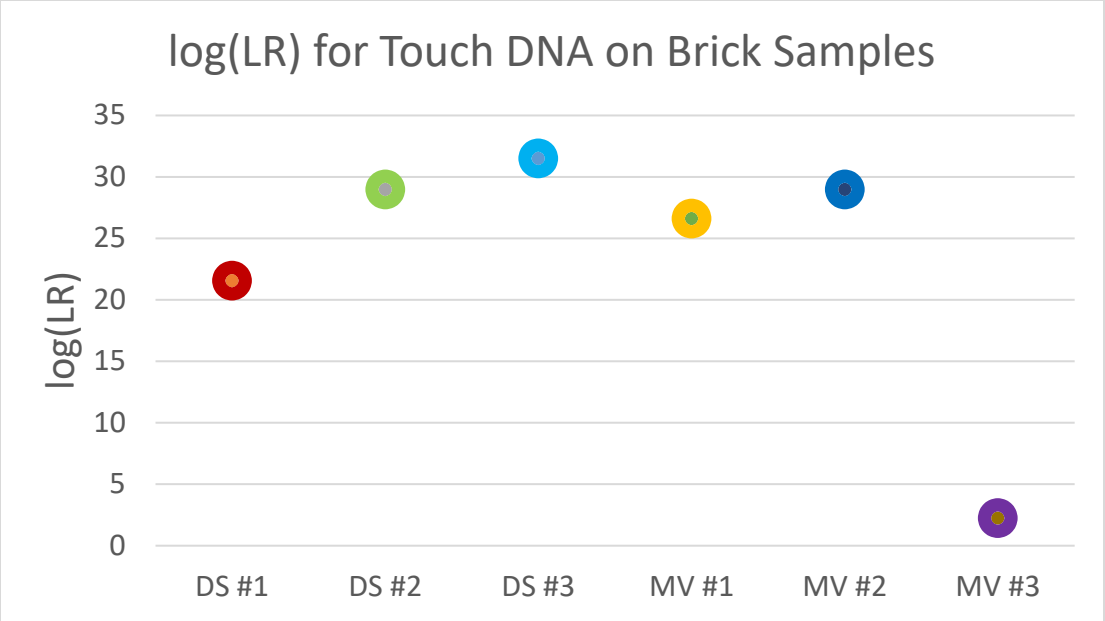


**Figure 19.** Dot plot for the logs of likelihood ratios calculated by STRmix™ for the contributor to each touch DNA on brick sample, for comparison across different sampling techniques. DS = double swab technique, MV = M-Vac®.

For all but one deconvolution of the DNA profiles obtained from samples of touch DNA on brick, the true donor to the touch DNA was included as a contributor according to the LRs assigned by STRmix™. All of these inclusions except one included the contributor with very strong support according to the SWGDAM verbal scale. One inclusion, MV #3, only included the contributor with moderate support.

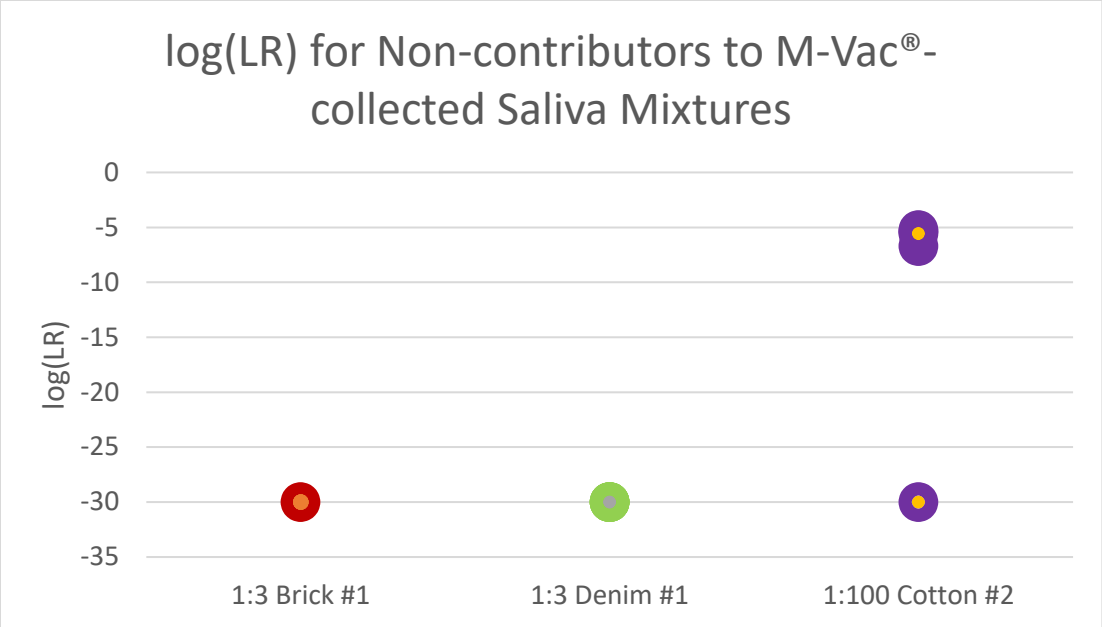


**Figure 20.** Dot plot for the logs of likelihood ratios calculated by STRmix™ for non-contributors to three samples of the four-person saliva mixture, for comparison across different substrates. These samples were collected using the M-Vac®.

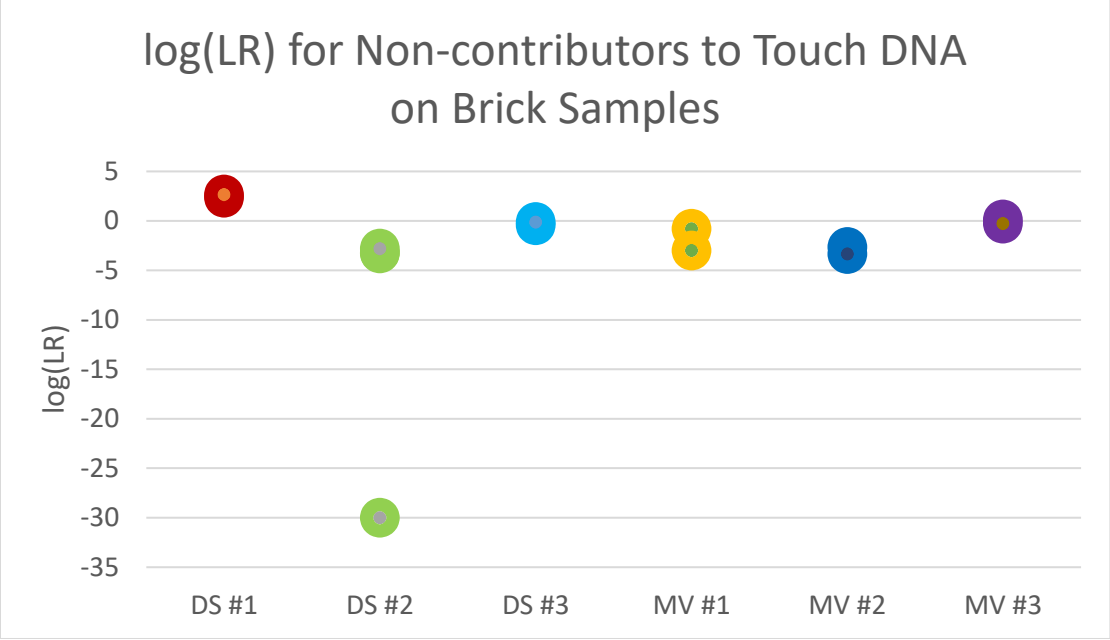


**Figure 21.** Dot plot for the logs of likelihood ratios calculated by STRmix™ for non-contributors for the touch DNA on brick samples, for comparison across different sampling methods. DS = double swab technique, MV = M-Vac®.

STRmix™'s ability to exclude noncontributors was tested by having it calculate likelihood ratios (LRs) by comparing sample DNA profiles to noncontributor reference profiles. In the dot plots depicted in Figures 20 and 21, a complete exclusion (a likelihood ratio of 0) is represented as log(LR) of -30.

STRmix™'s ability to exclude noncontributors was tested on three saliva mixture samples. For the two 1:3 saliva mixture dilution samples, STRmix™ assigned a LR of 0 for each noncontributor. For the 1:100 saliva mixture dilution sample, STRmix™ assigned LRs of 2.7 x $10^{-6}$ or less for each noncontributor.

STRmix™'s ability to exclude noncontributors was also tested on all of the touch DNA brick samples. For most of the touch DNA brick samples, STRmix™ calculated low LRs (depicted in Figure 21 as log(LR)s with negative values) for most non-contributors. For one of the touch DNA on brick samples, STRmix™ calculated a LR of 0 for one non-contributor to the double swab sample of Brick #2. For the M-Vac®-collected sample of Brick #3, STRmix™ calculated a LR of 1.3 for one non-contributor.

For the double swab sample of Brick #1, STRmix™ calculated a LR of 219.7 for one non-contributor and a LR of 438.1 for another non-contributor. However, it should be noted that very little DNA was able to amplified for this sample, which resulted in dropout at many loci and low peak heights for the few alleles that were able to called using GeneMapper *ID-X*. Because of this, STRmix™ had to take all possible allele combinations into consideration at some loci when calculating LRs for potential contributors. As a result, it is likely that, at multiple loci, the non-contributors' alleles happened to by chance match the possible allele combinations considered by STRmix™, which lead STRmix™ to calculate LRs with moderate support for inclusion of the non-contributors.

**Statistics**

Multiple statistical methods available in Excel (Microsoft, Redmond, WA) were used to test data from the third study, the M-Vac® performance investigation. First, a two-way ANOVA was conducted using two treatments (either the double swab technique or M-Vac® sample collection) divided among the three substrate types (cotton fabric, denim fabric, or brick) to check for any interactions between sample type and treatment. Given $p < .05$, a p-value of $1.01 \times 10^{-11}$ was calculated for the comparison between the two treatments, and a p-value of $1.84 \times 10^{-9}$ was calculated for the comparison across different substrate types. This indicates that significant differences in the amount of DNA collected were found between the two treatments, as well as between the different substrate types. In addition, a p-value of $1.97 \times 10^{-9}$ was calculated for potential interaction, which indicates that there may be an interaction between treatment and substrate type that affects the amount of DNA collected.

Next, a one-way ANOVA was conducted on samples from all substrate types (cotton fabric, denim fabric, or brick) collected using the double swab technique from the 1:3 dilution of the four-person saliva mixture. This one-way ANOVA was conducted to compare DNA yield of the 1:3 diluted four-person saliva mixture samples collected with the double swab technique across different substrates. Given $p < .05$, a p-value of .28 was obtained, which indicates that there were no significant differences in the quantity of DNA collected across the double swab samples.

A separate one-way ANOVA was conducted on samples from all substrate types (cotton fabric, denim fabric, or brick) collected using the M-Vac® on the 1:3 dilution of the four-person saliva mixture. This one-way ANOVA was conducted to compare DNA yield of the 1:3 diluted four-person saliva mixture samples collected with the M-Vac® across different substrates. Given

$p < .05$, a p-value of $5.68 \times 10^{-6}$ was obtained, which indicates that there were significant differences in the amount of DNA collected across the M-Vac®-collected samples.

| Sample Type | df | *t* | Two-tailed p (p < .05) |
|---|---|---|---|
| Cotton | 2 | -11.81 | 0.007098 |
| Denim | 2 | -41.63 | 0.0005764 |
| Brick | 3 | .4948 | 0.6547 |

**Table 5.** Summary table of results for t-tests comparing DNA yields from two sampling methods (double swab technique vs. M-Vac®) for each sample type. Significant results ($p < .05$) are highlighted in yellow.

Two-sample t-tests assuming unequal variances were also used for comparison. One set of two-sample t-tests assuming unequal variances compared the double swab technique to the M-Vac® across different substrates upon which samples of the 1:3 dilution of the four-person saliva were deposited. These samples were compared to determine whether there were significant differences in how much DNA the double swab technique and the M-Vac® collected on different substrates. Given $p < .05$, a p-value of .0071 was obtained for cotton fabric, which indicates that there were significant differences in the amount of DNA collected between the double swab technique and the M-Vac® for that substrate. Given $p < .05$, a p-value of .00058 was obtained for denim fabric, which indicates that there were significant differences in the amount of DNA collected between the double swab technique and the M-Vac® for that substrate. Given $p < .05$, a p-value of .65 was obtained for brick, which indicates that there were no significant differences in the amount of DNA collected between the double swab technique and the M-Vac® for that substrate.

| Sample Types Being Compared | Sample Method Used | df | *t* | Two-tailed p (p < .05) |
|---|---|---|---|---|
| Cotton vs. Denim | Double swab technique | 3 | -2.272 | .1077 |
| Cotton vs. Brick | Double swab technique | 2 | -.4771 | .6804 |
| Denim vs. Brick | Double swab technique | 4 | 1.058 | .3498 |
| Cotton vs. Denim | M-Vac® | 2 | -2.932 | .09927 |
| Cotton vs. Brick | M-Vac® | 2 | 11.81 | .007098 |
| Denim vs. Brick | M-Vac® | 2 | 42.09 | .0005641 |

**Table 6.** Summary table of results for t-tests comparing DNA yields between two of three sample types (cotton fabric, denim fabric, or brick) for each sampling method. Significant results ($p < .05$) are highlighted in yellow.

