Developmental Validation of the Casework Direct Kit, Custom: A Method for the Rapid Processing of Casework Samples

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Casework Direct System (Cat.# DC4560, DC4561)

The goal of extraction is to produce high-quality DNA of sufficient yield for use in downstream DNA typing applications.

This product is now available as the Casework Direct System (Cat.# DC4560, DC4561).

Introduction

The forensic casework DNA typing workflow consists of DNA extraction, DNA quantification, PCR amplification, detection of PCR products and analysis (1). DNA extraction involves cell lysis and release of DNA from the nucleus. Forensic samples also require separation of the DNA from a physical substrate, such as a swab. Conventional DNA extraction methods purify DNA by isolating it from other cellular components and potential PCR inhibitors. The goal of extraction is to produce high-quality DNA of sufficient yield for use in downstream DNA typing applications (1). One challenge to producing an optimal DNA extract is obtaining adequate human DNA yield and concentration from casework samples that normally contain minimal amounts of DNA.

Binding DNA to magnetic particles is a purification method commonly used in forensic laboratories. The amount of DNA recovered by using this technique is limited by both the number of magnetic particles and their surface capacity (1). The success of this method also depends on the ability of the DNA to bind effectively to the particles without interference; otherwise DNA can be lost during the washing process (1-2). Decreases in DNA yield have been observed in validation studies of paramagnetic bead purification methods (3).

The Casework Direct Kit, Custom (now available as the Casework Direct System, Cat.# DC4560, DC4561), contains a buffer and a reducing agent and is designed to rapidly produce a DNA lysate from forensic casework samples of varying substrates and size (4). The Casework Direct protocol generates amplification-ready lysates in approximately 35 minutes. The Casework Direct lysate is compatible with many Promega PowerPlex® amplification systems. No purification is required before using the lysate with downstream STR amplification unless inhibition is indicated by the quantification data. Since the method does not involve any binding or washing, there is less potential for loss of DNA during the process. This is particularly useful for touch samples and sexual assault-type evidence samples containing low quantities of male DNA. The protocol also reduces the amount of time the DNA analyst is handling the sample.

The Casework Direct lysate can be evaluated with PowerQuant® or Plexor® HY Systems to quantify the abundance of human DNA, determine the male/female DNA ratio and predict PCR inhibition (4). Additional information regarding the quality of DNA is obtained when the lysate is evaluated using the PowerQuant® System. Informed decisions about the next step in the workflow can be made about each Casework Direct sample. Therefore, the Casework Direct process is

ideal for screening sexual assault type evidence samples for the presence of male DNA, known as Y-screening. Traditional serology screening tests are less sensitive than DNA typing kits and are time consuming (5). Reliance on the results from serology tests could miss potential CODIS-eligible DNA profiles (5). A recent escalation in legislation requiring mandatory testing of all sexual assault evidence kits has resulted in an increase in laboratory backlogs and turnaround time (6). Use of the Casework Direct System, in conjunction with a DNA quantification system, allows the laboratory to make prompt decisions about which STR amplification system is most appropriate and can provide justification for ceasing work on a sample when no male DNA is present.

The experiments described here address the federal standards and guidelines established by the FBI Quality Assurance Standards (QAS) for Forensic DNA Testing Laboratories (7) and the Scientific Working Group on DNA Analysis Methods (SWGDAM) (8). The experiments included in the developmental validation examine sensitivity, reproducibility, stability, mixtures, contamination and non-probative sample criteria. These analyses demonstrate that the Casework Direct Kit, Custom, is a suitable, accurate and reproducible method for the rapid isolation of DNA.

Materials and Methods

Casework Direct Protocol

All swab and fabric cuttings were processed using a CW Microfuge Tube (Cat.# AS8201) and CW Spin Basket (Cat.# AS8101) assembly. For current consumable recommendations, see the *Casework Direct System Technical Manual* #TMD067. Casework Direct Solution (CDS) was prepared by mixing 100–400µl of Casework Direct Reagent with 0.5µl of tenfold-diluted 1-Thioglycerol added per 100µl of Casework Direct Reagent. Samples were briefly vortexed and then incubated in a heat block at 70°C for 30 minutes. After incubation, the tubes were again briefly vortexed and centrifuged at room temperature at maximum speed for approximately 5 minutes. The CW Spin Baskets were discarded, and the remaining lysate was used for quantification and amplification (Figure 1). Detailed processing instructions are available in the *Casework Direct System Technical Manual* #TMD067 (4)

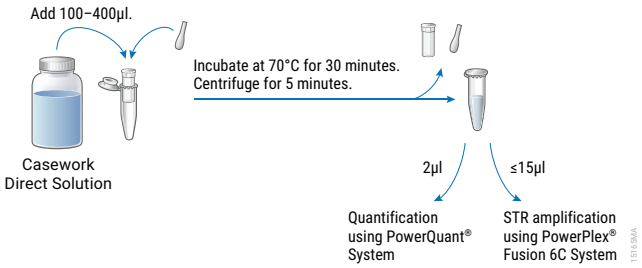

Figure 1. Protocol for Casework Direct Kit, Custom.

DNA Quantification

All Casework Direct lysates were quantified using the PowerQuant® System (Cat.# PQ5002) on the Applied Biosystems® 7500 Real-Time PCR System following the protocol in the *PowerQuant® System Technical Manual* #TMD047 (9). A total of 2µl of lysate was added to each quantification reaction. The following quality flags were monitored throughout the studies: [Autosomal]/[Degradation] ratio, [Autosomal]/[Y] ratio and internal PCR control (IPC) C_q shift. 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. An IPC C_q shift of 0.3 was used to flag a sample for possible inhibition. Quality thresholds were calculated and evaluated by the PowerQuant® Analysis Tool v. 1.0.0.0.

DNA Amplification

Samples were amplified with the PowerPlex® Fusion 6C System (Cat.# DC2705) using the amplification set-up and cycling parameters in the *PowerPlex® Fusion 6C System for Use on the Applied Biosystems® Genetic Analyzers Technical Manual* TMD045 (10). A total of 1ng of template DNA or a maximum of 15µl of lysate was added to each PowerPlex® Fusion 6C amplification reaction. DNA lysates needing further analysis were amplified with the PowerPlex® Y23 System (Cat.# DC2305) using the amplification setup and cycling parameters as described in the *PowerPlex® Y23 System for Use on the Applied Biosystems® Genetic Analyzers Technical Manual* #TMD035 (11). A total of 0.5ng of template DNA or a maximum of 15µl of lysate was added to each PowerPlex® Y23 amplification reaction. All samples for

both amplification systems were amplified on an Applied Biosystems® GeneAmp® PCR System 9700 thermal cycler.

Short Tandem Repeat (STR) Analysis

Separation of amplification products was performed on a 3500xL Genetic Analyzer (Applied Biosystems). Samples were prepared for separation by adding 1µl of amplified sample or allelic ladder to 9.5µl of Hi-Di™ Formamide (Applied Biosystems) and 0.5µl of internal lane standard. Samples were separated by electrophoresis using a 1.2kV, 24-second injection. GeneMapper® *ID*-X version 1.5 (Applied Biosystems) was used to determine fragment size and allele calls using analytical thresholds of 75RFU and 100RFU for PowerPlex® Fusion 6C and PowerPlex® Y23 Systems, respectively.

Sensitivity Study

Two male DNA samples (one blood and one semen) were used in the sensitivity study. Serial dilution of each neat body fluid in TE^{-4} buffer (10mM Tris-HCl [pH 8.0], 0.1mM EDTA) was prepared to obtain the following body fluid volumes: 100µl, 20µl, 4.0µl, 0.8µl, 0.16µl, 0.032µl and 0.0064µl. Each sample was created by drying 100µl of each dilution onto a whole cotton swab in triplicate. Each set of dilutions was extracted in 400µl of Casework Direct Solution (CDS) as described in the Casework Direct Kit, Custom, protocol.

Reproducibility Study

Two male DNA samples (one semen and one saliva) were used to verify reproducibility. Twenty microliters of neat saliva was diluted with TE^{-4} buffer to a final volume of 100µl, which was then dried on a whole cotton swab. Five microliters of semen was added directly to a pre-moistened cotton swab and allowed to dry. Each body fluid sample was prepared in triplicate and extracted in two separate extraction sets for a total of six replicates per fluid. Each extraction set was lysed in 400µl of CDS as described in the Casework Direct Kit, Custom, protocol.

Casework Direct Solution Volume and Substrate Cutting Size Study

Two DNA samples (one semen and one female saliva) were used in the CDS volume and substrate cutting size study. Cotton swabs were cut into the following sizes for each body fluid type: whole swab, half swab, quarter swab and eighth of a swab. Each whole swab had 40µl of respective body fluid added, each half swab had 20µl added, each quarter swab had 10µl added, and each eighth of a swab had 5µl added. Swabs were allowed to dry, and then each sample was extracted in duplicate using three different volumes of CDS (100µl, 200µl and 400µl) following the Casework Direct Kit, Custom, protocol.

