Developmental Validation of the Maxwell® FSC DNA IQ™ Casework Kit on the Maxwell® RSC 48 Instrument

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Maxwell® RSC 48 Instrument (Cat.# AS8500).

A reliable purification method is critical to forensic DNA laboratories that routinely encounter a variety of challenging samples.

Introduction

Biological evidence from crime scenes collected for the purposes of DNA testing is frequently mixed with substances that interfere with downstream analysis steps, such as quantification and short tandem repeat (STR) amplification. Reducing the amount of inhibitors present in extracted DNA can improve the quality of the generated DNA profile. A reliable purification method is therefore critical to forensic DNA laboratories that routinely encounter a variety of challenging samples.

The Maxwell® Rapid Sample Concentrator 48 (RSC 48) Instrument uses an automated workflow to purify DNA from preprocessed forensic samples. The instrument runs in conjunction with the Maxwell® FSC DNA IQ™ Casework Kit, which utilizes paramagnetic particles to prepare clean samples for STR analysis. The DNA IQ™ resin has been used extensively with manual methods, medium-throughput Maxwell systems, and large scale automation methods (1–6). The Maxwell® RSC 48 Instrument works with the same prefilled cartridges used with the Maxwell® FSC Instrument but can process up to 48 samples simultaneously, compared to 16 samples on the Maxwell® FSC. A set of studies showcasing the performance of the Maxwell® FSC Instrument with the Maxwell® FSC DNA IQ™ Casework Kit is also documented (7).

A series of validation studies are presented here to showcase the performance of the Maxwell® RSC 48 Instrument with the Maxwell® FSC DNA IQ™ Casework Kit for genomic DNA extraction and purification from forensic-type samples. These studies are based on requirements listed in the FBI Quality Assurance Standards for Forensic DNA Testing Laboratories (8) and guidelines outlined by the Scientific Working Group on DNA Analysis Methods (9).

Materials and Methods

Samples

Human blood, saliva, and buccal swabs were collected from donors. Semen samples were purchased from Lee Biosolutions, Inc (MO, USA). Casework-type samples were prepared from donated items or discarded items. Maxwell® FSC DNA IQ™ Casework Kit, Casework Extraction Kit, PowerQuant® System, and PowerPlex® Fusion System were obtained from Promega Corporation (WI, USA). All other chemicals used in this study were of analytical grade.

Preprocessing

Sample preprocessing was performed using the Casework Extraction Kit, which contains Casework Extraction Buffer, Proteinase K and 1-Thioglycerol.

The resulting lysate was purified using reagents and components in the Maxwell® FSC DNA IQ™ Casework Kit. Detailed preprocessing and purification protocols for solid and liquid samples are available in the *Maxwell® FSC DNA IQ™ Casework Kit Technical Manual* #TM499 (10).

DNA Extraction from Solid Substrates

All swab samples were processed using CW Spin Baskets. A CW Spin Basket was placed in a CW Microfuge Tube, and the solid substrate was placed at the bottom of the CW Spin Basket. The extraction mix was prepared by adding 286µl of Casework Extraction Buffer, 10µl of 18mg/ml Proteinase K and 4µl of 1-Thioglycerol, for a final volume of 300µl per sample. Each sample was incubated at 56°C for 30 minutes and centrifuged at maximum speed for 2 minutes at room temperature. The CW Spin Baskets and substrates were then discarded, and 200µl of Lysis Buffer was added to each tube containing extract. Following thorough mixing, the samples were added to the Maxwell® FSC Cartridge, and the protocol on the Maxwell® RSC 48 Instrument was initiated according to manufacturer instructions (11). All samples were eluted to a final volume of 50µl.

DNA Extraction from Liquid Samples

For the sensitivity study, 286µl of Casework Extraction Buffer, 10µl of Proteinase K (18mg/ml) and 4µl of 1-Thioglycerol were added to 100µl of liquid sample. For the repeatability study, 376µl of Casework Extraction Buffer, 10µl of Proteinase K and 4µl of 1-Thioglycerol were added to 10µl of liquid sample. Each sample was mixed thoroughly and incubated at 56°C for 30 minutes. Following incubation, 200µl of Lysis Buffer was added to each sample, which was then added to the Maxwell® FSC Cartridge. The protocol on the Maxwell® RSC 48 Instrument was initiated according to manufacturer instructions. All samples were eluted to a final volume of 50µl.