A second set of two-sample t-tests assuming unequal variances compared how much DNA the same sampling method (either the double swab technique or the M-Vac®) collected from the same samples of the 1:3 dilution of the four-person mixture across different substrates (cotton fabric, denim fabric, or brick). These samples were compared to determine whether there were significant differences in how much DNA the same sampling method collected across different substrates. Given $p < .05$, a p-value of .11 was obtained for the double swab technique on cotton fabric compared to denim fabric, which indicates that there were no significant differences in the amount of DNA collected using the double swab technique when comparing cotton fabric and denim fabric substrates. Given $p < .05$, a p-value of .68 was obtained for the double swab technique on cotton fabric compared to brick, which indicates that there were no significant differences in the amount of DNA collected using the double swab technique when comparing cotton fabric and brick substrates. Given $p < .05$, a p-value of .35 was obtained for the double swab technique on denim fabric compared to brick, which indicates that there were no significant differences in the amount of DNA collected using the double swab technique when comparing denim fabric and brick substrates. Given $p < .05$, a p-value of .10 was obtained for using the M-Vac® on cotton fabric compared to denim fabric, which indicates that there were no significant differences in the amount of DNA collected using the M-Vac® when comparing

cotton fabric and denim fabric substrates. Given $p < .05$, a p-value of .0071 was obtained for using the M-Vac® on cotton fabric compared to brick, which indicates that there were significant differences in the amount of DNA collected using the M-Vac® when comparing cotton fabric and brick substrates. Given $p < .05$, a p-value of .00056 was obtained for using the M-Vac® on denim fabric compared to brick, which indicates that there were significant differences in the amount of DNA collected using the M-Vac® when comparing denim fabric and brick substrates.

| Sample Dilutions Being Compared | df | *t* | Two-tailed p ($p < .05$) |
|---|---|---|---|
| 1:3 vs. 1:10 | 3 | 7.358 | .005188 |
| 1:3 vs. 1:30 | 2 | 10.48 | .008979 |
| 1:3 vs. 1:100 | 2 | 11.67 | .007259 |
| 1:10 vs. 1:30 | 3 | 6.771 | .006583 |
| 1:10 vs. 1:100 | 2 | 10.43 | .009074 |
| 1:30 vs. 1:100 | 2 | 3.831 | .06189 |

**Table 7.** Summary table of results for t-tests comparing DNA yields between different sample dilutions (1:3, 1:10, 1:30, or 1:100) for sample dilutions that were pipetted onto cotton fabric and later sampled using the M-Vac®. Significant results ($p < .05$) are highlighted in yellow.

A third set of two-sample t-tests assuming unequal variances compared how much DNA was collected by the M-Vac® from different sample dilutions (1:3, 1:10, 1:30, or 1:100) of the four-person saliva mixture on the same cotton fabric substrate. These samples were compared to determine whether there were significant differences in how much DNA the M-Vac® was able to collect from different dilutions of the four-person saliva mixture on the same substrate. Given $p < .05$, a p-value of .0051 was obtained for using the M-Vac® on the 1:3 dilution compared to the 1:10 dilution, which indicates that there were significant differences in the amount of DNA collected using the M-Vac® when comparing the 1:3 and 1:10 dilutions. Given $p < .05$, a p-value of .0090 was obtained for using the M-Vac® on the 1:3 dilution compared to the 1:30 dilution, which indicates that there were significant differences in the amount of DNA collected using the M-Vac® when comparing the 1:3 and 1:30 dilutions. Given $p < .05$, a p-value of .0073 was obtained for using the M-Vac® on the 1:3 dilution compared to the 1:100 dilution, which

indicates that there were significant differences in the amount of DNA collected using the M-Vac® when comparing the 1:3 and 1:100 dilutions. Given $p < .05$, a p-value of .0066 was obtained for using the M-Vac® on the 1:10 dilution compared to the 1:30 dilution, which indicates that there were significant differences in the amount of DNA collected using the M-Vac® when comparing the 1:10 and 1:30 dilutions. Given $p < .05$, a p-value of .0091 was obtained for using the M-Vac® on the 1:10 dilution compared to the 1:100 dilution, which indicates that there were significant differences in the amount of DNA collected using the M-Vac® when comparing the 1:10 and 1:100 dilutions. Given $p < .05$, a p-value of .062 was obtained for using the M-Vac® on the 1:30 dilution compared to the 1:100 dilution, which indicates that there were no significant differences in the amount of DNA collected using the M-Vac® when comparing the 1:30 and 1:100 dilutions.

## Discussion

### Initial Concentration Studies

The preparation of DNA samples in the SpeedVac™ has been found to lead to enhanced concentrations of DNA. This study clearly demonstrates the utility of the SpeedVac™ to increase DNA concentrations in M-Vac®-collected DNA samples, including those expected to have low concentrations of DNA. Increasing the concentrations of M-Vac®-collected DNA samples could lead to more complete DNA profiles being obtained through STR analysis, which would be invaluable for casework.

However, because the concentrations of DNA in the samples after concentrating them in the SpeedVac™ tended to be less than their expected concentrations, this indicates that samples are not being concentrated as much as they should theoretically be able to. This could prove problematic for M-Vac®-collected samples, which are expected to have low concentrations of DNA and therefore will need to be concentrated as much as possible. More research will need to conducted to find the cause of the decreased efficiency and remedy it.

### Investigating Potential DNA Loss Caused by the M-Vac® Pre-Filter System

A full DNA profile concordant with X19-63's DNA profile was obtained from one of the M-Vac®-collected samples. This demonstrates that the M-Vac® can successfully collect a donor's cells. This DNA profile did not show any signs of contamination, and no contamination was observed from the corresponding reagent blank. These results indicate that a donor's cells can be collected with the M-Vac®, vacuum-filtered through an M-Vac® Pre-Filter, vacuum-filtered through an M-Vac® Filter, and finally dried on the M-Vac® Filter without contamination occurring from the components or the physical process of the M-Vac®.

Therefore, DNA profiles collected in subsequent experiments can be expected not to contain contamination arising from the M-Vac®.

However, DNA was quantified on all of the M-Vac® Pre-Filters sampled in this experiment, as well as on all of the fibers collected off of each M-Vac® Pre-Filter. This indicates that, when the M-Vac® Pre-Filter is used to pre-filter samples, some cells get caught on the M-Vac® Pre-Filter and on debris caught by the M-Vac® Pre-Filter membrane. This suggests that, for casework samples that are expected to have very low amounts of DNA, it may not be advisable to use the M-Vac® Pre-Filter because DNA could be lost. If the M-Vac® Pre-Filter must be used, then it would advisable to extract for DNA both the M-Vac® Filter as well the M-Vac® Pre-Filter and the debris it collects. In addition, the results of the blood flow-through experiment showed that, according to the quantitation results, DNA passed through the filter and into the flow-through beneath. This indicates that, at least for sample types with high DNA concentrations such as blood, some DNA can pass through the M-Vac® Filter and be found in the flow-through.

Because the concentration of DNA in an M-Vac®-collected sample that was re-extracted with Casework Extraction Buffer was found to be far higher than that of an M-Vac®-collected sample that was only extracted with Incubation Buffer, Incubation Buffer was deemed to be not an ideal buffer for extracting DNA from M-Vac® Filters. Because of this, it is likely that significantly less DNA than was there was actually extracted from the earlier M-Vac®-collected samples extracted with Incubation Buffer. Therefore, the amount of DNA quantified from the M-Vac® Filters cannot accurately be compared to the amount quantified from the M-Vac® Pre-Filter membranes to determine how much DNA was caught on the M-Vac® Pre-Filters

compared to the M-Vac® Filters. In addition, it was decided that only Casework Extraction Buffer should be used to extract M-Vac®-collected samples in subsequent experiments.

Since the concentration of DNA increased in M-Vac®-collected samples that were concentrated as opposed to M-Vac®-collected samples that were not concentrated, and because the M-Vac® is intended to be used to sample large surface areas and therefore could result in low DNA concentrations, this suggests that M-Vac®-collected samples with low DNA concentrations should be concentrated. Again, this would prove invaluable for casework samples, which are expected to need to be concentrated because they may have low levels of DNA and since there is such a high volume of SRS.

**Investigation of M-Vac®'s Performance to Collect Cellular Material from Various Substrates Compared to the Double Swab Technique**

For the four-person saliva mixtures deposited on cotton and denim fabric, the M-Vac® was able to collect significantly more DNA than the double swab technique. This suggests that the M-Vac® would be a helpful alternative collection method for these substrates when the double swab technique yields little or no DNA.

However, neither the M-Vac® nor the double swab technique were able to collect much DNA from the four-person saliva mixtures deposited on brick substrate, and neither method performed significantly better than the other. This result is somewhat surprising since, given results from other studies, the M-Vac®'s wet-vacuum system was expected to work well on a porous substrate like brick (McLamb *et al.* 2020; Vickar *et al.* 2018). While using the M-Vac® on the bricks, it was noted that certain areas appeared to absorb the liquid SRS better than other areas within the same brick. In addition, within sets of bricks purchased together from the same manufacturer, individual bricks appeared to absorb the liquid SRS better or worse than other

bricks within that same set. Also, individual bricks appeared to be coated with more debris than other bricks within the same set of bricks. These observations suggest that different types of bricks may be better or worse substrates from which to collect cells using the M-Vac®. These observations also suggest that, within an individual brick, certain areas may be better substrates than others from which to collect DNA using the M-Vac®. Overall, it would appear that the M-Vac® is better at collecting DNA from certain types of bricks more than others. Our results with bricks are similar to the cinder block results observed by McLamb *et al.* (McLamb *et al.* 2020).

When comparing M-Vac®-collected samples of 1:3, 1:10, 1:30, and 1:100 dilutions of the four-person saliva mixture on cotton fabric, generally, a smaller amount of DNA is collected from the more dilute samples compared to less dilute samples. This was expected, since more diluted samples should contain less DNA. However, the percent DNA yield across these different dilutions generally was about 30 to 50%, regardless of how dilute the sample was. This shows that the M-Vac® has a fairly consistent recovery rate for DNA deposited on cotton fabric.

For all of the touch DNA on brick samples, not much DNA was yielded from either the double swab technique or the M-Vac®. Neither method appeared to yield more DNA than the other, and the M-Vac® may have yielded less DNA than the double swab technique for one sample. However, because these touch DNA on brick samples first had the double swab technique applied to them and then had the M-Vac® used on them, it may be possible that there were not many cells left after using the double swab technique for the M-Vac® to collect. Another possibility is that the wet swab used in the double swab technique left water behind that seeped into the brick and took cells with it, which would have left behind fewer cells for the M-Vac® to collect.

After amplifying the touch DNA on brick samples and obtaining electropherograms for each sample using capillary electrophoresis, most of the resulting DNA profiles appeared to be mixtures of two people: the expected donor and a very minor unknown contributor. However, this result was not entirely unexpected, since each donor was allowed to work at their desk in the hour before they gave their sample. During this time, they likely would have made contact with numerous surfaces and items, such as their computer keyboard, their desk, and any personal possessions they brought from home. Some of these surfaces and items could have also been touched by their co-workers, for example, and when the donors then touched these surfaces and items, transfer of the other person's DNA onto the donor's hands could have occurred (van Oorschot and Jones 1997). When the donor was later instructed to place their hands on the brick and apply pressure, the other person's DNA could have then been transferred onto the brick. The minor contributors to these DNA profiles were unable to be identified, though, since each minor contributor only contributed one allele to the entire DNA profile.

STRmix™ was able to perform deconvolutions of all of the M-Vac®-collected four-person saliva mixture samples and all of the M-Vac®-collected touch DNA samples, as well as calculate LRs for all of the samples compared to donors (Appendix B). Profiles with more than two contributors are not interpreted manually; STRmix™ can deconvolute more complex mixtures. For most of these samples, the LRs calculated by STRmix™ corresponded to the donors included in the four-person saliva mixture sample or the donor included in the touch DNA sample. In general, STRmix™ calculated very high LRs ($1.0 \times 10^6$ or higher) for all four donors that were expected to be present in the four-person saliva mixture samples and each donor of each touch DNA sample. Using the SWGDAM verbal qualifiers, most of the donors that were expected to be present in each sample had "very strong support" by STRmix™.