Inhibition Study

Three inhibitors commonly encountered in forensic casework were tested: hematin (Sigma-Aldrich, MO, USA), humic acid (Sigma-Aldrich) and tannic acid (Sigma-Aldrich). Twenty microliters of male saliva was added to a whole cotton swab along with a designated volume of inhibitor solution, allowed to dry and then extracted in duplicate using 400µl of CDS as described in the Casework Direct Kit, Custom, protocol. Hematin volumes tested were 60µl and 120µl of 2mM stock solution. Humic acid volumes tested were 3µl and 6µl of 5mg/ml stock solution. Tannic acid volumes tested were 3µl and 6µl of 5µg/µl stock solution.

Mixture Study

Two male and female DNA mixture sets were prepared (a blood/blood set and a semen/saliva set). Both sets of mixtures were prepared to obtain the following M:F ratios: 1:1, 1:10, 1:20, 1:50, 1:100, 1:500, 1:1,000, 1:2,500, 1:5,000 and 1:10,000. The blood mixture series was created by using a constant 20µl of female blood combined with 20µl of a designated dilution of male blood. The semen mixture series was created by using a constant 5µl of female saliva combined with 5µl of a designated dilution of semen. All body fluids were allowed to dry on their respective swabs, and then both mixture sets were processed in duplicate by extracting in 400µl of CDS as described in the Casework Direct Kit, Custom, protocol.

Correlation with Serology Testing

A serial dilution of a semen sample was performed in TE^{-4} buffer to obtain the following values: neat, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, 1:1,024, 1:2,048, 1:4,096, 1:8,192 and 1:16,384. Forty microliters of each

dilution were dried on a separate vaginal swab. One quarter of each swab was tested using an acid phosphatase (AP) presumptive test assay using α-naphthyl phosphate and Fast Blue B Salt reagents (Sigma-Aldrich). A sample was considered positive for AP if a purple color developed within 10 seconds of adding the Fast Blue B Salt reagent. Another quarter swab was used for microscopic (Micro) examination of spermatozoa using a Christmas tree stain (Serological Research Institute, CA, USA), as well as a prostate-specific antigen (PSA) test (SERATEC® PSA Semiquant, Germany) (12). The remaining half of each swab was extracted using 400µl of CDS following the Casework Direct protocol.

Casework Samples

Casework samples included a variety of mock evidence, such as touch skin cell samples and sexual assault type samples. Touch DNA was collected from ten different surfaces (Table 1). Each surface was swabbed evenly with two moistened cotton swabs. Each swab was split, and representative halves were extracted in duplicate with both the Casework Direct Kit, Custom, and the Maxwell® FSC DNA IQ™ Casework Kit (Cat.# AS1550) on the Maxwell® FSC Instrument (Cat.# AS4600). The Casework Direct swabs were extracted using 200µl of CDS following the Casework Direct protocol. The DNA IQ™ swabs were incubated in a volume of 300µl of extraction mix as described in the Extraction of Samples on a Solid Support with CW Spin Basket section of the *Maxwell® FSC DNA IQ™ Casework Kit Technical Manual* #TM499 (13). All DNA IQ™ samples were eluted in a final volume of 50µl following the instrument protocols in TM499.

Table 1. A variety of touch samples were collected from ten different surfaces as part of the mock casework study.

Touch DNA Sample Description	
Coffee Machine	Latex Glove
Car Keys	Computer Mouse
Cell Phone	TV Remote Control
Water Fountain Button	Telephone
Door Handle	Mouth of Water Bottle

A set of vaginal swabs was collected at varying post-coitus time intervals (8, 24, 48, 72 and 96 hours). A selection of mock sexual assault-type evidence samples was created using mixed body fluids dried on different substrates (Table 2). One quarter of each swab or stain was tested using the AP assay. Another quarter was used for Micro and PSA. The remaining half was extracted using 200µl of CDS as outlined in the Casework Direct protocol.

Table 2. A variety of sexual assault type samples were prepared using different substrates as part of the mock casework study.

Sample Description
Black Nylon Cutting ♀ Blood + Semen
Black Nylon Cutting ♀ Saliva + Semen
Red Nylon/Polyester Cutting with Semen
Red Nylon/Polyester Cutting ♀ Blood + Semen
Khaki Cutting with Semen
White Thermal Cutting with Semen
White Thermal Cutting ♀ Saliva + Semen
Denim Cutting ♀ Saliva + Semen
♀ Buccal Swab + Semen (1:50)
♀ Buccal Swab + Semen (1:100)
Swab of Condom with Semen

DNA IQ™ Purification of Inhibited Casework Direct Lysates

The following six blood mixture samples previously extracted with the Casework Direct Kit, Custom as part of the mixture study were used: 1:1, 1:10, 1:50, 1:100, 1:1,000 and 1:2,500. These samples demonstrated a shift of 0.3 or greater for the IPC C_q in the PowerQuant® data along with inhibition in the PowerPlex® Fusion 6C profiles. The Casework Direct lysates were additionally processed with the Maxwell® FSC DNA IQ™ Casework Kit by adding 200µl of Lysis Buffer to each lysate. Samples were then purified on the Maxwell® FSC Instrument as described in Section 4 of the *Maxwell® FSC DNA IQ™ Casework Kit Technical Manual* #TM499 (13). All DNA IQ™ samples were eluted in a final volume of 50µl.

Contamination Study

Reagent extraction blanks were processed along with each sample batch throughout the validation studies in order to assess potential DNA contamination of the Casework Direct Kit reagents. The reagent blank control consisted of all the reagents used during sample processing but contained no DNA. A total of twenty-five extraction blanks were analyzed for the presence of DNA as part of the validation.

Results

Sensitivity Study

A sensitivity study was performed to evaluate the linear range of extracted DNA. The data shows that the Casework Direct Kit, Custom, can extract DNA across a series of different body fluid input quantities. The average autosomal DNA concentration ranged from 0.0000 to 1.7213ng/µl extracted from 0.0064–100µl of blood (Table 3). The average autosomal DNA concentration ranged from 0.0004 to 10.9046ng/µl extracted from 0.0064–100µl of semen (Table 4). An average total yield of 0.8003ng was extracted from 0.032µl of blood. An average total yield of 0.1473ng was extracted from 0.0064µl of semen.

The results showed a linear correlation between the input volume of body fluid and the concentration of DNA, with the exception of the highest volume of blood (Figure 2). The average autosomal DNA concentration increases concurrently with an increase in body fluid volume.

However, we observed a leveling of the DNA concentration after the 20µl blood volume. The semen series exhibited a consistent proportional linear increase in DNA concentration as the input volume increased. No plateau of the DNA concentration was observed with the semen series. DNA yield extracted from body fluids may vary depending on the sample preparation, type, and donor.

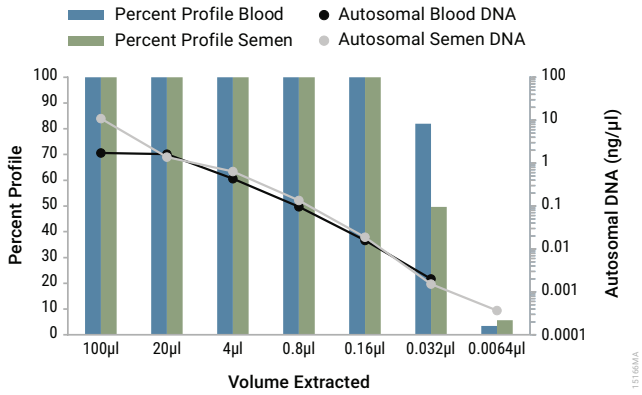

Figure 2. Linearity of DNA concentration and percent profile obtained from the blood and semen sensitivity series. The X axis represents the volume of body fluid extracted. The left Y axis represents the average percent profile obtained in the PowerPlex® Fusion 6C System (indicated by the bars). The right Y axis represents the average autosomal DNA concentration in logarithmic scale ten from the PowerQuant® System (indicated by the lines). Samples were extracted using the Casework Direct Kit, Custom, and quantified on the Applied Biosystems® 7500 Real-Time PCR System. Amplification was performed using a GeneAmp® 9700. One microliter of amplified product was electrophoresed on an Applied Biosystems® 3500xL instrument using a 1.2kV, 24-second injection.

Table 3. Average concentration, IPC C_q and total autosomal DNA yield across targets in the PowerQuant® System for various volumes of blood and semen extracted with the Casework Direct Kit, Custom, in the sensitivity study. Standard deviation of 3 replicates is indicated. [Auto] = autosomal target; [Deg] = degraded target; [Y] = male target.