DNA Quantification

The extracted DNA from each sample was quantified using the PowerQuant® System on the Applied Biosystems® 7500 Real-Time PCR System following the protocol detailed in the *PowerQuant® System Technical Manual* #TMD047 (12). The following sample quality flags were monitored throughout the studies: [Autosomal]/[Degradation] ratio, [Autosomal]/[Y] ratio and IPC (internal positive control) C_q. These quality flags provide information about the sample and probable STR profile quality. A threshold of 2 was used for the [Auto]/[D] and [Auto]/[Y] ratios to flag a sample as possibly degraded or as a potential mixture, respectively. A shift of 0.3 for the IPC C_q was used to flag a sample for possible inhibition.

STR Analysis

All samples except for those from the sensitivity and repeatability studies were amplified with PowerPlex® Fusion System following the protocol detailed in the *PowerPlex® Fusion System for Use on the Applied Biosystems® Genetic Analyzers Technical Manual* #TMD039 (13). A total of 0.5ng DNA or up to 15µl of sample was added per amplification reaction. Samples were amplified on a GeneAmp® PCR System 9700 thermal cycler, and 1µl of amplified product was used for electrophoresis. All samples were injected at 1.2kV for 24 seconds on the 3500xL Genetic Analyzer. Data were analyzed with GeneMapper® *ID-X* Software v1.5 using 100RFU as the analysis threshold.

Sensitivity Study

To evaluate the linear range of DNA extracted, a sensitivity study was performed. Serial dilutions of blood from two individuals were performed to obtain the following volumes: 0.008µl, 0.04µl, 0.2µl, 1.0µl, 5.0µl, 10µl, and 50µl. Each blood dilution was processed in triplicate as described in the Preprocessing and DNA Extraction from Liquid Samples sections.

Repeatability Study

The repeatability of DNA quantity and quality obtained from replicate samples were assessed. DNA from 10µl of body fluid (blood, saliva and semen) was extracted in triplicate as described in the Preprocessing and DNA Extraction from Liquid Samples sections to compare replicates within an extraction run. The extraction setup was performed a total of three times to compare replicates between extraction runs.

Mixture Study

A mixture study was performed to show that DNA from mixed donor cells in a variety of ratios can be effectively extracted and purified. Two mixture series were prepared by combining male and female blood (by volume) to obtain M:F ratios of 1:1, 1:5, 1:10, 1:20, 1:50 and 1:150. Each sample contained 10µl of the blood mixture dried on a cotton swab. Mixture samples were extracted in duplicate as described in the Preprocessing and DNA Extraction from Solid Substrates sections.

Inhibition Study

An inhibition study was performed to show that DNA can be effectively purified in the presence of inhibitors commonly encountered in forensic casework. Saliva (20µl) was added to swabs along with an appropriate volume of inhibitor solution (humic acid and hematin) and allowed to dry before extraction. The following volumes of inhibitors were tested: 3µl and 6µl of 5mg/ml stock humic acid; 60µl and 120µl of 2mM stock hematin. The samples were extracted in duplicate as described in the Preprocessing and DNA Extraction from Solid Substrates sections.

Contamination Study

To assess sample-to-sample contamination, extraction blanks were processed along with blood samples from the Mixture Study in an alternating pattern on the Maxwell® RSC 48 Instrument. At least three extraction blanks were also included with each extraction run throughout the validation.

Known and Casework Samples

Known buccal swabs and a variety of casework-type samples were included in this study to evaluate the performance in yielding pure and amplifiable DNA. Known samples consisted of duplicate buccal swabs from four female and three male individuals. Casework samples included mock evidence such as touch samples, as well as body fluid samples on various substrates. Known and casework samples were extracted as described in the Preprocessing and DNA Extraction from Solid Substrates sections.

Results and Discussion

Sensitivity Study

Quantifiable levels of DNA were detected from all extracted blood samples in the sensitivity study with the exception of one 0.008µl blood sample replicate from Individual 2. This sample was considered an outlier and not included in the calculated averages. The average total DNA recovered from the 0.008µl blood samples were 0.10ng and 0.18ng from Individual 1 and Individual 2, respectively (Table 1). A linear relationship was observed between the volume of extracted blood and the DNA concentration for samples ranging between 0.008µl and 5.0µl (Figure 1). Although there was an increase in DNA recovered from 10µl and 50µl blood samples, this increase was not proportional (Table 1). This may indicate the presence of sample components in the higher volumes of blood competing for binding to the resin (14). IPC C_q flags were not detected with the PowerQuant® System in any of the samples, indicating clean DNA extracts are recovered from a wide range of blood volumes (data not shown).

Table 1. Average total autosomal DNA yield ±1 standard deviation compared to volume of blood extracted.