There were a few four-person saliva mixtures samples that had contributors with LRs ranging from 3.94 to 2.75 x $10^4$. From the touch DNA samples, one sample had an LR of 166.8 calculated for the donor and one sample had an LR of 0 calculated for the donor. For all of these samples, the electropherograms showed varying levels of dropout (Appendix A), which likely contributed to the reduced LRs. In addition, the two touch DNA samples came from the same male donor, so there is a chance that that particular donor does not shed much DNA. Overall, though, the data indicate that STRmix™ can successfully include the correct donors to M-Vac®-collected saliva mixture samples and touch DNA samples.

STRmix™'s ability to calculate LRs that exclude non-contributors was also tested. For three of the four-person saliva mixtures samples, STRmix™ calculated LRs for five non-contributors to each sample, and all non-contributors were calculated to have LRs close to or equal to 0. This indicates that STRmix™ can successfully exclude non-contributors to M-Vac®-collected saliva mixture samples. For most of the touch DNA samples, STRmix™ calculated LRs of less than 1.32 or 0 for most of the non-contributors. This indicates that STRmix™ can successfully exclude non-contributors to M-Vac®-collected touch DNA samples. For one touch DNA sample collected with the double swab technique, though, STRmix™ calculated LRs of 219.7 and 438.1 for two non-contributors. However, it should be noted that the electropherogram of this particular touch DNA sample showed signs of dropout. For profiles with low levels of template DNA and dropout, non-contributors can end up being included by STRmix™ since STRmix™ has to consider many possible genotypes at some loci (Buckleton *et al.* 2019). Overall, though, STRmix™ was able to calculate LRs for non-contributors that tended to exclude them, as expected.

## Conclusion

Choosing the right sampling and analysis methods are important so that as many cells as possible can be collected and as much useful information as possible can be obtained from the resulting DNA profiles. One traditional approach is to sample evidence using the double swab technique and deconvolute any resulting DNA profiles using manual interpretation. However, the double swab technique may have trouble collecting cells from certain substrates, and manual interpretation can only be applied to samples containing up to three contributors and simple mixture samples. An alternate approach would be to sample evidence using the M-Vac® Wet-Vacuum System, which could collect more cells than the double swab technique for certain substrates, and to analyze DNA profiles using STRmix™, which can deconvolute more complex mixtures than manual interpretation allows.

This research was conducted to determine whether using the M-Vac® and STRmix™ would yield more information about samples compared to using the more traditional double swab technique combined with manual interpretation. The results of this research showed that collecting samples with the M-Vac® and then analyzing the resulting DNA profiles with STRmix™ gave more information than collecting samples with the double swab technique and then manually interpreting the resulting DNA profiles.

This research shows that the performance of both the M-Vac® and a traditional sampling method like the double swab technique will vary across different substrates and sometimes even within a category of substrate, such as brick. This demonstrates to crime laboratories that the type of substrate needs to be considered when choosing a sampling method. In addition, STRmix™ was able to accurately interpret complex DNA mixture profiles that would have been ineligible for manual interpretation. This demonstrates to crime laboratories how important it can

be to use probabilistic genotyping software, especially on complex DNA mixture profiles. In summary, the results of this research show that M-Vac®-collected samples can be successfully and accurately analyzed using STRmix™.

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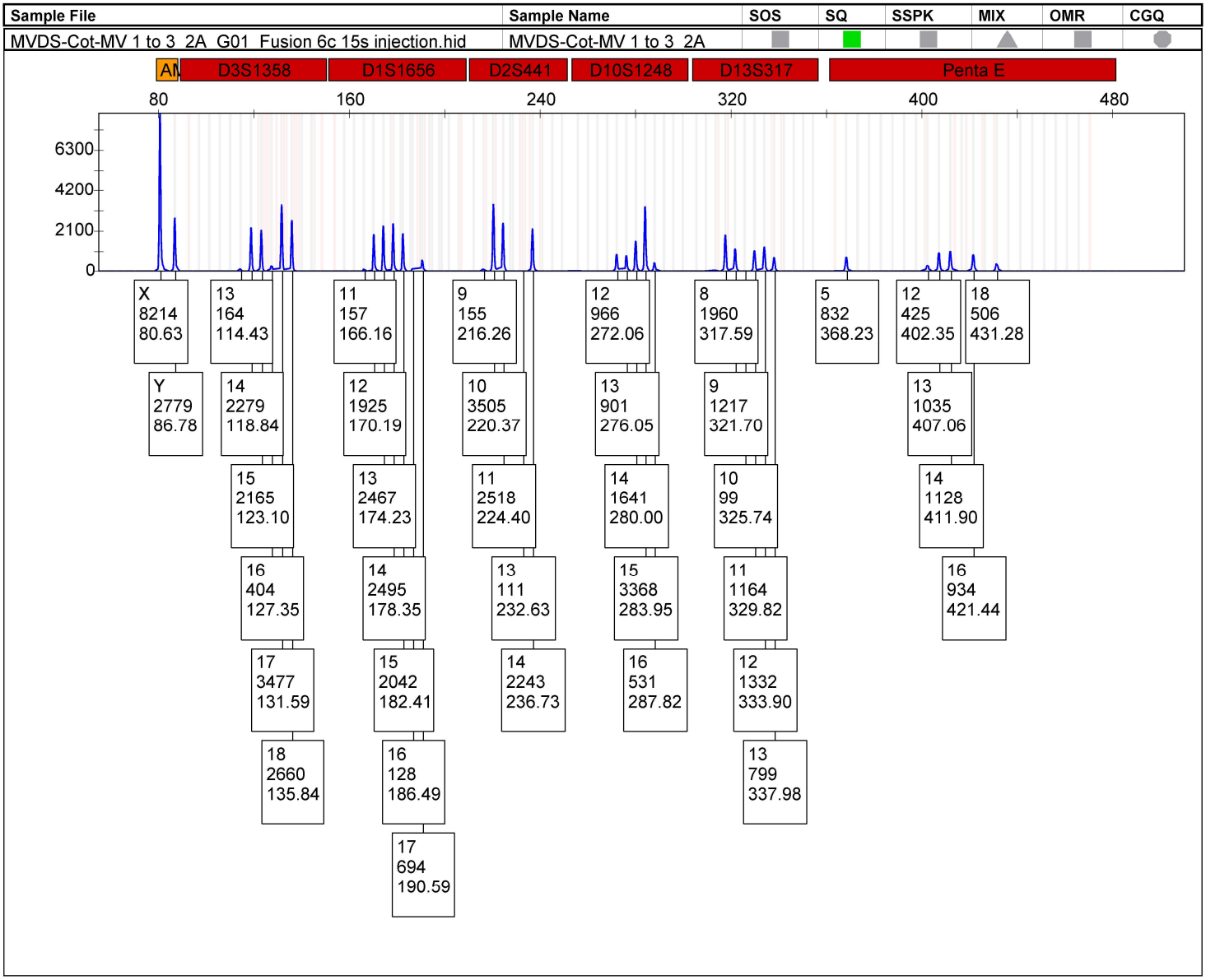
Sample File
Sample Name
SOS
SQ
SSPK
MIX
OMR
CGQ
MVDS-Cot-MV 1 to 3_2A_G01_Fusion 6c 15s injection.hid
MVDS-Cot-MV 1 to 3_2A
AM
D3S1358
D1S1656
D2S441
D10S1248
D13S317
Penta E
80
160
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320
400
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6300
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2100
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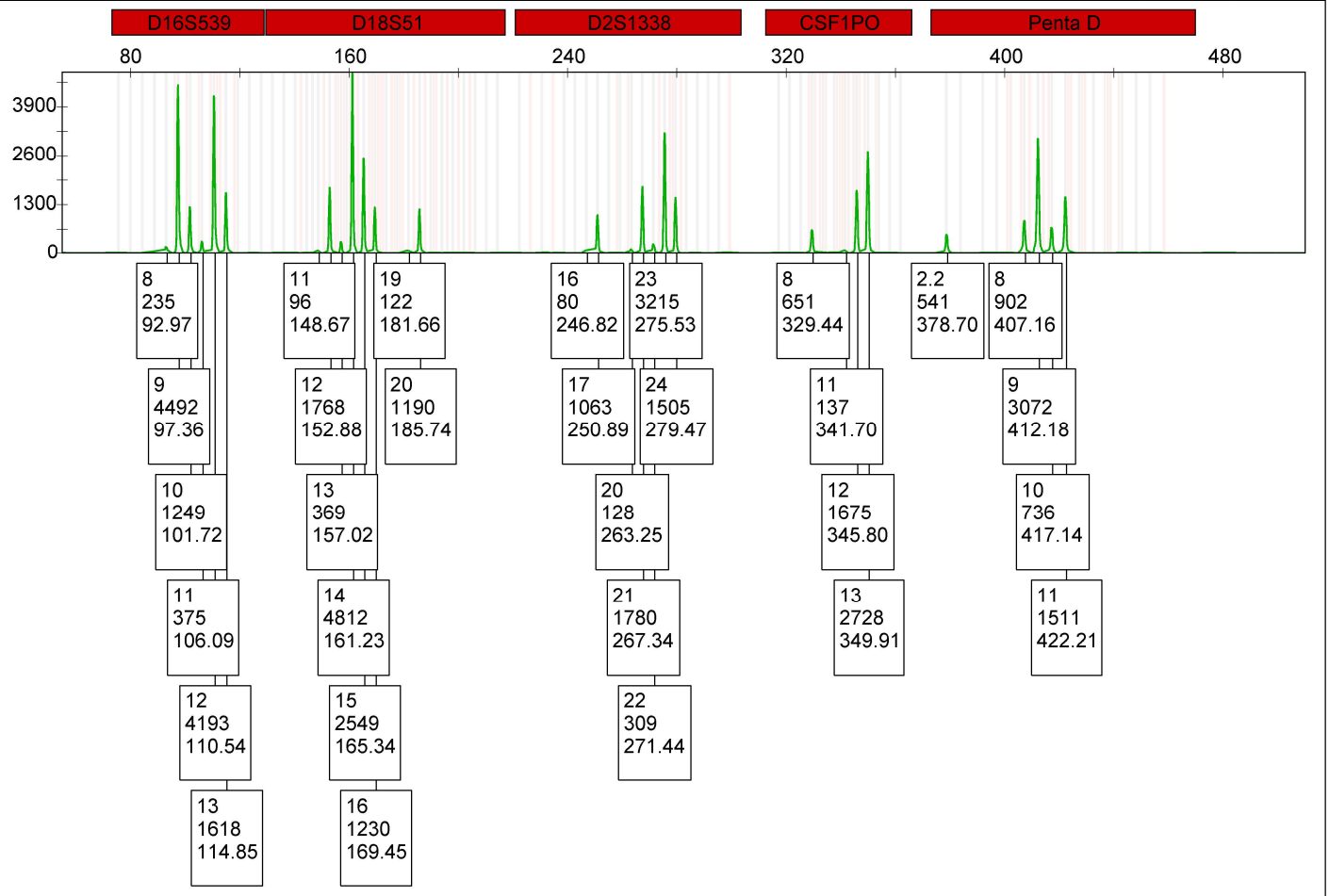
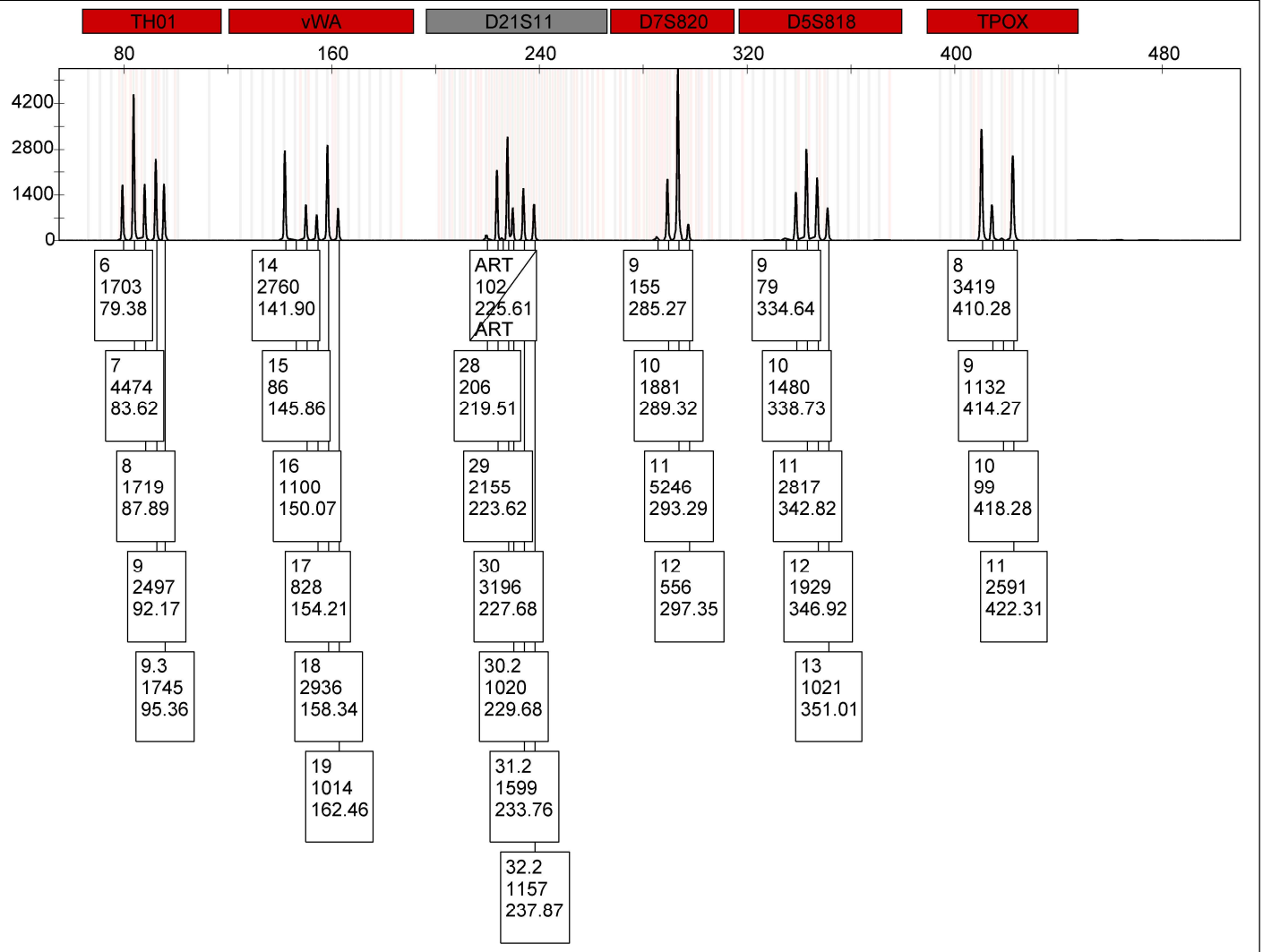
X 8214 80.63
Y 2779 86.78
13 164 114.43
14 2279 118.84
15 2165 123.10
16 404 127.35
17 3477 131.59
18 2660 135.84
11 157 166.16
12 1925 170.19
13 2467 174.23
14 2495 178.35
15 2042 182.41
16 128 186.49
17 694 190.59
9 155 216.26
10 3505 220.37
11 2518 224.40
13 111 232.63
14 2243 236.73
12 966 272.06
13 901 276.05
14 1641 280.00
15 3368 283.95
16 531 287.82
8 1960 317.59
9 1217 321.70
10 99 325.74
11 1164 329.82
12 1332 333.90
13 799 337.98
5 832 368.23
12 425 402.35
13 1035 407.06
14 1128 411.90
16 934 421.44
18 506 431.28