	[Auto] (ng/µl)	[Deg] (ng/µl)	[Y] (ng/µl)	IPC C_q	Total Autosomal DNA Yield (ng)
Blood Series					
100µl*	1.7213 ± 0.0519	2.7824 ± 0.1155	2.6397 ± 0.0672	21.39 ± 0.06	688.51
20µl*	1.6237 ± 0.2646	1.9159 ± 0.2964	2.0998 ± 0.3073	21.06 ± 0.06	649.49
4µl	0.4349 ± 0.0246	0.3440 ± 0.0322	0.5072 ± 0.0484	20.88 ± 0.16	173.95
0.8µl	0.0966 ± 0.0069	0.0666 ± 0.0038	0.1028 ± 0.0046	20.66 ± 0.10	38.634
0.16µl	0.0158 ± 0.0025	0.0097 ± 0.0004	0.0176 ± 0.0024	20.58 ± 0.08	6.3382
0.032µl	0.0020 ± 0.0005	0.0011± 0.0003	0.0022 ± 0.0003	20.52 ± 0.03	0.8003
0.0064µl	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0003 ± 0.0002	20.59 ± 0.04	0.0000
Semen Series					
100µl	10.9046 ± 1.1332	6.6106 ± 0.6945	13.3023 ± 1.8256	20.66 ± 0.05	4361.8
20µl*	1.3873 ± 0.2904	0.9451 ± 0.2006	1.4136 ± 0.2941	21.15 ± 0.08	554.92
4µl	0.6395 ± 0.1994	0.3766 ± 0.1122	0.6479 ± 0.1597	20.75 ± 0.08	255.81
0.8µl	0.1351 ± 0.0203	0.0877 ± 0.0119	0.1480 ± 0.0118	20.62 ± 0.01	54.020
0.16µl	0.0189 ± 0.0018	0.0108 ± 0.0006	0.0210 ± 0.0005	20.52 ± 0.07	7.5543
0.032µl	0.0015 ± 0.0005	0.0009 ± 0.0001	0.0021 ± 0.0003	20.61 ± 0.11	0.6100
0.0064µl	0.0004 ± 0.0003	0.0001 ± 0.0002	0.0003 ± 0.0000	20.50 ± 0.05	0.1473

*IPC C_q shift flag present in at least one replicate.

Complete PowerPlex® Fusion 6C profiles were obtained with as little as 0.16µl of blood and semen (Figure 2). The average peak heights of the 20µl and 100µl blood samples were approximately half the average peak heights of the 4µl and 0.8µl blood samples (Figure 3). These peak height results in conjunction with IPC C_q shift flags present in the PowerQuant® System data (not shown) indicate that the 20µl and 100µl blood samples had inhibitors present to impact the quantification and amplification results. The 20µl semen samples were flagged for possible inhibition with PowerQuant® System (data not shown); however, no signs of inhibition were apparent in the PowerPlex® Fusion 6C profile.

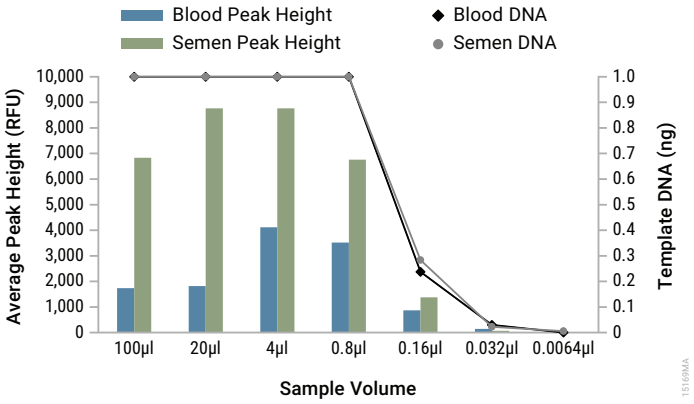

Figure 3. Average peak heights of sensitivity samples with corresponding amount of DNA template amplified with PowerPlex® Fusion 6C System. The X axis represents the volume of body fluid extracted. The left Y axis represents the average peak heights (indicated by the bars). The right Y axis represents the amount of template DNA used in the amplification reaction (indicated by the lines). Samples were extracted using the Casework Direct Kit, Custom, and quantified using PowerQuant® System on the Applied Biosystems® 7500 Real-Time PCR System. Amplification was performed using a GeneAmp® 9700. One microliter of amplified product was electrophoresed on an Applied Biosystems® 3500xL instrument using a 1.2kV, 24-second injection.

Reproducibility Study

The reproducibility of quantity and quality of DNA obtained from replicate samples was assessed. The results from replicates were compared within an extraction set and between extraction sets. The DNA concentration within sets and between sets was similar for each of the body fluids tested (Table 4 and Figure 4). No IPC C_q shift flag was detected in any of the sample replicates. Amplification with PowerPlex® Fusion 6C System resulted in complete STR profiles with reproducible genotypes (data not shown). Average peak heights ranged between 3,345RFU and 7,365RFU for the saliva samples (Figure 5). Average peak heights of semen samples ranged between 3,681RFU and 5,577RFU (Figure 5).

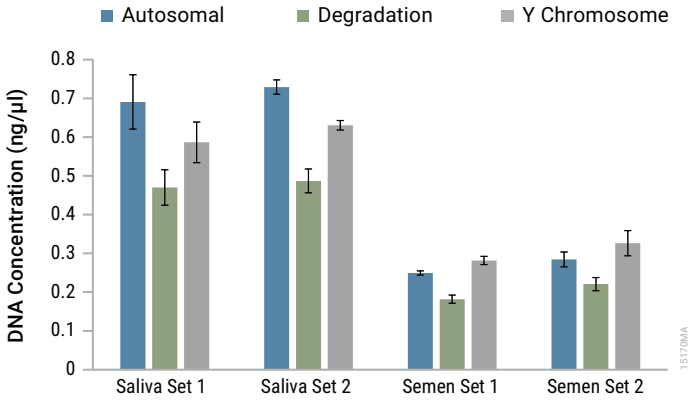

Figure 4. PowerQuant® quantification results for the saliva and semen reproducibility samples. The X axis represents the extraction set. The Y axis represents the average DNA concentration in ng/µl. Error bars represent +/– 1 standard deviation. Samples were extracted using the Casework Direct Kit, Custom, and quantified on the Applied Biosystems® 7500 Real-Time PCR System.

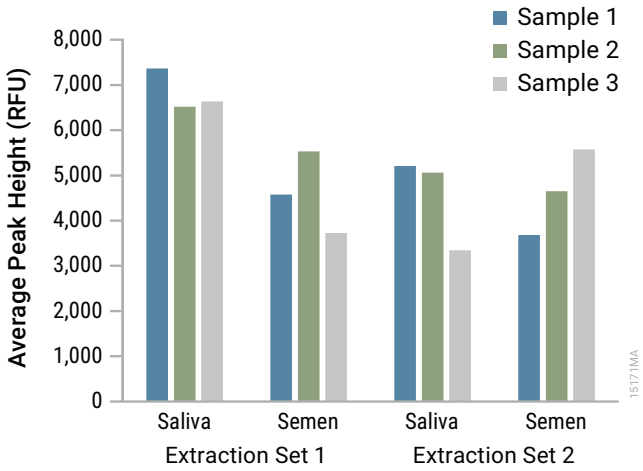

Figure 5. Average peak heights of the saliva and semen reproducibility samples. The X axis represents the extraction set. The Y axis represents the average peak height of each sample. Samples were extracted using the Casework Direct Kit, Custom, and quantified using PowerQuant® System on the Applied Biosystems® 7500 Real-Time PCR System. Amplification was performed with the PowerPlex® Fusion 6C System using a GeneAmp® 9700. One microliter of amplified product was electrophoresed on an Applied Biosystems® 3500xL instrument using a 1.2kV, 24-second injection.

Casework Direct Solution Volume and Substrate Cutting Size Study

The compatibility of the Casework Direct protocol with varying volumes of CDS and different sizes of swab cuttings was investigated. The results show that the Casework Direct Kit, Custom, yields amplifiable DNA over a range of swab cutting sizes and CDS volume combinations. CDS volumes from 100–400µl combined with whole, half, quarter, and eighth of a swab cuttings were successfully tested. The results exhibited a general positive correlation between CDS volume and resulting total human DNA yield.

Table 4. Average concentration and Coefficient of Variation (%) across targets in PowerQuant® System for replicates within extraction sets and between extraction sets for saliva and semen processed with the Casework Direct Kit, Custom, in the reproducibility study. Standard deviation is indicated.