	Average Total Yield (ng)	
Volume of Blood (µl)	Individual 1	Individual 2
50	344.19 ± 17.58	535.52 ± 90.11
10	191.54 ± 13.30	219.59 ± 11.75
5	144.87 ± 9.10	193.05 ± 25.63
1	32.32 ± 2.76	59.54 ± 7.80
0.2	4.73 ± 0.76	11.47 ± 1.85
0.04	0.69 ± 0.06	1.67 ± 0.10
0.008	0.10 ± 0.03	0.18 ± 0.01

Repeatability Study

The variability within extraction runs and between extraction runs was similar for all body fluids tested. The autosomal coefficient of variation (CV) ranged from 4.5% from within Semen Run #1 to 24.5% from between all saliva runs (Table 2). Some variation in DNA concentration is likely attributable to irregularities inherent to pipetting viscous body fluids. IPC C_q flags were not detected with the PowerQuant® System in any of the samples (data not shown).

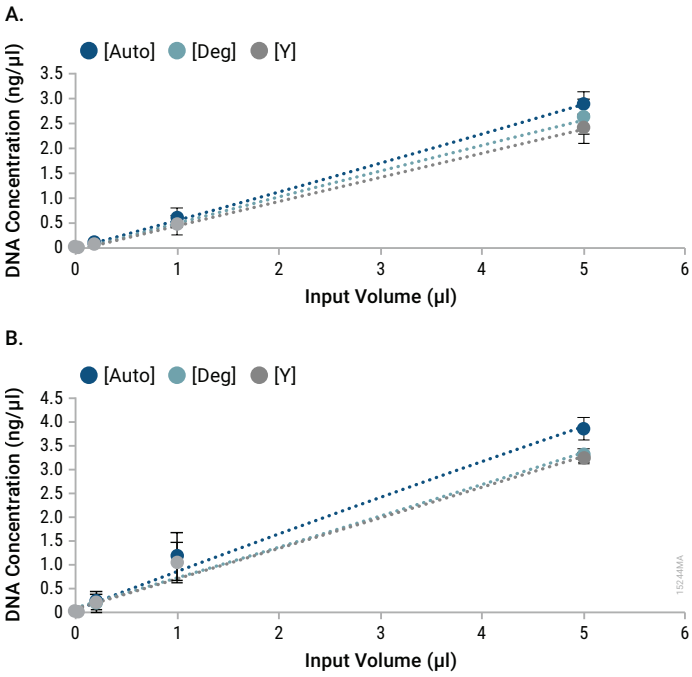

Figure 1. Linearity of DNA concentration from each extraction for Individual 1 (Panel A) and Individual 2 (Panel B). The X axis represents the volume of blood used for sample extraction. The Y axis represents the average DNA concentration (Autosomal, Degradation and Y DNA targets). The error bars represent ±1 standard deviation. Samples were extracted using the Maxwell® RSC 48 Instrument with the Maxwell® FSC DNA IQ™ Casework Kit and quantified using the PowerQuant® System on the Applied Biosystems® 7500 Real-Time PCR System.

Mixture Study

Both male and female components of all mixture samples were successfully extracted. The results showed an increase in the [Auto]/[Y] ratio as the amount of the male component in the mixture decreased. Full male profiles were obtained down to the 1:10 samples of Mixture 1 and the 1:5 samples of Mixture 2. Partial male, minor profiles were detected down to the 1:150 samples for both mixture series (Figure 2).

Inhibition Study

No IPC C_q flags were detected from the saliva samples with added inhibitors, indicating that most of the inhibitors were removed during purification (data not shown). Full profiles for all samples were obtained using the PowerPlex® Fusion System. The samples containing hematin displayed more inter-locus peak height imbalance than all other samples, indicating possible minor inhibition (Figure 3–4).

Contamination Assessment

Twenty-six reagent blanks were assessed throughout the validation. No DNA was detected in any of the samples using the PowerQuant® System. When amplified with PowerPlex® Fusion, seven of the reagent blanks exhibited one or two low-level peaks (less than 200RFU). None of those peaks were reproducible upon re-amplification (data not shown).

Table 2. Average concentration, standard deviation, and %CV across PowerQuant® System targets for replicates within runs and between runs for body fluids. Auto = autosomal target; Deg = degradation target; Y = Y target.