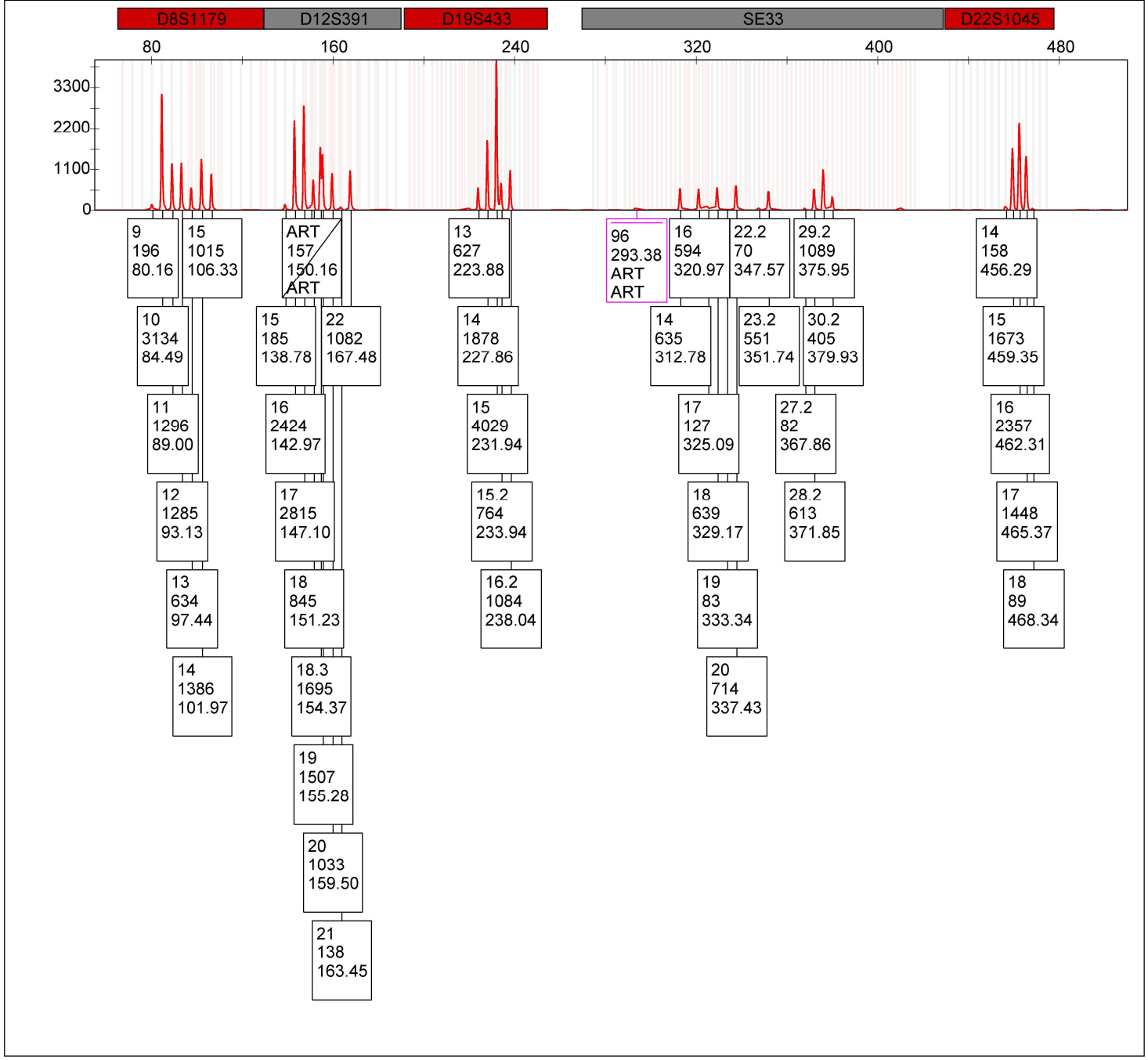
| Sample File | Sample Name | SOS | SQ | SSPK | MIX | OMR | CGQ |
|---|---|---|---|---|---|---|---|
| MVDS-Cot-MV 1 to 3_2A_G01_Fusion 6c 15s injection.hid | MVDS-Cot-MV 1 to 3_2A | | | | | | |



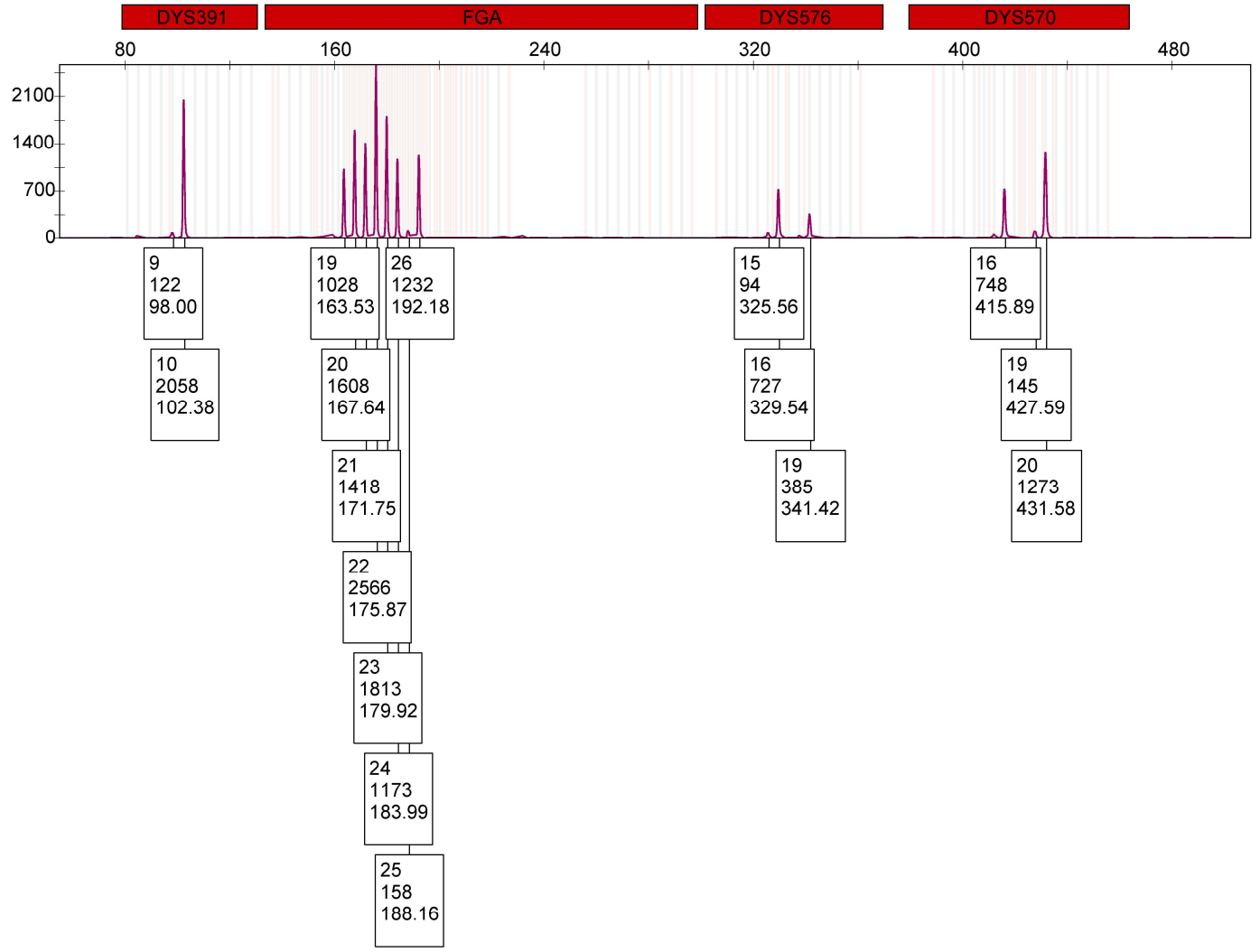
| Sample File | Sample Name | SOS | SQ | SSPK | MIX | OMR | CGQ |
|---|---|---|---|---|---|---|---|
| MVDS-Cot-MV 1 to 3_2A_G01_Fusion 6c 15s injection.hid | MVDS-Cot-MV 1 to 3_2A | | | | | | |