	[Auto] (ng/µl)	[Auto] CV (%)	[Deg] (ng/µl)	[Deg] CV (%)	[Y] (ng/µl)	[Y] CV (%)	IPC C_q	IPC C_q CV (%)
Saliva Extraction								
Extraction Set #1 (N=3)	0.6910 ± 0.0700	10.1	0.4702 ± 0.0457	9.7	0.5866 ± 0.0526	9.0	20.65 ± 0.12	0.6
Extraction Set #2 (N=3)	0.7293 ± 0.0184	2.5	0.4870 ± 0.0308	6.3	0.6307 ± 0.0121	1.9	20.77 ± 0.06	0.3
Inter-Run (N=6)	0.7101 ± 0.0546	7.7	0.4786 ± 0.0399	8.3	0.6086 ± 0.0440	7.2	20.71 ± 0.11	0.5
Semen Extraction								
Extraction Set #1 (N=3)	0.2495 ± 0.0055	2.2	0.1816 ± 0.0106	5.8	0.2817 ± 0.0104	3.7	20.83 ± 0.11	0.5
Extraction Set #2 (N=3)	0.2846 ± 0.0192	6.8	0.2208± 0.0171	7.7	0.3262 ± 0.0324	9.9	20.83 ± 0.10	0.5
Inter-Run (N=6)	0.2670 ± 0.0225	8.4	0.2012 ± 0.0242	12.0	0.3039± 0.0328	10.8	20.83 ± 0.10	0.5

There is also a negative correlation between total human DNA yield and autosomal DNA concentration (Figure 6). Occasionally, use of 100µl of CDS necessitated longer spin times in the basket assembly. Internal studies are recommended to confirm the CDS volumes appropriate for the sample type and size desired for use in each individual laboratory.

For saliva samples, the decrease in DNA concentration was more pronounced for the eighth of a swab size than the whole swab when the volume of CDS was increased (Figure 6). A 1.7-fold decrease in concentration was observed with the whole swab between the 100µl CDS sample and the 400µl CDS sample, whereas a 3.6-fold decrease was observed with the eighth of a swab cutting. The increase in total human DNA yield was more pronounced for saliva tested with a whole swab (Figure 6), which resulted in a 2.5-fold increase in total human DNA yield between the 100µl and 400µl CDS samples. The increase in total human DNA yield was minimal with the eighth of a swab cutting, resulting in a 1.2-fold increase in DNA between the 100µl and 400µl CDS samples. No IPC C_q shift flag was detected in any of the saliva sample replicates. Amplification with the PowerPlex® Fusion 6C System resulted in STR profiles with average peak heights ranging from 2,590RFU to 4,832RFU for the saliva samples (Figure 7).

The results for semen samples exhibited a trend similar to the saliva samples with some exceptions (Figure 6). On average, semen on half of a swab yielded the most total human DNA and highest autosomal concentration of DNA when extracted with 200µl of CDS. Additionally, semen on a whole swab resulted in higher autosomal DNA concentration when extracted with 400µl of CDS. Five semen sample replicates extracted with 400µl of CDS (half swab and quarter swab cutting sizes) displayed IPC C_q shift flags with the PowerQuant® System. Two semen sample replicates extracted with 200µl of CDS (quarter and eighth of a swab cutting sizes) displayed IPC C_q shift flags. This data set showed inconsistency in the IPC C_q shift between extraction replicates of the same sample. However, all semen samples produced STR profiles with average peak heights that ranged between 7,207RFU and 11,235RFU (Figure 8).

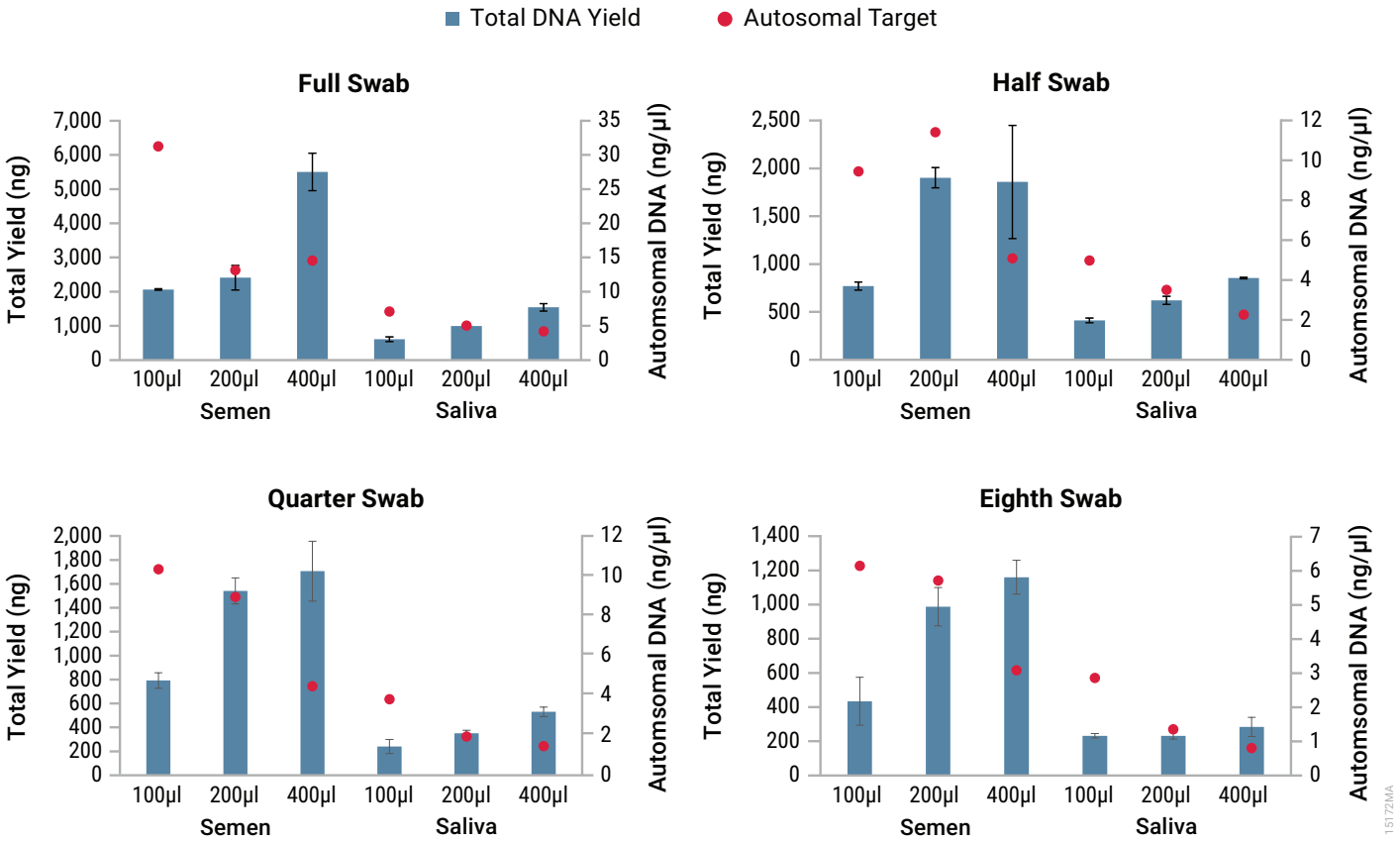

Figure 6. Average total human DNA yield and average autosomal DNA concentration for the saliva and semen samples used in the CDS volume and substrate cutting size study. The X axis represents the body fluid and volume of CDS used. The left Y axis represents the average total human DNA yield of each sample (indicated by the bars). The right Y axis represents the average autosomal DNA concentration (indicated by the dots). The top left graph represents the data obtained with a full swab. The top right graph represents the data obtained with half of a swab. The bottom left graph represents the data obtained using a quarter of a swab. The bottom right graph represents the data obtained using an eighth of a swab. The error bars represent +/– 1 standard deviation. Samples were extracted using the Casework Direct Kit, Custom, and quantified using PowerQuant® System on the Applied Biosystems® 7500 Real-Time PCR System.

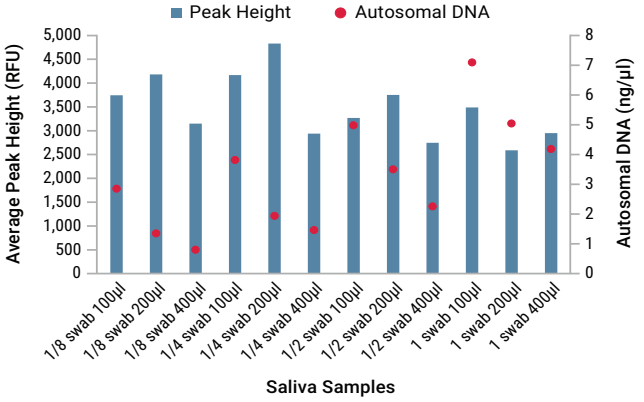

Figure 7. Average peak heights and average autosomal DNA concentration for the saliva samples used in the CDS volume and substrate cutting size study. The X axis represents the size of swab cutting and volume of CDS used. The left Y axis represents the average peak height (indicated by the bars). The right Y axis represents the average autosomal DNA concentration (indicated by the dots). Samples were quantified using PowerQuant® System on the Applied Biosystems® 7500 Real-Time PCR System. Amplification was performed with the PowerPlex® Fusion 6C System using a GeneAmp® 9700. One microliter of amplified product was electrophoresed on an Applied Biosystems® 3500xL instrument using a 1.2kV, 24-second injection.