	Average [Auto] (ng/µl)	[Auto] CV	Average [Deg] (ng/µl)	[Deg] CV	Average [Y] (ng/µl)	[Y] CV	Average IPC C_q	IPC C_q CV
Blood Extraction								
Run #1 (N=3)	4.07 ± 0.24	5.9%	3.84 ± 0.19	4.9%	3.51 ± 0.14	3.8%	21.03 ± 0.05	0.24%
Run #2 (N=3)	3.91 ± 0.37	9.6%	3.25 ± 0.34	10.6%	3.28 ± 0.33	10.1%	20.91 ± 0.03	0.15%
Run #3 (N=3)	3.53 ± 0.22	6.1%	2.97 ± 0.29	9.7%	2.75 ± 0.20	7.1%	20.86 ± 0.01	0.03%
Inter-Run (N=9)	3.84 ± 0.36	9.5%	3.35 ± 0.46	13.6%	3.18 ± 0.40	12.4%	20.93 ± 0.08	0.38%
Saliva Extraction								
Run #1 (N=3)	1.33 ± 0.12	9.3%	1.00 ± 0.06	6.0%	1.09 ± 0.08	7.5%	20.98 ± 0.02	0.08%
Run #2 (N=3)	1.65 ± 0.30	18.3%	1.01 ± 0.18	18.1%	1.37 ± 0.22	15.8%	20.85 ± 0.05	0.24%
Run #3 (N=3)	1.03 ± 0.16	15.8%	0.63 ± 0.13	21.2%	0.82 ± 0.12	15.2%	20.74 ± 0.03	0.13%
Inter-Run (N=9)	1.33 ± 0.33	24.5%	0.88 ± 0.22	25.1%	1.09 ± 0.27	24.9%	20.86 ± 0.10	0.49%
Semen Extraction								
Run #1 (N=3)	10.90 ± 0.49	4.5%	9.64 ± 0.93	9.6%	9.55 ± 0.66	6.9%	20.99 ± 0.16	0.77%
Run #2 (N=3)	11.18 ± 0.69	6.2%	10.18 ± 1.03	10.2%	10.17 ± 0.64	6.3%	20.99 ± 0.01	0.04%
Run #3 (N=3)	8.86 ± 0.46	5.2%	7.83 ± 0.20	2.6%	7.20 ± 0.39	5.5%	20.93 ± 0.01	0.05%
Inter-Run (N=9)	10.31 ± 1.18	11.4%	9.22 ± 1.29	14.0%	8.98 ± 1.40	15.6%	20.97 ± 0.10	0.46%

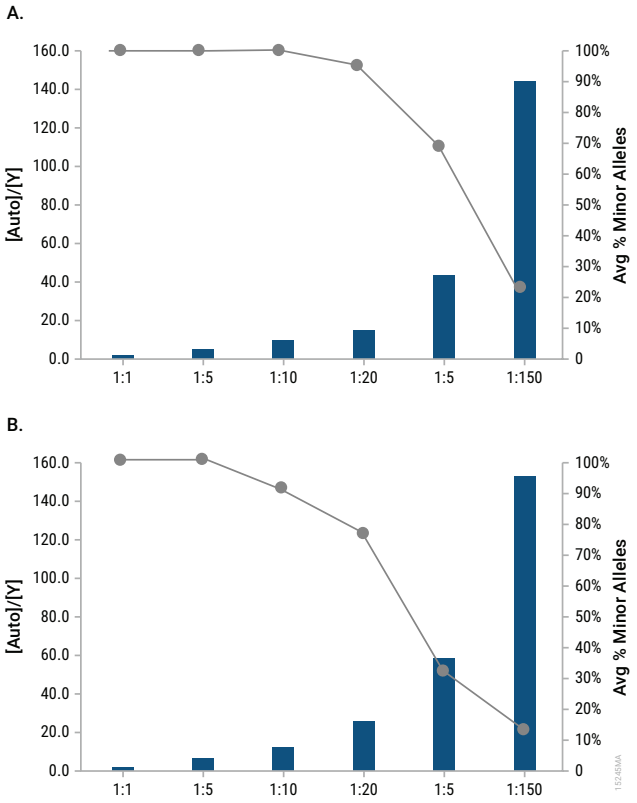

Figure 2. Quantification and STR results for two male/female mixture sets. The X axis represents the ratio of male to female blood that was used in the sample. The left Y axis represents the average ratio of autosomal DNA to male DNA (indicated by the bars). The right Y axis represents the average % minor alleles detected (indicated by the line). Samples were extracted using the Maxwell® RSC 48 Instrument with the Maxwell® FSC DNA IQ™ Casework Kit and quantified using the PowerQuant® System on the Applied Biosystems® 7500 Real-Time PCR System. Amplification was performed using PowerPlex® Fusion on a GeneAmp® PCR System 9700 thermal cycler, and 1µl of amplified product was subjected to electrophoresis on an Applied Biosystems® 3500xL series instrument using a 1.2kV, 24-second injection.