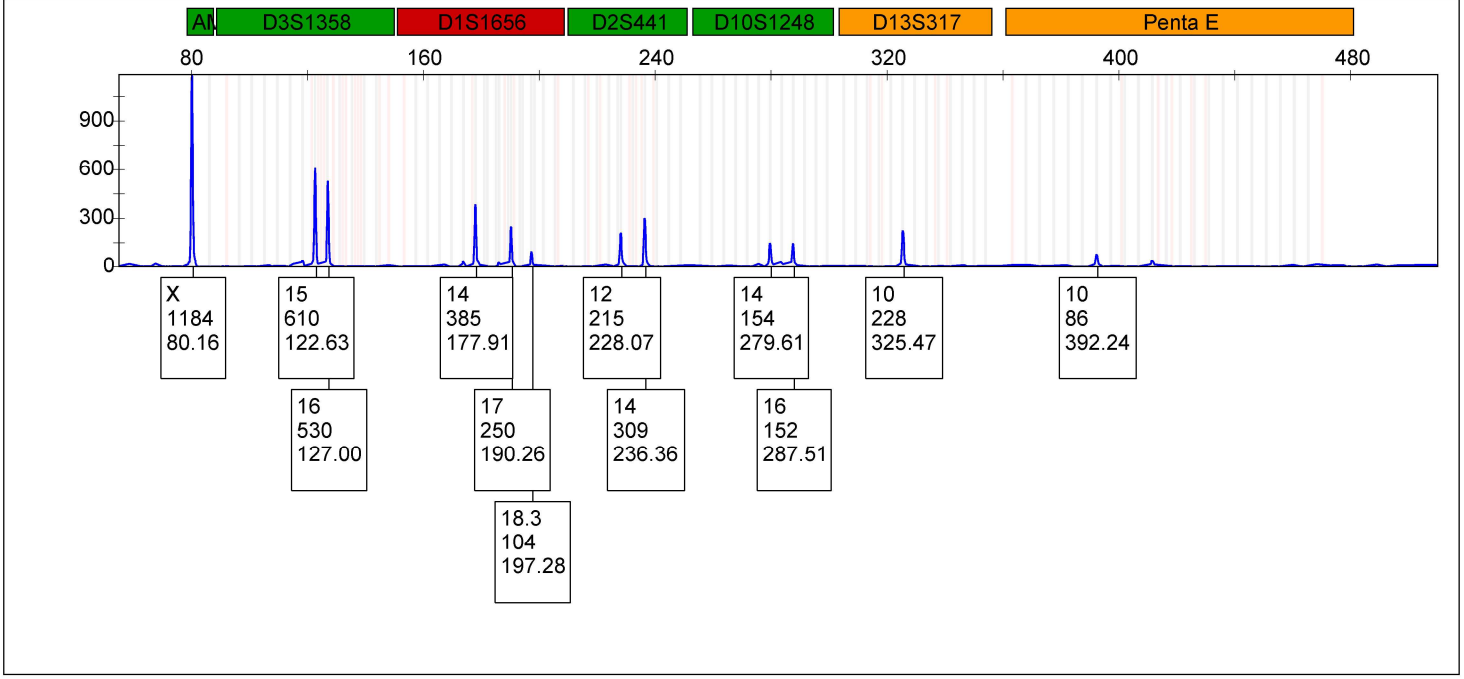
| Sample File | Sample Name | SOS | SQ | SSPK | MIX | OMR | CGQ |
|---|---|---|---|---|---|---|---|
| MVDS-Cot-MV 1 to 3_2A_G01_Fusion 6c 15s injection.hid | MVDS-Cot-MV 1 to 3_2A | | | | | | |

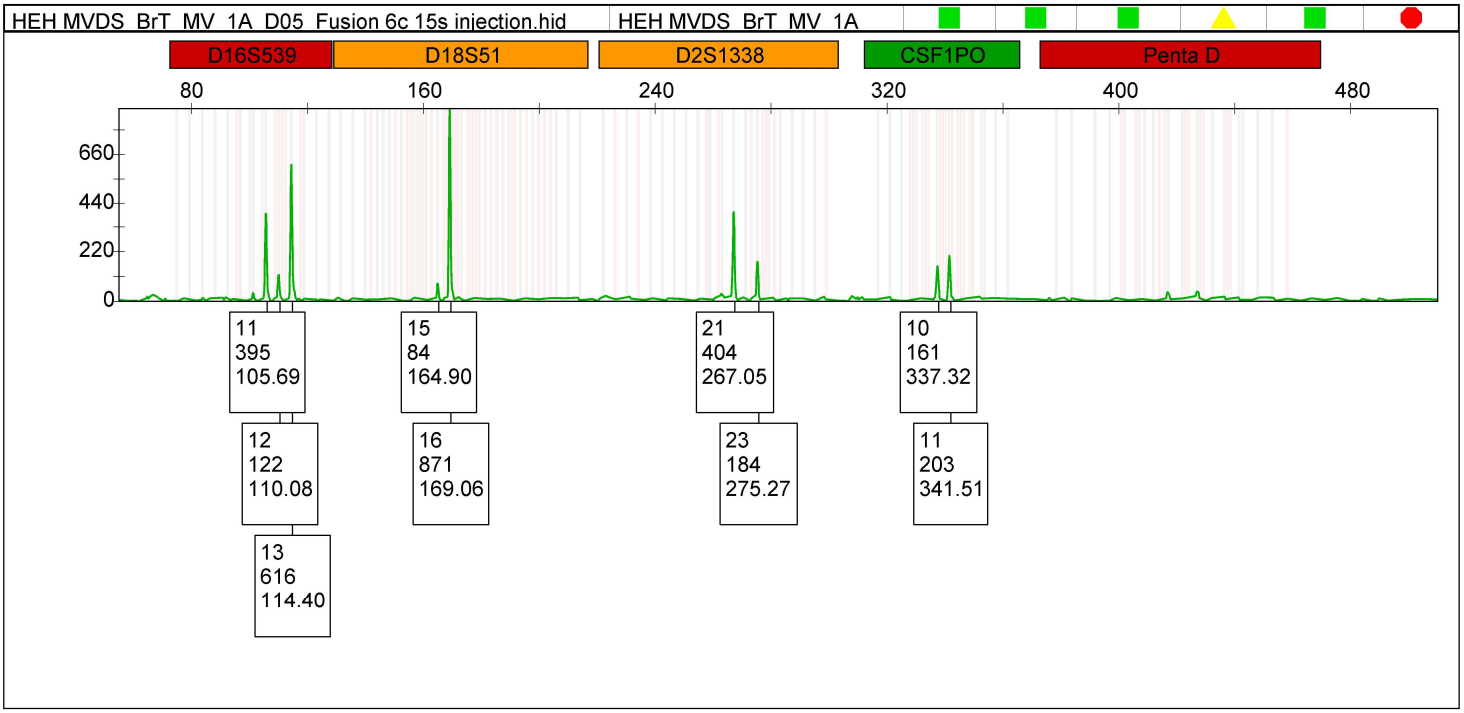


| Sample File | Sample Name | SOS | SQ | SSPK | MIX | OMR | CGQ |
|---|---|---|---|---|---|---|---|
| MVDS-Cot-MV 1 to 3_2A_G01_Fusion 6c 15s injection.hid | MVDS-Cot-MV 1 to 3_2A | | | | | | |

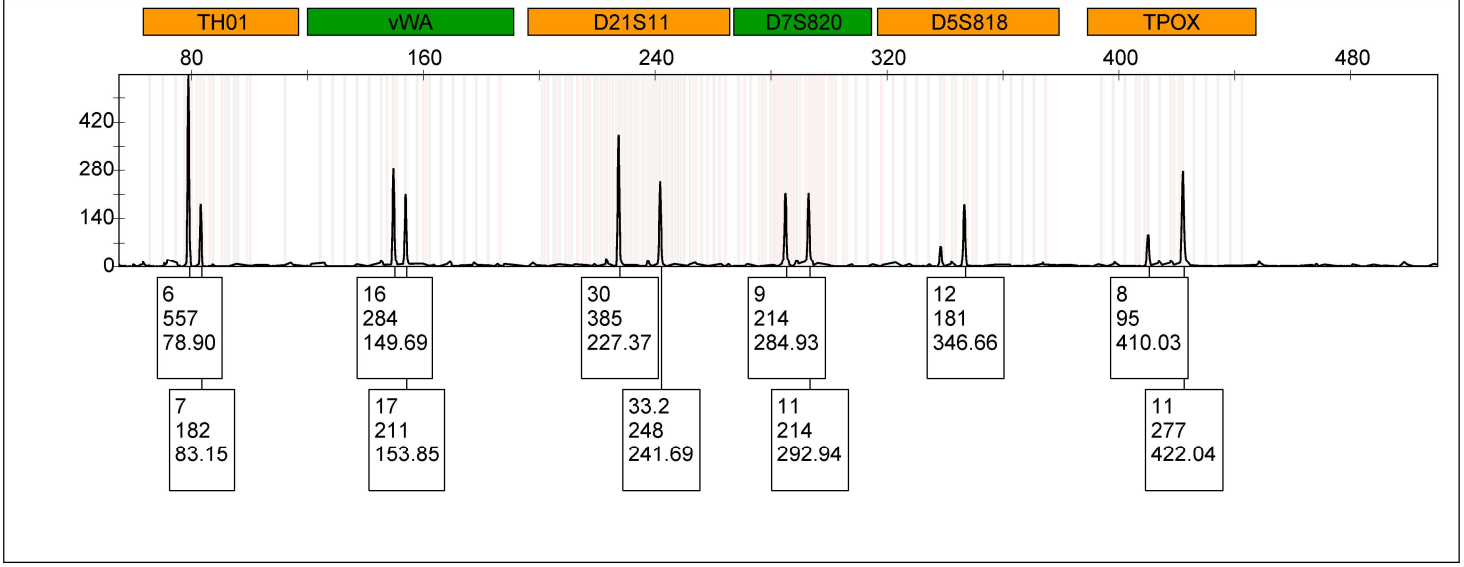


| Sample File | Sample Name | SOS | SQ | SSPK | MIX | OMR | CGQ |
|---|---|---|---|---|---|---|---|
| HEH MVDS_BrT_MV_1A_D05_Fusion 6c 15s injection.hid | HEH MVDS_BrT_MV_1A | | | | | | |