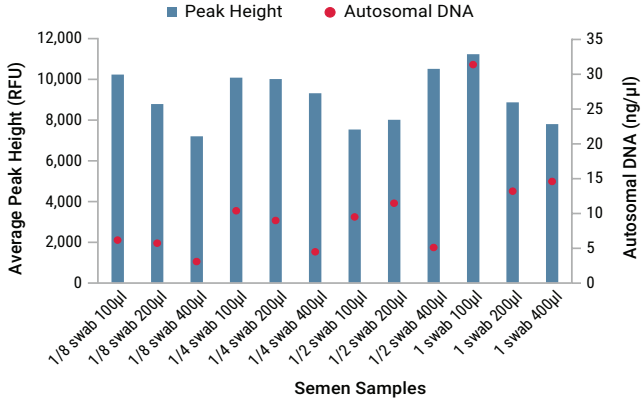

Figure 8. Average peak heights and average autosomal DNA concentration for the semen samples used in the CDS volume and substrate cutting size study. The X axis represents the size of swab cutting and volume of CDS used. The left Y axis represents the average peak height (indicated by the bars). The right Y axis represents the average autosomal DNA concentration (indicated by the dots). Samples were quantified using PowerQuant® System on the Applied Biosystems® 7500 Real-Time PCR System. Amplification was performed with the PowerPlex® Fusion 6C System using a GeneAmp® 9700. One microliter of amplified product was electrophoresed on an Applied Biosystems® 3500xL instrument using a 1.2kV, 24-second injection.

Inhibition Study

An inhibition study was performed to show that non-purified DNA in a Casework Direct lysate can be effectively utilized in downstream quantification and amplification applications. Quantification values were obtained for all samples in this study using the PowerQuant® System and amplification resulted in full STR profiles using PowerPlex® Fusion 6C and PowerPlex® Y23 Systems (Table 5). IPC C_q shift flags were observed in the 120µl hematin samples, which corresponded to a reduction in average PowerPlex® Fusion 6C peak heights for this sample. Average peak heights for the samples containing 120µl of hematin were similar to those containing 60µl of hematin (Figure 9). The average peak heights of samples containing humic acid and tannic acid were similar to those with no inhibitors added (Figures 10 and 11); however, one replicate of 6µl of tannic acid displayed a threshold flag for IPC C_q shift (data not shown). The samples containing 60µl of hematin resulted in the lowest average peak heights with the PowerPlex® Y23 System (data not shown). The results demonstrate that the Casework Direct Kit, Custom, may be used with samples containing common PCR inhibitors. Since no purification is performed, profile quality may be affected by high levels of inhibitors, as was observed with the samples containing hematin.

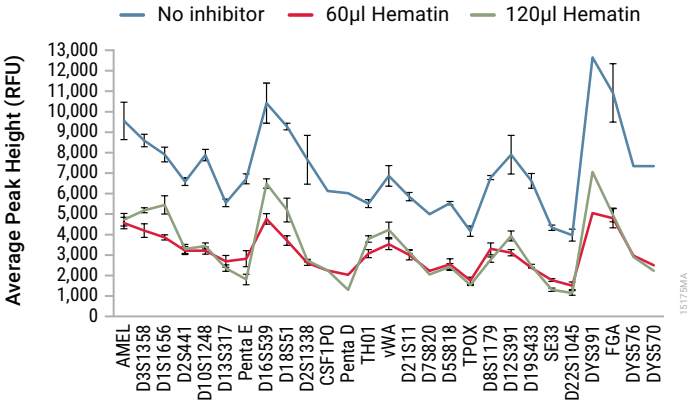

Figure 9. Average peak heights obtained by locus for hematin samples using PowerPlex® Fusion 6C System. The error bars represent +/– 1 standard deviation. Samples were extracted using the Casework Direct Kit, Custom, and quantified using the PowerQuant® System on the Applied Biosystems® 7500 Real-Time PCR System. Amplification was performed using a GeneAmp® 9700. One microliter of amplified product was electrophoresed on an Applied Biosystems® 3500xL instrument using a 1.2kV, 24-second injection.

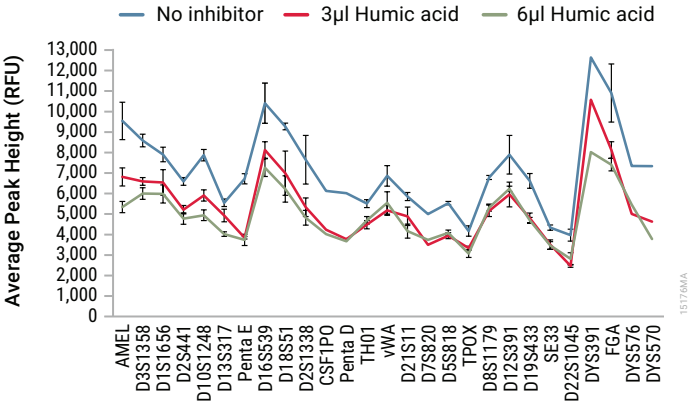

Figure 10. Average peak heights obtained by locus for humic acid samples using PowerPlex® Fusion 6C System. The error bars represent +/– 1 standard deviation. Samples were extracted using the Casework Direct Kit, Custom, and quantified using PowerQuant® System on the Applied Biosystems® 7500 Real-Time PCR System. Amplification was performed using a GeneAmp® 9700. One microliter of amplified product was electrophoresed on an Applied Biosystems® 3500xL instrument using a 1.2kV, 24-second injection.

Table 5. Average PowerQuant® System results +/– 1 standard deviation and percent profile results for PowerPlex® Fusion 6C and PowerPlex® Y23 Systems for inhibition study samples. Orange cells represent a threshold flag with the PowerQuant® System.

Inhibitor Series	[Auto] (ng/µl)	[Deg] (ng/µl)	[Y] (ng/µl)	IPC C_q Shift	IPC Threshold	% Profile PowerPlex® Fusion 6C System	% Profile PowerPlex® Y23 System
No Inhibitor	0.1630 ± 0.0049	0.1160 ± 0.0044	0.1366 ± 0.0074	–0.211 ± 0.008	Below	100%	100%
60µl of 2mM Hematin	0.1841 ± 0.0906	0.1535 ± 0.0760	0.1687 ± 0.0796	0.203 ± 0.055	Below	100%	100%
120µl of 2mM Hematin	0.1068 ± 0.0170	0.1006 ± 0.0173	0.1072 ± 0.0123	0.462 ± 0.134	At or Above	100%	100%
3µl of 5mg/ml Humic Acid	0.1478 ± 0.0149	0.0920± 0.0154	0.1282 ± 0.0133	0.023 ± 0.103	Below	100%	100%
6µl of 5mg/ml Humic Acid	0.1789 ± 0.0206	0.1086 ± 0.0089	0.1543 ± 0.0178	0.175 ± 0.004	Below	100%	100%
3µl of 5µg/µl Tannic Acid	0.1723 ± 0.0075	0.1174± 0.0033	0.1466 ± 0.0039	0.137 ± 0.048	Below	100%	100%
6µl of 5µg/µl Tannic Acid	0.1385 ± 0.0194	0.0915 ± 0.0140	0.1314 ± 0.0186	0.270 ± 0.092	Below	100%	100%

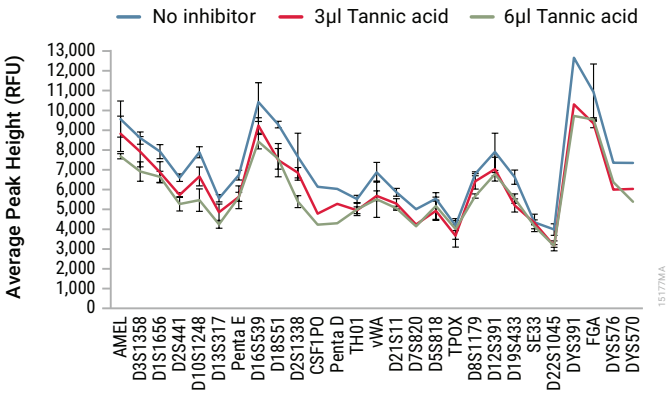

Figure 11. Average peak heights obtained by locus for tannic acid samples using PowerPlex® Fusion 6C System. The error bars represent +/– 1 standard deviation. Samples were extracted using the Casework Direct Kit, Custom, and quantified using PowerQuant® System on the Applied Biosystems® 7500 Real-Time PCR System. Amplification was performed using a GeneAmp® 9700. One microliter of amplified product was electrophoresed on an Applied Biosystems® 3500xL instrument using a 1.2kV, 24-second injection.

Mixture Study

A mixture study was performed to show that male DNA can be obtained in the presence of large amounts of female DNA in a variety of donor sample types. Mixture Series 1 consisted of male and female blood. These samples generated large IPC C_q shifts with the PowerQuant® System, with averages ranging from 0.77 to 3.73. These shifts, combined with PowerPlex® Fusion 6C profiles exhibiting sloping peak heights and dropout, suggest inhibition may be caused by the large volume of blood used in sample setup (data not shown). Some of these mixture samples were purified and data presented in the DNA IQ™ Purification results section.