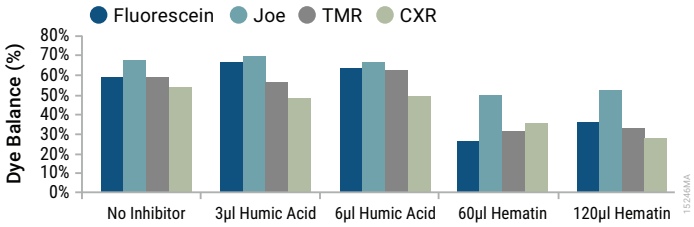

Figure 3. Inter-locus peak height balance by dye channel for samples that contained hematin and humic acid inhibitors. The X axis represents the sample and the inhibitor present in the sample. The Y axis represents the average dye balance calculated by dividing the lowest average peak height at that dye channel by the highest average peak height at that dye channel. Samples were extracted using the Maxwell® RSC 48 Instrument with the Maxwell® FSC DNA IQ™ Casework Kit and quantified using the PowerQuant® System on the Applied Biosystems® 7500 Real-Time PCR System. Amplification was performed using PowerPlex® Fusion on a GeneAmp® PCR System 9700 thermal cycler, and 1µl of amplified product was subjected to electrophoresis on an Applied Biosystems® 3500xL series instrument using a 1.2kV, 24-second injection.

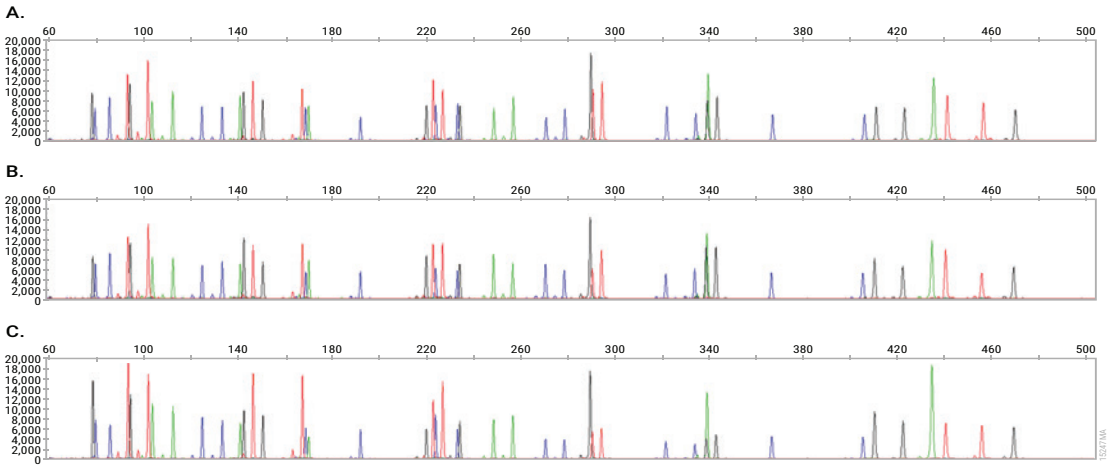

Figure 4. Representative DNA profiles of samples that contained humic acid and hematin inhibitors (scaled to 20,000RFU). Panel A. A DNA profile from a saliva sample containing no added inhibitor. **Panel B.** A DNA profile from a saliva sample containing 6µl of 5mg/ml humic acid. **Panel C.** A DNA profile from a saliva sample containing 120µl of 2mM hematin. Samples were extracted using the Maxwell® RSC 48 Instrument with the Maxwell® FSC DNA IQ™ Casework Kit and quantified using the PowerQuant® System on the Applied Biosystems® 7500 Real-Time PCR System. Amplification was performed using PowerPlex® Fusion on a GeneAmp® PCR System 9700 thermal cycler, and 1µl of amplified product was subjected to electrophoresis on an Applied Biosystems® 3500xL series instrument using a 1.2kv, 24-second injection.

Known Samples

Average autosomal DNA concentrations ranged between 30–48ng/µl for the known buccal swab samples (Figure 5). No male DNA was detected in the female buccal samples (K1, K3, K6 and K7), and no IPC C_q flags, [Auto]/[Y] or degradation flags were detected with the PowerQuant® System. Full profiles were obtained for all samples and average overall peak heights ranged between 5457–7147RFU (data not shown).