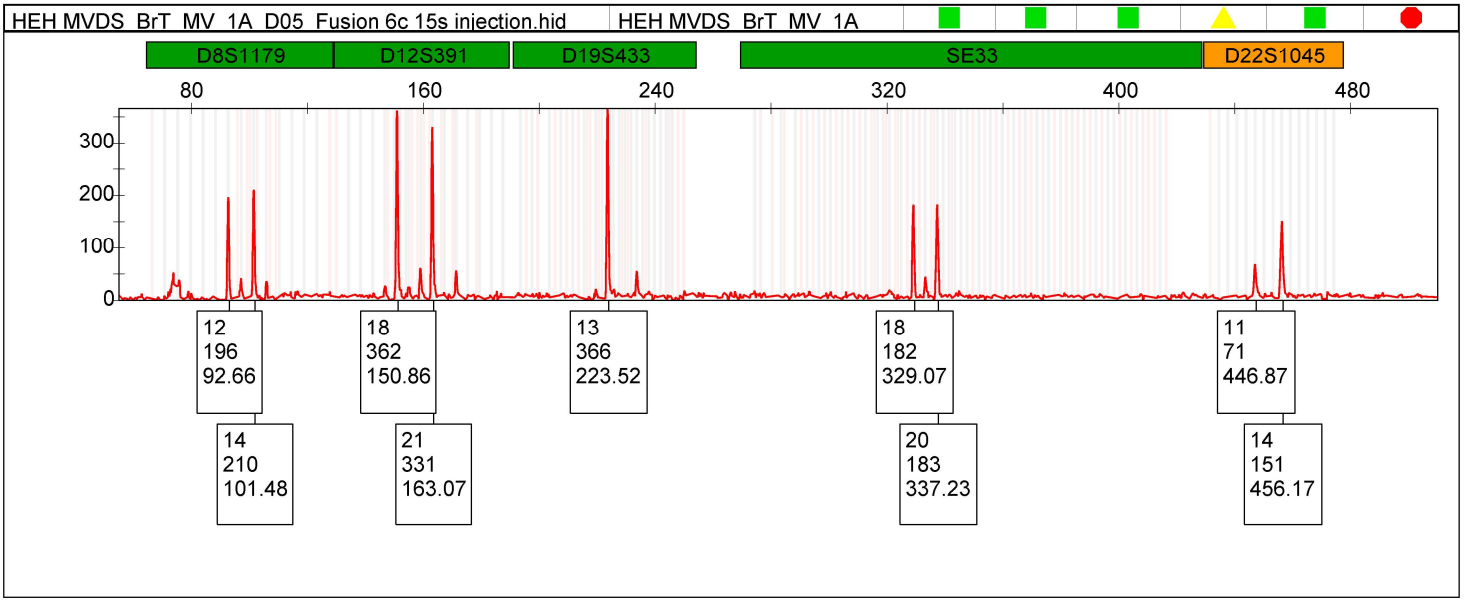


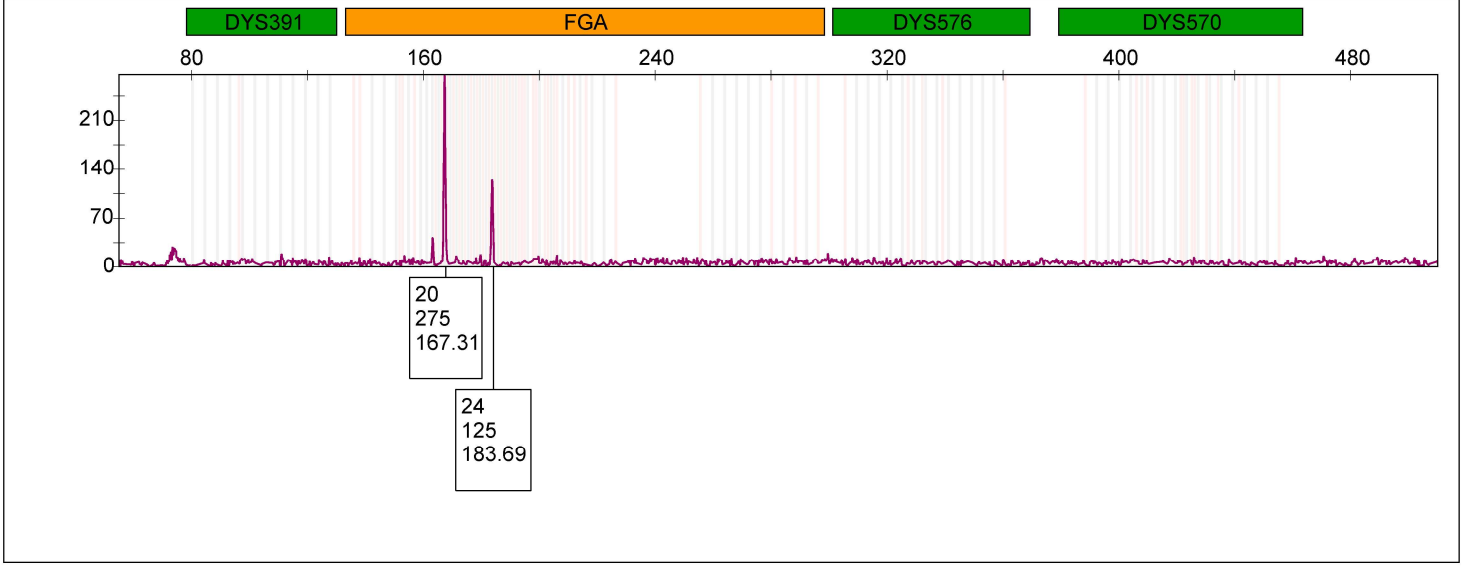
| Sample File | Sample Name | SOS | SQ | SSPK | MIX | OMR | CGQ |
|---|---|---|---|---|---|---|---|
| HEH MVDS_BrT_MV_1A_D05_Fusion 6c 15s injection.hid | HEH MVDS_BrT_MV_1A | | | | | | |




| HEH MVDS_BrT_MV_1A_D05_Fusion 6c 15s injection.hid | HEH MVDS_BrT_MV_1A | | | | | | |
|---|---|---|---|---|---|---|---|



| Sample File | Sample Name | SOS | SQ | SSPK | MIX | OMR | CGQ |
|---|---|---|---|---|---|---|---|
| HEH MVDS_BrT_MV_1A_D05_Fusion 6c 15s injection.hid | HEH MVDS_BrT_MV_1A | ■ | ■ | ■ | ▲ | ■ | ● |



# Deconvolution Report

## DETAILS

| | |
|---|---|
| **STRMIX VERSION:** | STRmix V2.5.11 |
| **USER:** | heh2013 |
| **RUN DATE:** | 09 Nov 2020 12:37:37 |
| **TOTAL RUN TIME:** | 10 mins, 50 secs |
| **REPORT RUN:** | 09 Nov 2020 12:48:28 |

## RUN PARAMETERS

| | |
|---|---|
| **CONTRIBUTORS:** | 4 |
| **PROFILING KIT:** | Fusion6C_3500_Alameda |
| **SAMPLE FILE:** | MVDS-Cot-MV 1 to 3_2A_EV.csv |

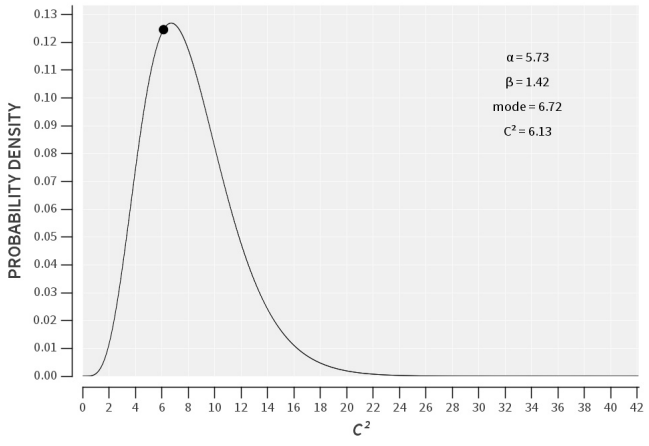
| | |
|---|---|
| **CASE NUMBER:** | MVDS |
| **SAMPLE NAME:** | Cot-MV 1 to 3_2 |
| **COMMENTS:** | |
| **SEED:** | 227966 |

## SUMMARY OF CONTRIBUTORS

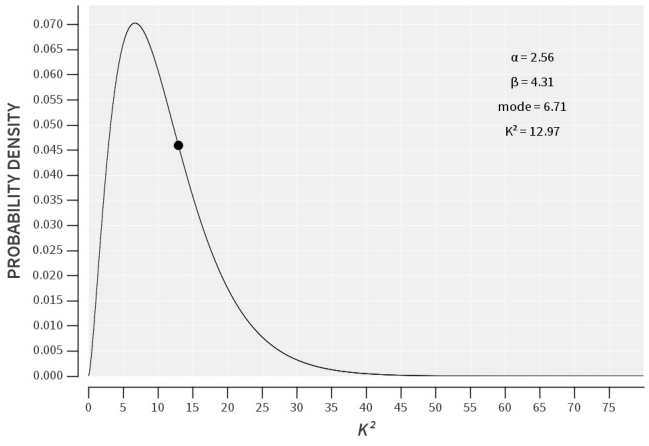
| CONTRIBUTORS | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Template (rfu) | 1939 | 1638 | 1414 | 840 |
| Mixture Proportion | 33% | 28% | 24% | 14% |
| Degradation starts at 79bp (rfu/bp) | 4.724 | 3.631 | 2.394 | 0.908 |

## VARIANCE CHARTS

ALLELE VARIANCE




STUTTER VARIANCE



| LOCUS | ALLELE | HEIGHT | SIZE |
|---|---|---|---|
| D22S1045 | 14 | 158 | 456 |
| | 15 | 1673 | 459 |
| | 16 | 2357 | 462 |
| | 17 | 1448 | 465 |
| | 18 | 89 | 468 |
| DYS391 | 9 | 122 | 98 |
| | 10 | 2058 | 102 |
| FGA | 19 | 1028 | 163 |
| | 20 | 1608 | 167 |
| | 21 | 1418 | 171 |
| | 22 | 2566 | 175 |
| | 23 | 1813 | 179 |
| | 24 | 1173 | 183 |
| | 25 | 158 | 188 |
| | 26 | 1232 | 192 |
| DYS576 | 15 | 94 | 325 |
| | 16 | 727 | 329 |
| | 19 | 385 | 341 |
| DYS570 | 16 | 748 | 415 |
| | 19 | 145 | 427 |
| | 20 | 1273 | 431 |

## POST BURN-IN SUMMARY

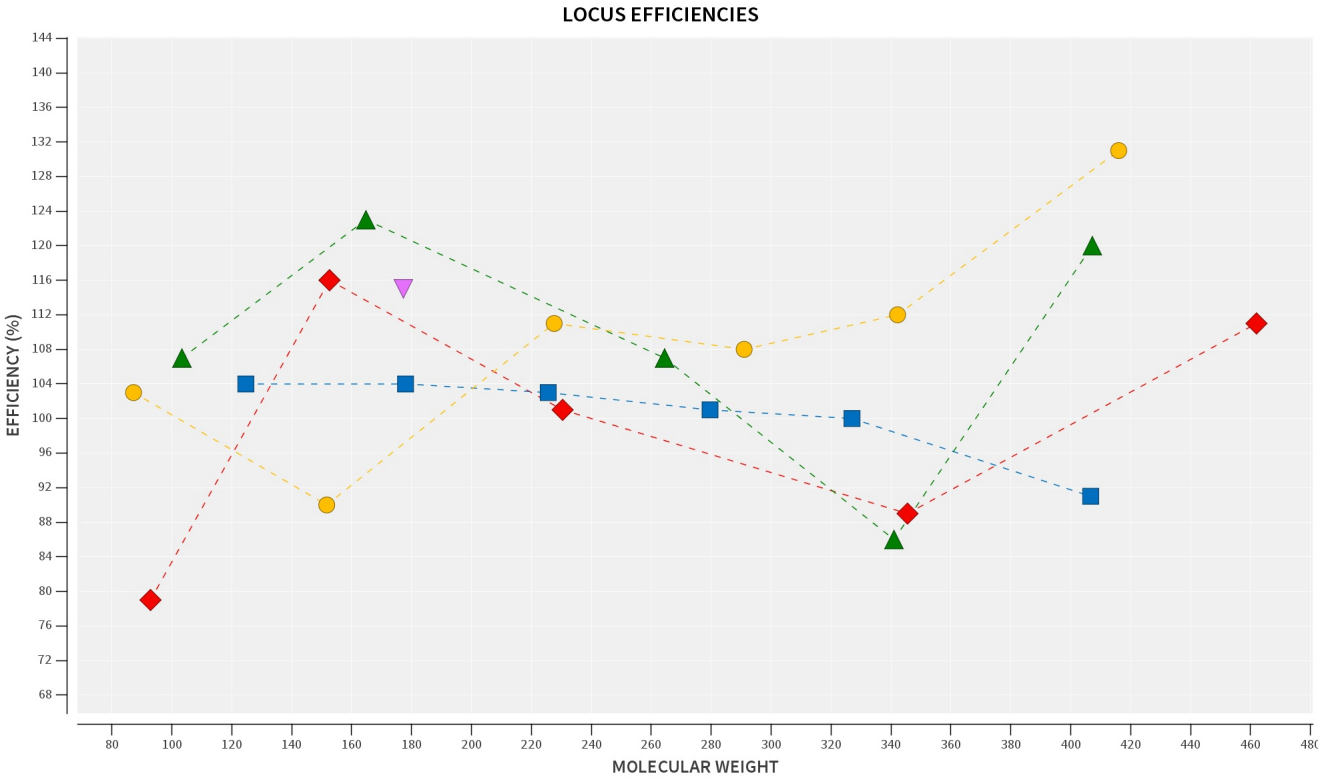
| | | | |
|---|---|---|---|
| Total iterations | 11184647 | Acceptance rate | 1 in 27.96 |
| Effective sample size | 5797.65 | log(likelihood) | 92.57 |
| Gelman-Rubin convergence diagnostic | 1.19 | | |
| Allele variance (mode = 6.715) | 6.133 | Stutter variance (mode = 6.713) | 12.968 |

## STUTTER FILES USED IN RUN

| | |
|---|---|
| Stutter File | Alameda_Fusion6c_Stutter_Amended.txt |
| Stutter Exceptions File | Alameda_Fusion6c_Exceptions.csv |
| Forward Stutter File | Alameda_Fusion6c_Forward Stutter_amended.txt |

## LOCUS EFFICIENCIES

| LOCUS | LOCUS EFFICIENCY | DETECTION THRESHOLD |
|---|---|---|
| D3S1358 | 104% | 70 |
| D1S1656 | 104% | 70 |
| D2S441 | 103% | 70 |
| D10S1248 | 101% | 70 |
| D13S317 | 100% | 70 |
| Penta E | 91% | 70 |
| D16S539 | 107% | 70 |
| D18S51 | 123% | 70 |
| D2S1338 | 107% | 70 |
| CSF1PO | 86% | 70 |
| Penta D | 120% | 70 |
| TH01 | 103% | 70 |
| vWA | 90% | 70 |
| D21S11 | 111% | 70 |
| D7S820 | 108% | 70 |
| D5S818 | 112% | 70 |
| TPOX | 131% | 70 |
| D8S1179 | 79% | 70 |
| D12S391 | 116% | 70 |
| D19S433 | 101% | 70 |
| SE33 | 89% | 70 |
| D22S1045 | 111% | 70 |
| DYS391 | | |
| FGA | 115% | 70 |
| DYS576 | | |
| DYS570 | | |



# SETTINGS

## CASE SETTINGS

| | |
|---|---|
| Case number | MVDS |
| Sample ID | Cot-MV 1 to 3_2 |
| Comments | |
| Seed | 227966 |
| Extended output | N |

## MCMC SETTINGS

| | |
|---|---|
| Number of contributors | 4 |
| Burn-in accepts per chain | 100000 |
| Post burn-in accepts per chain | 50000 |
| Use Mx priors | N |
| Number of chains | 8 |
| Random walk SD | 0.005 |
| Post burn-in shortlist | 9.0 |
| Auto continue on Gelman-Rubin | N |