Despite inhibition, the PowerQuant® System was able to consistently detect male DNA in the Casework Direct Mixture Series 1 lysates down to the 1:5,000 mixture ratio, which corresponded to an average [Auto]/[Y] ratio of 2,596. Minor male alleles that were unique and not in stutter positions relative to the major female component were detected down to the 1:100 mixture with the PowerPlex® Fusion 6C System. Approximately 31% of the minor male alleles were still detected at this ratio. The 1:100 mixture had an average [Auto]/[Y] ratio of 31 with 0.0058ng/µl male DNA. A full PowerPlex® Y23 profile was detected down to the 1:10 mixture. Approximately 41% of the Y-STR profile was detected at the 1:100 ratio (Table 6).

Mixture Series 2 consisted of semen and female saliva and did not exhibit inhibition at quantification or STR-amplification. Male DNA was consistently detected with the PowerQuant® System down to the 1:500 mixture ratio, which corresponded to an average [Auto]/[Y] ratio of 2,884. Minor male alleles that were unique and not in stutter positions relative to the major female component were detected down to the 1:20 mixture ratio with the PowerPlex® Fusion 6C System. This corresponded to an average [Auto]/[Y] ratio of 82 and 0.0115ng/µl male DNA. Approximately 23% of the minor male alleles were still detected at this ratio. A full PowerPlex® Y23 profile was detected down to the 1:20 mixture. Approximately 76% of the Y-STR profile was detected at the 1:100 ratio, which had an average of 0.0026ng/µl male DNA and an average [Auto]/[Y] ratio of 341 (Table 6 and Figure 12).

Correlation with Serology Testing

A Y-screening approach utilizing the Casework Direct Kit, Custom, with the PowerQuant® System was compared to conventional serology testing. The 1:64 semen dilution did not produce serology or quantification results, in concordance with the data trend obtained from the other samples, so it was removed from the results with the assumption that a pipetting error occurred during sample preparation.

The AP test was demonstrated to be the least sensitive protocol used in this study for the detection of semen on a vaginal swab. Positive AP tests were obtained for semen dilutions down to 1:32 (Table 7). PSA was detected down to the 1:8,192 dilution of semen. Spermatozoa were detected microscopically down to the 1:1,024 dilution of semen. The Casework Direct Y-screening method detected male DNA down to the 1:4,096 dilution of semen. The AP test indicated a negative semen result with the 1:128 dilution, which produced a full Y-STR profile with PowerPlex® Y23. Both the PSA test and the Casework Direct Y-screening method produced positive results at the 1:4,096 semen dilution, which generated no-STR profiles. The male quantification result from the 1:4,096 semen dilution generated from the Y-screening method (0.0004ng/µl) is consistent with previous no-STR profile results. The 1:8,192 semen dilution tested positive for PSA and negative for all other screening methods and corresponded to one allele detected with PowerPlex® Y23. Overall, the Casework Direct Y-screening method was best suited to predict male STR profile potential.

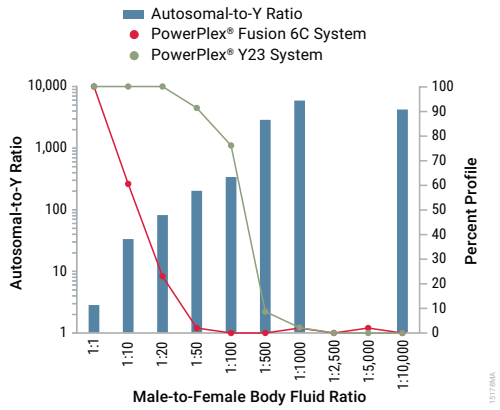

Figure 12. Average [Auto]/[Y] ratio and STR percent profile results for Mixture Series 2 (semen/saliva). The X axis represents the ratio of male to female body fluid that was used to prepare the sample. The left Y axis represents the average ratio of [Auto] to [Y] detected by PowerQuant® System (indicated by the bars) in logarithmic scale ten. The right Y axis represents the average % unique minor profile obtained with PowerPlex® Fusion 6C System (indicated by the red line) and the average % profile obtained with PowerPlex® Y23 System (indicated by the green line). Samples were extracted using the Casework Direct Kit, Custom, and quantified on the Applied Biosystems® 7500 Real-Time PCR System. Amplification was performed using a GeneAmp® 9700. One microliter of amplified product was electrophoresed on an Applied Biosystems® 3500xL instrument using a 1.2kV, 24-second injection.

The [Auto]/[Y] ratio value determined from the Casework Direct Y-screening method, in conjunction with the PowerQuant® System, gave predictive information regarding the percent male profile obtained with PowerPlex® Fusion 6C. As expected, the greater the [Auto]/[Y] ratio, the fewer male alleles were detected with the PowerPlex® Fusion 6C System. Full male PowerPlex® Fusion 6C profiles were obtained with the 1:1 and 1:2 semen dilutions, which corresponded to [Auto]/[Y] ratios of 4.09 and 3.31, respectively. A majority of male alleles could be detected with the PowerPlex® Fusion 6C System down to the 1:16 semen dilution, which corresponded to an [Auto]/[Y] ratio of 25.57 and 76% of unshared minor male alleles detected (Table 7).

Casework Samples

A variety of forensic casework type samples were included in the study to evaluate the kit's ability to yield lysates with

Table 6. Average PowerQuant® quantification results for Mixture Series 1 (blood/blood) and Mixture Series 2 (semen/saliva).

Male:Female	[Auto] (ng/µl)	[Deg] (ng/µl)	[Y] (ng/µl)	[Auto]/[Y]	% Profile Unique Minor PowerPlex® Fusion 6C System	% Profile PowerPlex® Y23 System
Mixture Series 1 (Blood)						
1:1	0.3228	0.4164	0.2097	1.54	95%	100%
1:10	0.3494	0.4573	0.0567	6.17	43%	100%
1:20	0.3226	0.3496	0.0298	10.83	57%	74%
1:50	0.1825	0.2051	0.0102	17.91	54%	54%
1:100	0.1783	0.1986	0.0058	30.99	31%	41%
1:500	0.2642	0.3117	0.0014	184.70	3%	7%
1:1000	0.2119	0.2692	0.0006	356.65	0%	2%
1:2500	0.3498	0.3967	0.0004	918.84	4%	7%
1:5000	0.4726	0.4809	0.0002	2596.32	2%	0%
1:10000	0.2601	0.2795	ND	–	5%	0%
Mixture Series 2 (Semen and Saliva)						
1:1	1.1805	0.7229	0.4153	2.84	100%	100%
1:10	0.8102	0.3871	0.0242	33.53	61%	100%
1:20	0.9462	0.4693	0.0115	82.24	23%	100%
1:50	0.8222	0.3996	0.0040	203.58	2%	91%
1:100	0.8775	0.4597	0.0026	340.56	0%	76%
1:500	0.9046	0.3891	0.0003	2884.35	0%	9%
1:1000	0.8210	0.3782	0.0001	5936.49	2%	2%
1:2500	0.7916	0.4044	ND	–	0%	0%
1:5000	0.7835	0.3632	ND	–	2%	0%
1:10000	0.8612	0.3918	0.0001	4242.82	0%	0%

human DNA concentration appropriate for use with the PowerPlex® amplification kits. The quantity and quality of DNA obtained from casework samples were assessed.

Ten touch DNA samples were processed with the Casework Direct Kit, Custom and Maxwell® FSC DNA IQ™ Casework Kit on the Maxwell® FSC Instrument. Total human DNA yield as well as autosomal DNA concentration were compared (Figures 13 and 14). The Casework Direct Kit yielded more total human DNA than the Maxwell® FSC DNA IQ™ Casework Kit for nine out of the ten samples. The average concentration of autosomal DNA detected with the PowerQuant® System was similar between the two extraction methods with the exception of the telephone swab, which yielded more DNA with the Maxwell® FSC DNA IQ™ Casework Kit than with the Casework Direct Kit (Figure 14). No IPC C_q shift flag was detected in any of the samples. The PowerPlex® Fusion 6C profiles obtained from the two extraction methods were similar and exhibited differences consistent with stochastic effects expected from low-level, mixed DNA samples (representative example Figure 15). One exception was the telephone swab sample extracted with the Maxwell® FSC DNA IQ™ Casework Kit, which generated more alleles at higher peak heights than the Casework Direct lysate.

Five post-coital swabs were screened for semen using the AP test, PSA test and microscopic sperm search, in addition to Y-screening via Casework Direct Kit and PowerQuant® System (Table 8). The Casework Direct Y-screening method was the most sensitive method for predicting male DNA. At 72 hours post-coitus the vaginal swab tested negative for AP, PSA and sperm identification. However, the Casework Direct lysate contained 0.0030ng/µl male DNA, which resulted in 91% of alleles being called with the PowerPlex® Y23 System. All screening methods were negative for the 96-hour post-coital swab, and no STR results were obtained (Figure 16).