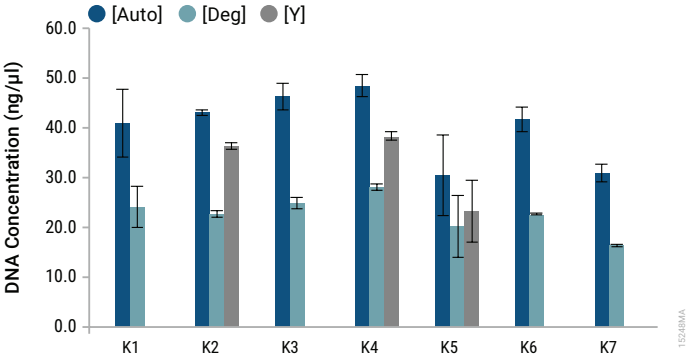

Figure 5. Quantification results for known buccal samples. The X axis represents the samples. The Y axis represents the average DNA concentration in ng/µl. Error bars represent ±1 standard deviation. Samples were extracted using the Macwell® RSC 48 Instrument with the Maxwell® FSC DNA IQ™ Casework Kit and quantified using the PowerQuant® System on the Applied Biosystems® 7500 Real-Time PCR System.

Casework Samples

A variety of sample types were successfully extracted using the Maxwell® RSC 48 Instrument (Table 3). Autosomal DNA was detected in all samples using the PowerQuant® System. The samples with the highest quantification values included the buccal swab with semen (34ng/µl) and vaginal swab with semen (39ng/µl). The samples with the lowest quantification values included a swab of a steering wheel (0.0007ng/µl) and a cutting of cigarette butt filter paper (0.0003ng/µl). It should be noted that the cigarette butt was recovered from outside and may have been exposed to prolonged sunlight and/or rain. No IPC C_q flags were detected with the PowerQuant® System. Several samples generated flags for the [Auto]/[Y] ratio and [Auto]/[Deg] ratio. With the exception of the two cigarette butts, the major source(s) of the DNA was known and compared to the obtained DNA profiles. All casework profiles generated were consistent with the expected source of DNA. Several samples included minor unknown contributors as expected with casework type samples. We show representative electropherograms for a mixed sample (Figure 6); a single-source, high quantification sample (Figure 7); and a degraded sample (Figure 8).

Conclusion

A series of validation studies were conducted to test the performance of the Maxwell® RSC 48 Instrument used in conjunction with the Maxwell® FSC DNA IQ™ Casework Kit for extracting genomic DNA from common forensic samples. The results demonstrate the system's ability to reliably generate high quality DNA extracts from a variety of samples types, making the Maxwell® RSC 48 Instrument suitable for use in a forensic laboratory.

Table 3. Casework sample quantification and STR results. The asterisk (*) represents a flagged quality threhold in the PowerQuant® System. The STR data denote whether the PowerPlex® Fusion System profile generated was of a single source or mixed composition and the percent profile obtained of the known source contributor. Auto = autosomal target; Deg = degradation target; Y = Y target.

Sample Description	[Auto] (ng/µl)	[Deg] (ng/µl)	[Y] (ng/µl)	[Auto]/[Y]	[Auto]/[D]	STR Profile (% Expected Contributor Profile)
Buccal swab + semen	33.6839	15.6499	4.4636	7.55*	2.15*	Mixture. Major ♀ (100%) and Minor ♂ (100%)
Vaginal swab + semen	38.6281	10.565	1.9611	19.7*	3.66*	Mixture. Major ♀ (100%) and Minor ♂ (74%)
Swab of exterior condom	9.8333	2.8118	0.0537	182.98*	3.5*	Mixture. Major ♀ (100%)
Swab of interior condom	17.499	12.8102	15.0447	1.16	1.37	Single source ♂ (100%)
Swab of ♂ saliva on ♀ skin	2.1542	1.1128	1.6795	1.28	1.94	Mixture. Major ♂ (100%)
Swab of saliva on ♂ skin	0.8003	0.3255	0.0046	173.66*	2.46*	Single source ♀ (100%)
Cutting semen stain on cloth (black cotton)	20.5984	14.6003	17.7043	1.16	1.41	Single source ♂ (100%)
Cutting blood stain on cloth (white cotton)	10.6133	5.745	0.0062	1724.54*	1.85	Single source ♀ (100%)
Cutting saliva stain on cloth (black cotton)	14.8053	7.8215	11.1268	1.33	1.89	Single source ♂ (100%)
Cutting blood and semen on cloth (pink cotton)	12.1606	6.8451	2.5315	4.8*	1.78	Mixture. ♂ (100%) and ♀ (100%)
Cigarette Butt 1 (from outside)	0.0003	–	–	–	–	One allele (Donor Unknown)
Cigarette Butt 2 (from outside)	0.0341	0.0004	0.0088	3.89*	95.87*	Partial profile (Donor Unknown)
Swab of mouth of coffee mug	4.5377	1.893	0.0131	347.47*	2.4*	Mixture. Major ♀ (100%)
Steering wheel swab (red leather cover)	0.0007	–	–	–	–	Single source ♀ (24%)
Swab of gun	0.3737	0.0309	0.1552	2.41*	12.1*	Mixture. Major ♂ (90%)
Swab of hat	0.6911	0.1618	0.4241	1.63	4.27*	Mixture. Major ♂ (100%)
Swab of black sandal	0.1316	0.0328	0.0746	1.76	4.02*	Mixture. Major ♂ (100%)
Swab of knife handle	0.4749	0.055	0.2022	2.35*	8.63*	Mixture. Major ♂ (98%)
Cutting of drinking straw	0.2564	0.1172			2.19*	Single source ♀ (100%)
Cutting of chewing gum	3.3524	1.8196	0.0002	17486.03*	1.84	Single source ♀ (100%)