## KIT SETTINGS

| | | |
|---|---|---|
| Ignored loci | DYS391, DYS576, DYS570 | |
| Detection thresholds | D3S1358 | 70 |
| | D1S1656 | 70 |
| | D2S441 | 70 |
| | D10S1248 | 70 |
| | D13S317 | 70 |
| | Penta E | 70 |
| | D16S539 | 70 |
| | D18S51 | 70 |
| | D2S1338 | 70 |
| | CSF1PO | 70 |
| | Penta D | 70 |
| | TH01 | 70 |
| | vWA | 70 |
| | D21S11 | 70 |
| | D7S820 | 70 |
| | D5S818 | 70 |
| | TPOX | 70 |
| | D8S1179 | 70 |
| | D12S391 | 70 |
| | D19S433 | 70 |
| | SE33 | 70 |
| | D22S1045 | 70 |
| | FGA | 70 |
| Stutter max | 0.3 | |
| Forward stutter max | 0.15 | |
| Saturation | 28000 | |
| Degradation starts at | -1.0 | |
| Degradation max | 0.01 | |
| Drop-in cap | 200 | |
| Drop-in frequency | 0.0039 | |
| Drop-in gamma parameters | 12.74, 3.75 | |
| Allelic variance (α, β) | 5.729, 1.42 | |
| Stutter variance (α, β) | 2.556, 4.314 | |

| | |
|---|---|
| Min variance factor | 0.5 |
| Locus amplification variance | 0.008 |

**PROFILE SETTINGS**

| | |
|---|---|
| Number of evidence profiles | 1 |
| Evidence profile filenames | MVDS-Cot-MV 1 to 3_2A_EV.csv |
| Number of Hp knowns | 0 |
| Number of Hd knowns | 0 |

**PERFORMANCE SETTINGS**

| | |
|---|---|
| Number of threads | 8 |
| Low memory mode | N |

# LR From Previous Report

## DETAILS

| | |
|---|---|
| **STRMIX VERSION:** | STRmix V2.5.11 |
| **USER:** | heh2013 |
| **RUN DATE:** | 10 Nov 2020 17:00:57 |
| **TOTAL RUN TIME:** | 5 mins, 26 secs |
| **REPORT RUN:** | 10 Nov 2020 17:06:25 |

## RUN PARAMETERS

| | |
|---|---|
| **CONTRIBUTORS:** | 4 |
| **PROFILING KIT:** | Fusion6C_3500_Alameda |
| **SAMPLE FILE:** | MVDS-Cot-MV 1 to 3_2A_EV.csv |
| **KNOWN CONTRIBUTORS UNDER HP:** | X19-15_REF.csv |

| | |
|---|---|
| **CASE NUMBER:** | MVDS |
| **SAMPLE NAME:** | Cot-MV 1 to 3_2-LRPrev_X19-15 |
| **COMMENTS:** | LR calculated comparing to ref X19-15_REF.csv<br>LR calculated from previous analysis in: S:\10. STRMix\MVDS_Cot-MV 1 to 3_2_2020-11-09-11-03-48 |
| **SEED:** | 330506 |

## SUMMARY OF CONTRIBUTORS

| CONTRIBUTORS | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| CONTRIBUTOR ORDER GIVING HIGHEST LR | | | | |
| All Populations | X19-15_REF.csv | Unknown | Unknown | Unknown |

## SUMMARY OF LR

*TABLE 1 OF 2*

Factor-of-N! enabled. 99% 1-sided lower HPD interval calculated from 1000 iterations, MCMC uncertainty on.

| LR | NIST1036_CAUC | NIST1036_AFAM | NIST1036_ASIAN |
|---|---|---|---|
| PROPORTION | 0.25 | 0.25 | 0.25 |
| Children per family | 0 | 0 | 0 |
| Population size | 0 | 0 | 0 |
| Total LR | 6.19207E14 | 4.86205E12 | 2.43230E16 |
| Sibling | 2.42062E5 | 3.17659E4 | 2.52281E5 |
| Parent/Child | 2.87602E7 | 1.93854E6 | 1.43561E8 |
| Half Sibling | 1.70573E10 | 4.72022E8 | 9.81643E10 |
| Grandparent/Grandchild | 1.70573E10 | 4.72022E8 | 9.81643E10 |
| Uncle or Aunt/Niece or Nephew | 1.70573E10 | 4.72022E8 | 9.81643E10 |
| Cousin | 1.30231E12 | 2.16127E10 | 1.14885E13 |
| Unified | | | |

*TABLE 2 OF 2*

Factor-of-N! enabled. 99% 1-sided lower HPD interval calculated from 1000 iterations, MCMC uncertainty on.

| LR | NIST1036_HISP | STRATIFIED |
|---|---|---|
| PROPORTION | 0.25 | |
| Children per family | 0 | |
| Population size | 0 | |
| Total LR | 2.95906E14 | 3.12335E14 |
| Sibling | 1.85268E5 | 1.97009E5 |
| Parent/Child | 2.51552E7 | 2.68487E7 |
| Half Sibling | 1.18541E10 | 1.27668E10 |
| Grandparent/Grandchild | 1.18541E10 | 1.27668E10 |
| Uncle or Aunt/Niece or Nephew | 1.18541E10 | 1.27668E10 |
| Cousin | 7.95995E11 | 8.74205E11 |
| Unified | | |

## PER LOCUS LIKELIHOOD RATIOS

*TABLE 1 OF 2*

| | NIST1036_CAUC 0.01b(1.0, 1.0) | | | NIST1036_AFAM 0.01b(1.0, 1.0) | | |
|---|---|---|---|---|---|---|
| LOCUS | Pr(E\|Hp) | Pr(E\|Hd) | LR | Pr(E\|Hp) | Pr(E\|Hd) | LR |
| D3S1358 | 4.83616E-5 | 1.04939E-5 | 4.60856E0 | 2.52121E-5 | 1.93603E-6 | 1.30226E1 |
| D1S1656 | 2.45179E-7 | 4.86886E-8 | 5.03566E0 | 4.41823E-7 | 2.85822E-7 | 1.54580E0 |
| D2S441 | 3.99303E-4 | 1.25367E-4 | 3.18507E0 | 9.77124E-5 | 1.58702E-5 | 6.15698E0 |
| D10S1248 | 3.90377E-5 | 6.87689E-6 | 5.67664E0 | 7.05573E-5 | 1.26374E-5 | 5.58319E0 |
| D13S317 | 3.47333E-6 | 4.00150E-6 | 8.68007E-1 | 1.04349E-7 | 2.16033E-7 | 4.83023E-1 |
| Penta E | 5.10058E-8 | 8.97097E-9 | 5.68566E0 | 1.60023E-8 | 4.06778E-9 | 3.93392E0 |
| D16S539 | 2.14419E-5 | 3.92586E-6 | 5.46171E0 | 3.82848E-5 | 7.39790E-6 | 5.17509E0 |
| D18S51 | 9.40250E-7 | 3.02256E-7 | 3.11077E0 | 1.22741E-7 | 1.00924E-7 | 1.21617E0 |
| D2S1338 | 5.70162E-7 | 7.16856E-8 | 7.95364E0 | 7.00928E-7 | 1.83207E-7 | 3.82588E0 |
| CSF1PO | 9.29501E-6 | 2.14890E-7 | 4.32547E1 | 1.42475E-6 | 9.94244E-8 | 1.43299E1 |
| Penta D | 1.51940E-6 | 6.83914E-8 | 2.22163E1 | 1.16292E-6 | 1.59775E-6 | 7.27850E-1 |
| TH01 | 3.17498E-5 | 9.62573E-6 | 3.29843E0 | 3.34420E-5 | 3.68663E-5 | 9.07117E-1 |
| vWA | 6.27240E-6 | 4.16698E-6 | 1.50526E0 | 2.44404E-6 | 9.25333E-7 | 2.64126E0 |
| D21S11 | 3.83355E-6 | 1.97036E-6 | 1.94561E0 | 2.71198E-7 | 1.01953E-7 | 2.66004E0 |
| D7S820 | 6.06636E-5 | 2.27010E-5 | 2.67229E0 | 4.11931E-5 | 1.98790E-5 | 2.07219E0 |
| D5S818 | 2.14777E-4 | 5.73654E-5 | 3.74401E0 | 1.55643E-4 | 3.49320E-5 | 4.45559E0 |
| TPOX | 9.31607E-4 | 6.02890E-4 | 1.54523E0 | 4.25734E-4 | 1.79171E-4 | 2.37614E0 |
| D8S1179 | 8.83136E-6 | 1.30641E-6 | 6.76002E0 | 3.73685E-6 | 1.60372E-7 | 2.33011E1 |
| D12S391 | 7.55882E-6 | 4.03221E-8 | 1.87461E2 | 7.94848E-6 | 4.24255E-8 | 1.87351E2 |
| D19S433 | 1.63661E-5 | 5.96669E-7 | 2.74290E1 | 2.87731E-6 | 9.10736E-8 | 3.15932E1 |
| SE33 | 1.76446E-9 | 5.03456E-10 | 3.50470E0 | 8.97000E-10 | 1.58706E-10 | 5.65198E0 |
| D22S1045 | 2.21803E-4 | 7.41930E-5 | 2.98954E0 | 5.86641E-5 | 3.09665E-5 | 1.89444E0 |
| DYS391 | | | | | | |
| FGA | 1.40567E-7 | 2.93110E-7 | 4.79571E-1 | 1.80682E-7 | 2.57134E-7 | 7.02674E-1 |
| DYS576 | | | | | | |
| DYS570 | | | | | | |
| LR TOTAL | | | 7.01406E15 | | | 4.79134E13 |
| FACTOR OF N! LR | | | 2.86101E15 | | | 1.85825E13 |
| 99% 1-SIDED LOWER HPD INTERVAL | | | 6.19207E14 | | | 4.86205E12 |

*TABLE 2 OF 2*

| | NIST1036_ASIAN 0.01b(1.0, 1.0) | | | NIST1036_HISP 0.01b(1.0, 1.0) | | |
|---|---|---|---|---|---|---|
| LOCUS | Pr(E\|Hp) | Pr(E\|Hd) | LR | Pr(E\|Hp) | Pr(E\|Hd) | LR |
| D3S1358 | 3.06809E-5 | 6.88708E-7 | 4.45485E1 | 3.65507E-5 | 4.65709E-6 | 7.84839E0 |
| D1S1656 | 6.42680E-7 | 2.04426E-7 | 3.14383E0 | 5.44883E-7 | 1.44916E-7 | 3.75999E0 |
| D2S441 | 2.35240E-4 | 3.40931E-5 | 6.89992E0 | 5.85969E-4 | 2.51143E-4 | 2.33321E0 |
| D10S1248 | 6.55524E-5 | 1.28608E-5 | 5.09705E0 | 4.69386E-5 | 9.27827E-6 | 5.05899E0 |
| D13S317 | 1.69679E-5 | 8.39081E-6 | 2.02220E0 | 4.09701E-6 | 3.82930E-6 | 1.06991E0 |
| Penta E | 3.69289E-8 | 3.93655E-9 | 9.38102E0 | 7.01130E-8 | 7.17502E-9 | 9.77181E0 |
| D16S539 | 1.13411E-4 | 3.50161E-5 | 3.23882E0 | 5.82413E-5 | 1.17997E-5 | 4.93583E0 |
| D18S51 | 1.42859E-6 | 6.05936E-7 | 2.35766E0 | 1.28367E-6 | 5.14817E-7 | 2.49345E0 |
| D2S1338 | 4.00324E-7 | 3.33713E-8 | 1.19961E1 | 8.07856E-7 | 6.21196E-8 | 1.30048E1 |
| CSF1PO | 1.34195E-5 | 2.20768E-7 | 6.07856E1 | 4.65478E-6 | 8.19533E-8 | 5.67979E1 |
| Penta D | 1.28563E-5 | 2.26308E-7 | 5.68087E1 | 4.01464E-6 | 3.30281E-7 | 1.21552E1 |
| TH01 | 6.25124E-5 | 1.77176E-5 | 3.52826E0 | 6.49077E-5 | 2.65159E-5 | 2.44787E0 |
| vWA | 2.94851E-5 | 1.60443E-5 | 1.83773E0 | 2.58179E-6 | 1.41309E-6 | 1.82705E0 |
| D21S11 | 3.91814E-7 | 2.49788E-7 | 1.56858E0 | 2.89969E-6 | 2.09613E-6 | 1.38335E0 |
| D7S820 | 5.16882E-4 | 3.04640E-4 | 1.69670E0 | 2.12640E-4 | 1.20540E-4 | 1.76406E0 |
| D5S818 | 6.53962E-5 | 4.93364E-5 | 1.32552E0 | 1.36950E-4 | 4.20913E-5 | 3.25363E0 |
| TPOX | 8.71085E-4 | 6.57661E-4 | 1.32452E0 | 5.40288E-4 | 3.23897E-4 | 1.66809E0 |
| D8S1179 | 7.58995E-6 | 1.98978E-6 | 3.81447E0 | 1.03004E-5 | 1.01024E-6 | 1.01960E1 |
| D12S391 | 7.37878E-6 | 7.51232E-9 | 9.82224E2 | 4.40829E-6 | 2.54421E-8 | 1.73267E2 |
| D19S433 | 6.87492E-6 | 2.02299E-7 | 3.39840E1 | 1.53793E-5 | 7.39760E-7 | 2.07896E1 |
| SE33 | 2.95648E-11 | 5.11015E-12 | 5.78551E0 | 1.81390E-9 | 5.70318E-10 | 3.18051E0 |
| D22S1045 | 1.38742E-4 | 9.39104E-5 | 1.47738E0 | 3.04302E-4 | 1.50703E-4 | 2.01922E0 |
| DYS391 | | | | | | |
| FGA | 2.36704E-7 | 3.42937E-7 | 6.90224E-1 | 2.47669E-7 | 3.66141E-7 | 6.76431E-1 |
| DYS576 | | | | | | |
| DYS570 | | | | | | |
| LR TOTAL | | | 6.72656E17 | | | 3.80311E15 |
| FACTOR OF N! LR | | | 2.02096E17 | | | 1.44575E15 |
| 99% 1-SIDED LOWER HPD INTERVAL | | | 2.43230E16 | | | 2.95906E14 |