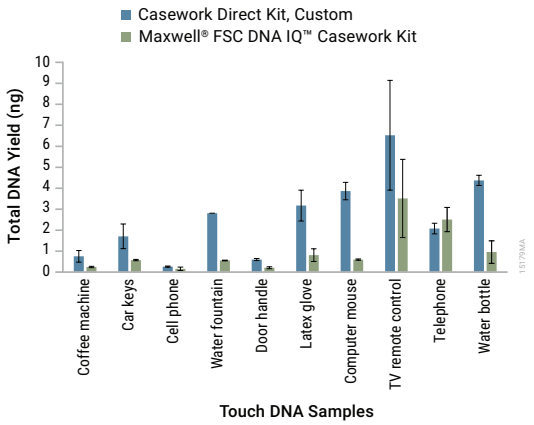

Figure 13. Average total human DNA yield obtained for ten touch DNA samples extracted with both Casework Direct Kit, Custom, and Maxwell® FSC DNA IQ™ Casework Kit. The X axis represents the sample type. The Y axis represents average total DNA yield. The error bars represent +/– 1 standard deviation. Samples were quantified with PowerQuant® System on the Applied Biosystems® 7500 Real-Time PCR System.

Table 7. Serology, PowerQuant® System quantification and STR percent profile results for a series of semen dilutions on vaginal swabs. AP = acid phosphatase test; PSA = prostate-specific antigen test; Micro = microscopic detection of spermatozoa.

Semen Dilution	AP	PSA	Micro	[Auto] (ng/µl)	[Y] (ng/µl)	[Auto]/[Y]	% Male Profile PowerPlex® Fusion 6C System	% Profile PowerPlex® Y23 System
1:1	+	+	+	21.1606	5.1680	4.09	100%	100%
1:2	+	+	+	18.1976	5.4907	3.31	100%	100%
1:4	+	+	+	25.0649	2.9709	8.44	94%	100%
1:8	+	+	+	18.5494	1.4588	12.72	91%	100%
1:16	+	+	+	18.0361	0.7052	25.57	76%	100%
1:32	+	+	+	20.0539	0.2744	73.08	24%	100%
1:128	–	+	–	16.8790	0.0645	261.78	9%	100%
1:256	–	+	–	25.2289	0.0247	1,023.28	9%	78%
1:512	–	+	+	18.1153	0.0119	1,517.11	0%	78%
1:1,024	–	+	+	17.8833	0.0027	6,604.19	0%	39%
1:2,048	–	+	–	14.5056	0.0020	7,248.01	0%	17%
1:4,096	–	+	–	20.9346	0.0004	50,120.68	0%	0%
1:8,192	–	+	–	21.6256	–	–	0%	4%
1:16,384	–	–	–	14.0106	–	–	0%	0%

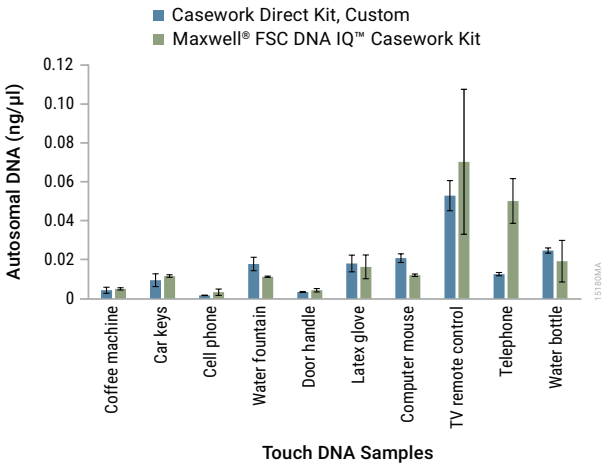

Figure 14. Average autosomal quantification results for ten touch DNA samples extracted with both Casework Direct Kit, Custom, and Maxwell® FSC DNA IQ™ Casework Kit. The X axis represents the sample type. The Y axis represents average autosomal DNA concentration. The error bars represent +/– 1 standard deviation. Samples were quantified with PowerQuant® System on the Applied Biosystems® 7500 Real-Time PCR System.

A variety of mock casework samples containing semen and prepared on various substrates were processed. The samples were tested using serology screening methods, Casework Direct Y-screening and the PowerPlex® Fusion 6C and PowerPlex® Y23 Systems (Table 9). All samples tested positive for PSA and male DNA via Y-screening with the Casework Direct Kit, Custom. Full Y-STR profiles and partial or full autosomal STR profiles were obtained from all samples except where inhibition is stated. The blood/semen mixture on black nylon and the blood/semen mixture on red nylon/polyester generated STR profiles that were inhibited and exhibited a noisy baseline. Both of these samples exhibited IPC C_q shift flags with the PowerQuant® System. The inconclusive AP results were due to substrate color masking reagent color change. Substrates containing heavy soil, such as fingerprint powder, may necessitate longer spin times in the basket.

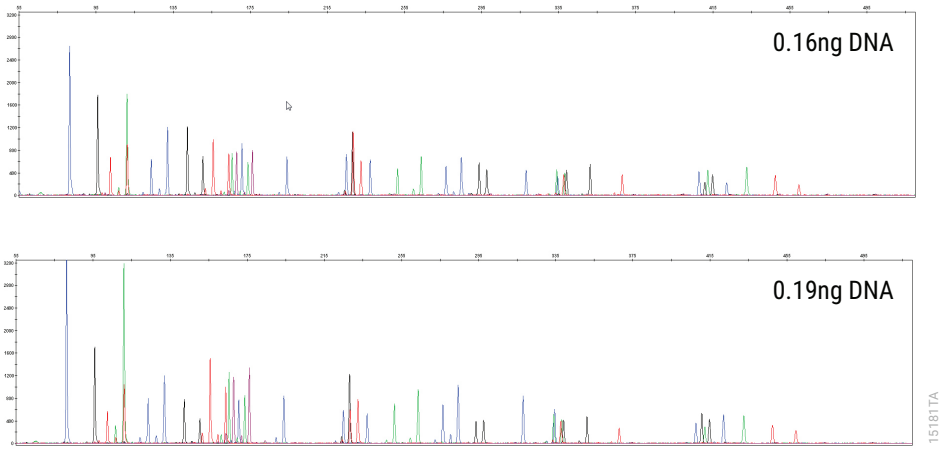

Figure 15. A female DNA profile developed from the swab of a set of car keys (scaled to 3,200RFU). The top panel represents the profile from the sample extracted using the Maxwell® FSC Instrument with the DNA IQ™ Casework Extraction Kit. The bottom panel represents the profile from the sample extracted using Casework Direct Kit, Custom. Samples were quantified using the PowerQuant® System on the Applied Biosystems® 7500 Real-Time PCR System. Amplification was performed using the PowerPlex® Fusion 6C System on a GeneAmp® 9700. One microliter of amplified product was electrophoresed on an Applied Biosystems® 3500xL instrument using a 1.2kv, 24-second injection.

Table 8. Serology, PowerQuant® System quantification and STR percent profile results for vaginal swabs obtained at varying post-coitus time intervals.

Sample	AP	PSA	Micro	[Auto] (ng/µl)	[Y] (ng/µl)	[Auto]/[Y]	% Male Profile PowerPlex® Fusion 6C System	% Profile PowerPlex® Y23 System
8 hours	+	+	+	25.2410	1.7294	14.59	100%	100%
24 hours	+	+	+	126.4271	0.4382	288.50	11%	100%
48 hours	–	–	+	178.0133	0.0271	6,572.58	8%	87%
72 hours	–	–	–	58.2087	0.0030	19,626.22	0%	91%
96 hours	–	–	–	24.5149	–	–	0%	0%

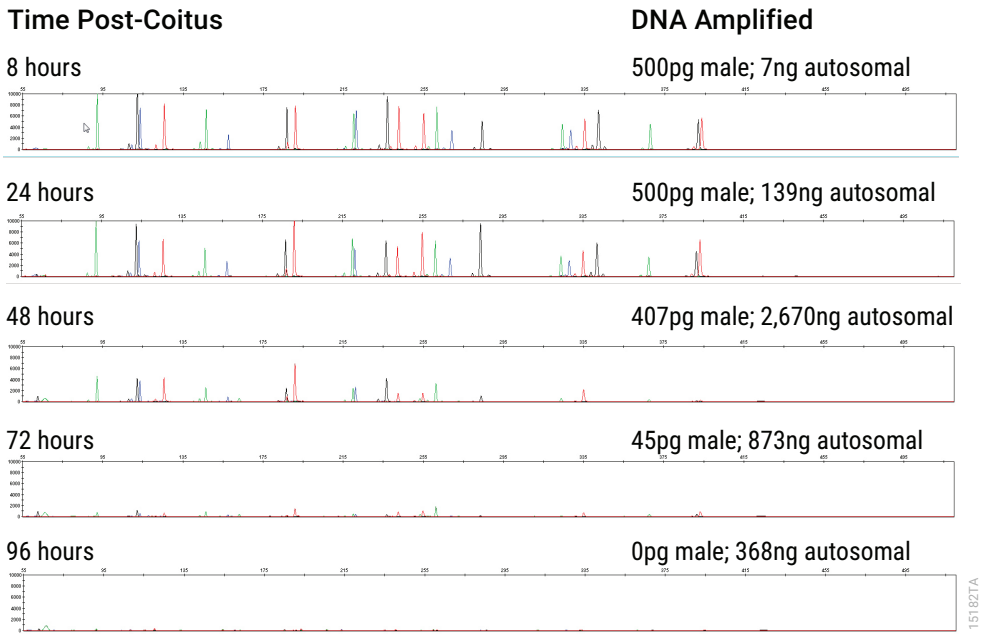

Figure 16. Male DNA profiles developed from a set of vaginal swabs collected from a donor at varying post-coitus intervals (scaled to 10,000RFU). Samples were quantified using the PowerQuant® System on the Applied Biosystems® 7500 Real-Time PCR System. Amplification was performed using the PowerPlex® Y23 System on a GeneAmp® 9700. One microliter of amplified product was electrophoresed on an Applied Biosystems® 3500xL instrument using a 1.2kv, 24-second injection.