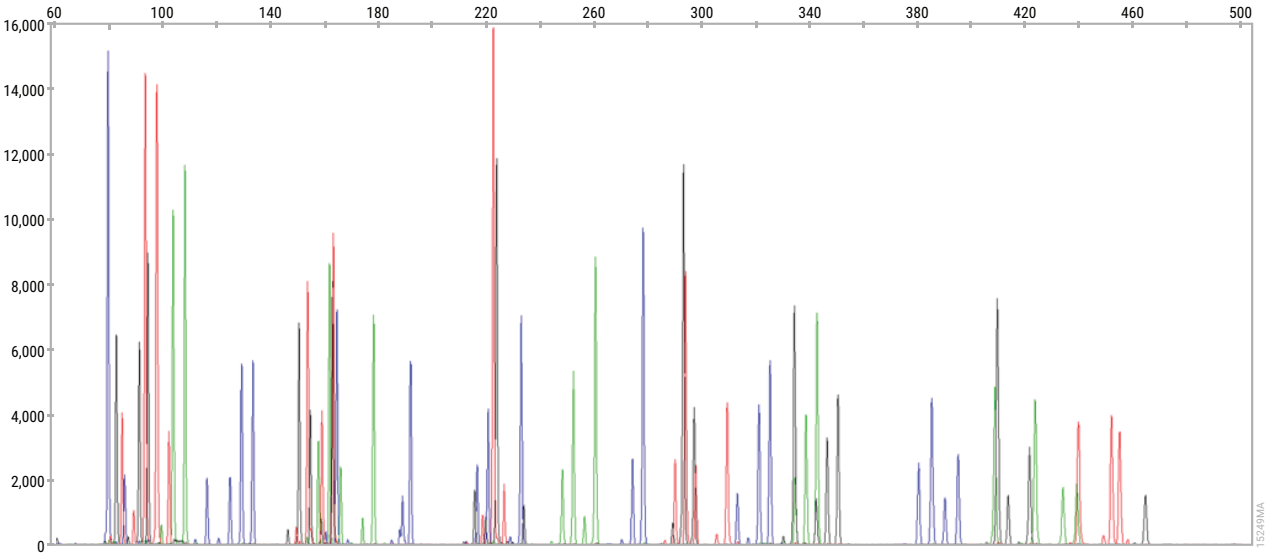

Figure 6. A mixed DNA profile from a cutting of cloth containing blood and semen (scaled to 16,000RFU). Sample was extracted using the Maxwell® RSC 48 Instrument with the Maxwell® FSC DNA IQ™ Casework Kit and quantified using the PowerQuant® System on the Applied Biosystems® 7500 Real-Time PCR System. Amplification was performed using PowerPlex® Fusion on a GeneAmp® PCR System 9700 thermal cycler, and 1µl of amplified product was subjected to electrophoresis on an Applied Biosystems® 3500xL series instrument using a 1.2kV, 24-second injection.