## REFERENCE FILES

| LOCUS | X19-15_REF.CSV |
|---|---|
| D3S1358 | 14, 18 |
| D1S1656 | 14, 15 |
| D2S441 | 10, 14 |
| D10S1248 | 15, 15 |
| D13S317 | 12, 13 |
| Penta E | 5, 13 |
| D16S539 | 9, 12 |
| D18S51 | 15, 20 |
| D2S1338 | 21, 24 |
| CSF1PO | 8, 13 |
| Penta D | 2.2, 8 |
| TH01 | 7, 8 |
| vWA | 18, 18 |
| D21S11 | 29, 32.2 |
| D7S820 | 10, 11 |
| D5S818 | 10, 11 |
| TPOX | 8, 11 |
| D8S1179 | 10, 11 |
| D12S391 | 16, 18.3 |
| D19S433 | 15, 16.2 |
| SE33 | 18, 30.2 |
| D22S1045 | 15, 17 |
| DYS391 | |
| FGA | 21, 22 |
| DYS576 | |
| DYS570 | |

# SETTINGS

## CASE SETTINGS

| | |
|---|---|
| Case number | MVDS |
| Sample ID | Cot-MV 1 to 3_2-LRPrev_X19-15 |
| Comments | LR calculated comparing to ref X19-15_REF.csv<br>LR calculated from previous analysis in: S:\10. STRMix\MVDS_Cot-MV 1 to 3_2_2020-11-09-11-03-48 |
| **Seed** | **330506** |
| Extended output | N |

## KIT SETTINGS

| | | |
|---|---|---|
| Ignored loci | DYS391, DYS576, DYS570 | |
| Detection thresholds | D3S1358 | 70 |
| | D1S1656 | 70 |
| | D2S441 | 70 |
| | D10S1248 | 70 |
| | D13S317 | 70 |
| | Penta E | 70 |
| | D16S539 | 70 |
| | D18S51 | 70 |
| | D2S1338 | 70 |
| | CSF1PO | 70 |
| | Penta D | 70 |
| | TH01 | 70 |
| | vWA | 70 |
| | D21S11 | 70 |
| | D7S820 | 70 |
| | D5S818 | 70 |
| | TPOX | 70 |
| | D8S1179 | 70 |
| | D12S391 | 70 |
| | D19S433 | 70 |
| | SE33 | 70 |
| | D22S1045 | 70 |
| | FGA | 70 |
| Stutter max | 0.3 | |
| Forward stutter max | 0.15 | |
| Saturation | 28000 | |
| Degradation starts at | -1.0 | |
| Degradation max | 0.01 | |
| Drop-in cap | 200 | |
| Drop-in frequency | 0.0039 | |
| Drop-in gamma parameters | 12.74, 3.75 | |
| Allelic variance (α, β) | 5.729, 1.42 | |
| Stutter variance (α, β) | 2.556, 4.314 | |
| Min variance factor | 0.5 | |
| Locus amplification variance | 0.008 | |

## PROFILE SETTINGS

| | |
|---|---|
| Number of evidence profiles | 1 |
| Evidence profile filenames | MVDS-Cot-MV 1 to 3_2A_EV.csv |
| Number of Hp knowns | 1 |
| Hp reference filenames | X19-15_REF.csv |
| Number of Hd knowns | 0 |

## LR SETTINGS

| | |
|---|---|
| Number of populations | 4 |
| Factor of N! LR | Y |
| HPD Iterations | 1000 |
| Use MCMC uncertainty | Y |
| HPD quantile | 99% |
| HPD sides | 1 |

***NIST1036_CAUC***

| | |
|---|---|
| Proportion | 0.25 |
| FST | 0.01b(1.0, 1.0) |
| Allele frequency file | NIST1036_Cauc_July2017_ESR.csv |

***NIST1036_AFAM***

| | |
|---|---|
| Proportion | 0.25 |
| FST | 0.01b(1.0, 1.0) |
| Allele frequency file | NIST1036_AfAm_July2017_ESR.csv |

***NIST1036_ASIAN***

| | |
|---|---|
| Proportion | 0.25 |
| FST | 0.01b(1.0, 1.0) |
| Allele frequency file | NIST1036_Asian_July2017_ESR.csv |

***NIST1036_HISP***

| | |
|---|---|
| Proportion | 0.25 |
| FST | 0.01b(1.0, 1.0) |
| Allele frequency file | NIST1036_Hisp_July2017_ESR.csv |

## PERFORMANCE SETTINGS

| | |
|---|---|
| Number of threads | 8 |
| Low memory mode | N |

# Alameda County Sheriff's Office Crime Laboratory
# Forensic Biology Unit – Technical Procedures Manual
# 3.1 - DNA Extraction - Maxwell® 16

**PRINCIPLE:** DNA extraction is performed on substrates containing suspected biological material. DNA extraction consists of two steps: digestion and purification. Following cell lysis and protein degradation, the DNA IQ™ Casework Pro Kit for Maxwell® 16 is used with the Maxwell® 16 Instrument to purify DNA from forensic casework samples through magnetic DNA IQ™ Resin and a series of washes.

**SAMPLES:** Swabs or stains that may contain biological material.

NOTE: Samples suspected to contain only semen (e.g., liquid semen swabbed from a surface) may use this protocol.

**REAGENTS, SUPPLIES, and EQUIPMENT:**

Reagents

- Casework Extraction Kit (Promega Corporation, Madison, WI)
  - Casework Extraction Buffer (CEB)
  - Proteinase K, 18 mg/mL
  - 1-Thioglycerol
  - Nuclease-Free Water
- DNA IQ™ Casework Pro Kit for Maxwell® 16 (Promega Corporation) includes:
  - Lysis Buffer
  - Elution Buffer
  - Maxwell 16 LEV Cartridges (reagent cartridges)
  - 0.5 mL Elution Tubes
  - LEV Plungers

Supplies

- Nucleospin® Forensic Filters (Forensic Filter) (Macherey-Nagel, Düren, Germany)
- Costar® 2 mL Snap Cap Microcentrifuge Polypropylene Tubes ("dolphin nose" tubes) (Corning Life Sciences, Corning, NY)
- Pipettes

Equipment

- Maxwell® 16 Instrument (Maxwell 16) (Promega Corporation)
- Heat block
- Centrifuge (5424 Eppendorf, Hamburg, Germany)
- Vortexer

**SAFETY**:

- DNA IQ extraction chemistry contains guanidinium thiocyanate, which is harmful by inhalation, in contact with skin, and if swallowed. Upon contact with acids or bleach, guanidinium thiocyanate will liberate toxic gas. After use, place Maxwell 16 LEV Cartridge in labeled hazardous waste container.
- Discard all waste appropriately.

**PROCEDURE:**

Cell Lysis

1. Ensure all appropriate samples tubes have a Forensic Filter in place prior to extraction.

   NOTE: If samples were previously screened, digest the cell pellet and substrate in the bottom of the tube.

   NOTE: Do not use the Forensic Filter-supplied 2 mL tube; it does not seat properly in the heat block. Use "dolphin nose" tube with the Forensic Filter.

2. Prepare Digestion Master Mix for each sample that contains the following plus one (1) additional aliquot:

   - 386 µL of Casework Extraction Buffer (CEB)
   - 10 µL of Proteinase K
   - 4 µL of 1-Thioglycerol

     NOTE: 1-thioglycerol is viscous; pipette slowly.

3. Add 400 µL Digestion Master Mix to each sample and incubate at 56°C for 30 to 60 minutes.
4. Vortex all samples and centrifuge at maximum speed for 10 minutes.

   NOTE: Vortex and centrifuge immediately after removing from the heat block.

   NOTE: Transfer any remaining liquid from the Forensic Filter to the collection tube.

5. Remove and discard the Forensic Filter containing the substrate.

   NOTE: For consumed samples, retain the substrate.

6. Add 200 µL of Lysis Buffer to each tube. Mix contents.

Maxwell 16 Extraction

7. Reagent cartridge contents:

| Well # | Well Contents | User Adds |
|---|---|---|
| 1 | Lysis Buffer | Sample + Lysis Buffer |
| 2 | DNA IQ Resin | |
| 3 | Lysis Buffer | |
| 4 | Wash Buffer | |
| 5 | Wash Buffer | |
| 6 | Wash Buffer | |
| 7 | Empty | |
| 8 | Empty | Plunger |

8. Place reagent cartridges into the Maxwell 16 cartridge rack.

   NOTE: It is vital that the reagent cartridges be properly seated in the rack.

9. Peel off the foil.

   NOTE: Ensure that all sealing tape and residual are removed from the reagent cartridges.

10. Load plunger in Well #8 of each reagent cartridge.
11. Label each 0.5 mL Elution Tube with sample identity. Load Elution Tubes in the front of the Maxwell 16 cartridge rack. Add 40 to 100 µL of Elution Buffer to the bottom of each Elution Tube.
12. Transfer digested samples into Well #1 of each reagent cartridge.
13. Turn on Maxwell 16. Press "RUN/STOP" to extend the Maxwell 16 platform. Place Maxwell 16 cartridge rack on the platform.

    NOTE: Ensure the Maxwell 0.5 mL Elution Tubes are OPEN before proceeding.

14. Press "RUN/STOP" to retract the Maxwell 16 platform. Close the workstation door. Extraction will start automatically.
15. When the protocol ends, open workstation door. Verify all plungers have been removed from the rod assembly (manually remove if necessary).
16. Press "RUN/STOP" to extend the Maxwell 16 platform. Remove Maxwell 16 cartridge rack before pressing "RUN/STOP" to retract the platform.

    NOTE: Never allow liquids to sit on Maxwell surfaces for extended periods of time.

17. Close Elution Tubes.

    NOTE: Close tubes as soon as possible to avoid evaporation.

18. Discard the reagent cartridges and plungers in the appropriate disposal containers.
19. Remove Elution Tubes with extracted DNA and store at 4°C or proceed directly to DNA quantification.
20. Select "Yes" for additional run or "No" to retract the platform. If no further runs are to be performed, power off the instrument.
21. Clean the sample tray using a cloth dampened with purified water or 70% ethanol.

    NOTE: Do not use other solvents or abrasive cleaners; do **NOT** use 10% bleach on Maxwell parts.

References

Baute DT, Hoover HE, and Roby RK. Alameda County Sheriff's Office Crime Laboratory, Validation of Forensic Filters in Non-Differential Extractions, dated April 17, 2019.

Promega Corporation, DNA IQ™ Casework Pro Kit for Maxwell® 16, TM332, Revised 12/16.

Ridolfi D. Alameda County Sheriff's Office Crime Laboratory, Maxwell Extraction Efficiency Validation-New Magnetic Rods and 4.8 Version Firmware, dated August 2, 2011.

Roby RK, Newman AN, and Hoover HE. Alameda County Sheriff's Office Crime Laboratory, Modification of the DNA Extraction Procedure Using the Casework Extraction Kit for Cell Digestion, dated January 27, 2021.