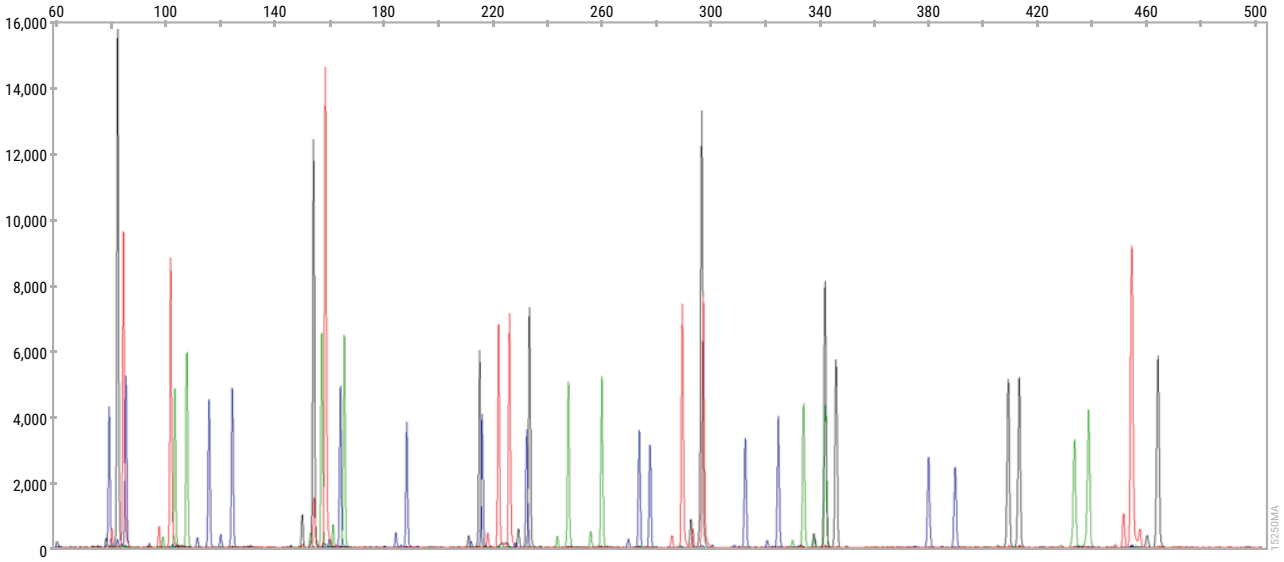

Figure 7. A single-source, high quantification DNA profile from a swabbing of the interior of a condom (scaled to 16,000RFU). Sample was extracted using the Maxwell® RSC 48 Instrument with the Maxwell® FSC DNA IQ™ Casework Kit and quantified using the PowerQuant® System on the Applied Biosystems® 7500 Real-Time PCR System. Amplification was performed using PowerPlex® Fusion on a GeneAmp® PCR System 9700 thermal cycler, and 1µl of amplified product was subjected to electrophoresis on an Applied Biosystems® 3500xL series instrument using a 1.2kV, 24-second injection.

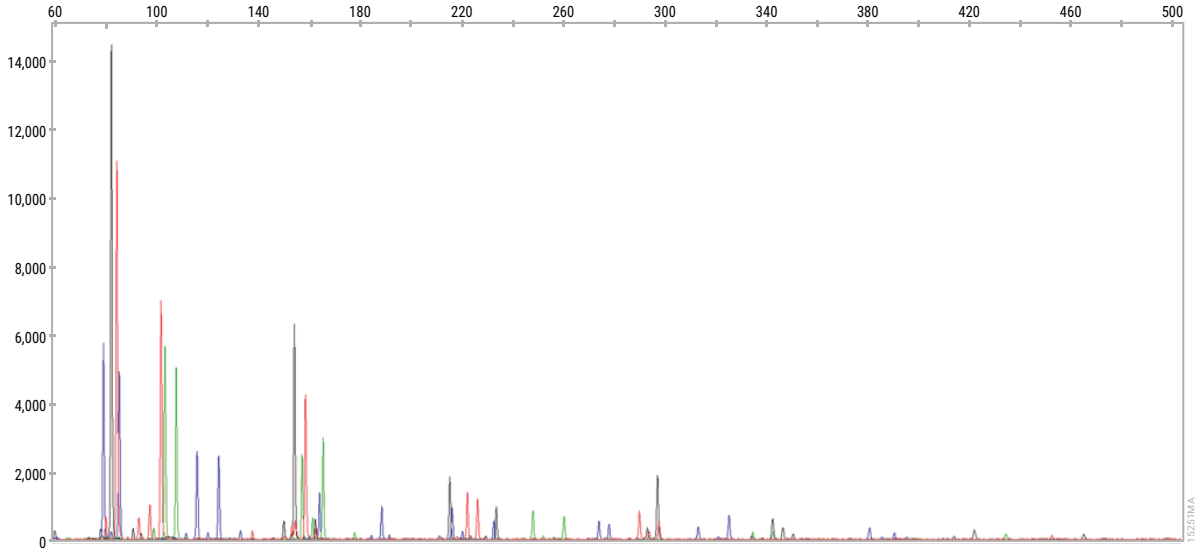

Figure 8. A degraded DNA profile from a swabbing of a gun (scaled to 15,000RFU). Sample was extracted using the Maxwell® RSC 48 Instrument with the Maxwell® FSC DNA IQ™ Casework Kit and quantified using the PowerQuant® System on the Applied Biosystems® 7500 Real-Time PCR System. Amplification was performed using PowerPlex® Fusion on a GeneAmp® PCR System 9700 thermal cycler, and 1µl of amplified product was subjected to electrophoresis on an Applied Biosystems® 3500xL series instrument using a 1.2kV, 24-second injection.

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