Table 9. Serology, PowerQuant® System quantification and STR percent profile results for a variety of mock casework samples containing semen. Orange cells represent a threshold flag with the PowerQuant® System. Inc = inconclusive.

Sample Description	AP	PSA	Micro	[Auto] (ng/µl)	[Y] (ng/µl)	[Auto]/ [Y]	IPC Shift (C_q)	IPC Threshold	% Male Profile PowerPlex® Fusion 6C System	% Profile PowerPlex® Y23 System
Black Nylon Cutting ♀ Blood + Semen	Inc	+	+	0.8743	0.6019	1.45	0.68	At or Above	Inhibited	Inhibited
Black Nylon Cutting ♀ Saliva + Semen	Inc	+	+	1.5108	0.0706	21.40	0.02	Below	88%	100%
Red Nylon/Polyester Cutting with Semen	Inc	+	+	0.0494	0.0480	1.03	0.40	At or Above	100%	100%
Red Nylon/Polyester Cutting ♀ Blood + Semen	Inc	+	–	0.3428	0.1132	3.03	0.80	At or Above	Inhibited	Inhibited
Khaki Cutting with Semen	+	+	+	0.9281	1.0667	0.87	–0.11	Below	100%	100%
White Thermal Cutting with Semen	+	+	+	3.7283	3.9832	0.94	–0.07	Below	100%	100%
White Thermal Cutting ♀ Saliva + Semen	+	+	–	1.9708	0.6491	3.04	–0.32	Below	100%	100%
Denim Cutting ♀ Saliva + Semen	+	+	+	3.0404	0.6425	4.73	–0.17	Below	100%	100%
♀ Buccal Swab + Semen (1:50)	–	+	+	9.6436	0.2630	36.66	–0.22	Below	67%	100%
♀ Buccal Swab + Semen (1:100)	–	+	+	6.5178	0.1204	54.15	–0.27	Below	48%	100%
Swab of Condom with Semen	+	+	+	4.3510	5.4050	0.80	0.70	At or Above	100%	100%

DNA IQ™ Purification of Inhibited Casework Direct Lysates

This study was performed to demonstrate that Casework Direct lysates indicating inhibition in the quantification data can be subsequently cleaned up using the Maxwell® FSC DNA IQ™ Casework Kit on a Maxwell® Instrument. After purification with the Maxwell® FSC Instrument, the IPC C_q shift improved and autosomal DNA concentration increased for six blood samples previously extracted in the mixture study (Figures 17 and 18). Prior to purification, the PowerPlex® Fusion 6C profiles displayed sloping peak heights and drop-out consistent with inhibition. After purification, the profiles exhibited no indication of inhibition (Figure 19).

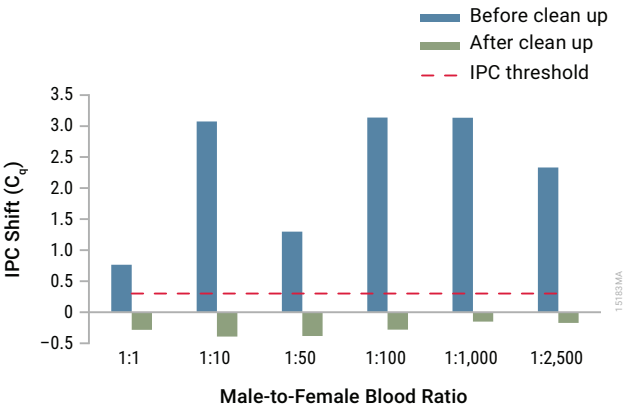

Figure 17. IPC C_q shift results for six blood/blood mixture samples before and after clean-up with the Maxwell® FSC DNA IQ™ Casework Kit. The X axis represents the ratio of male to female blood used to prepare the sample. The Y axis represents the IPC C_q shift. The red dotted line represents the default C_q shift value for which a sample is flagged for inhibition (0.3). Initial lysates were produced with the Casework Direct Kit, Custom. Samples were quantified with PowerQuant® System on the Applied Biosystems® 7500 Real-Time PCR System.

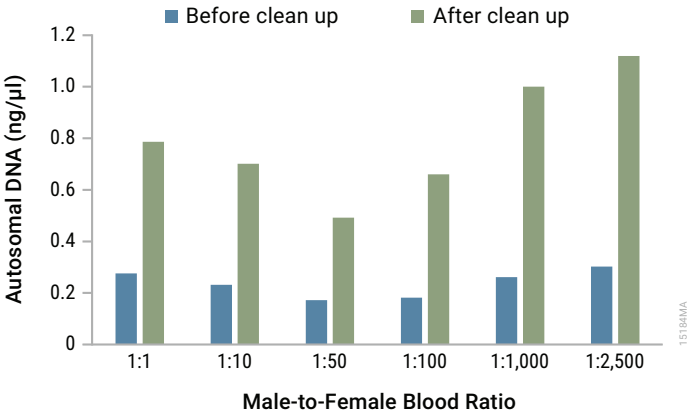

Figure 18. Quantification results for six blood/blood mixture samples before and after cleanup with the Maxwell® FSC DNA IQ™ Casework Kit. The X axis represents the ratio of male to female blood used to prepare the sample. The Y axis represents the autosomal DNA concentration. Initial lysates were produced with the Casework Direct Kit, Custom. Samples were quantified with PowerQuant® System on the Applied Biosystems® 7500 Real-Time PCR System.

Contamination Study

Twenty-five extraction blanks were assessed for possible contamination. Quantification values were obtained for two of the extraction blanks. An extraction blank used with the serology correlation study had an autosomal DNA concentration of 0.0002ng/µl, and one allele at 88RFU was detected with the PowerPlex® Fusion 6C System. No alleles were detected with the PowerPlex® Y23 System. An extraction blank used with the casework study had an autosomal DNA concentration of 0.0008ng/µl; however, no alleles were detected with the PowerPlex® Fusion 6C or PowerPlex® Y23 Systems. The remaining twenty-three extraction blanks did not have DNA detected at either the quantification or STR amplification stage.

Conclusions

The Casework Direct System was developed for the rapid and efficient extraction of low-template DNA forensic casework samples. The protocol minimizes potential loss of DNA from samples and reduces the amount of time a DNA analyst handles a sample. The Casework Direct lysates can be coupled with downstream quantification and PCR amplification using Promega systems.

The extraction of genomic DNA with the Casework Direct Kit, Custom, was evaluated with a comprehensive set of experiments that meet the criteria of current validation standards and guidelines. The developmental validation study results corroborate that the kit provides a reliable and reproducible method to extract DNA from a range of sample types encountered within a forensic setting. This includes compatibility with different body fluid volumes and mixtures, and varying substrate types and sizes. The study also showed that DNA lysates extracted with the kit produce STR profiles when directly amplified using a Promega amplification system. Casework Direct Solution volumes from 100–400µl and small cuttings to whole swabs were successfully tested.

Shifts in the IPC C_q value of PowerQuant® reactions were occasionally not as consistent with the Casework Direct lysate. This was observed in data from a few samples in the sensitivity study, CDS volume/cutting size study, inhibition study, and casework samples. Some low-level artifacts were observed in the PowerPlex® Fusion 6C and Y23 amplifications. These artifacts appeared to be sample-dependent and were most prevalent in samples containing blood or semen. Body fluid type and amount, individual donor, substrate material and sample preparation can influence extraction methodologies. Casework Direct lysates still retain substrate chemicals, cellular material and body fluid proteins that may influence the PCR.

The Casework Direct protocol in conjunction with the PowerQuant® System provided a sensitive, quick and informative Y-screening method for male DNA. The Casework Direct screening approach provided reliable and predictive information about the potential quality of a male STR profile as compared to acid phosphatase, PSA

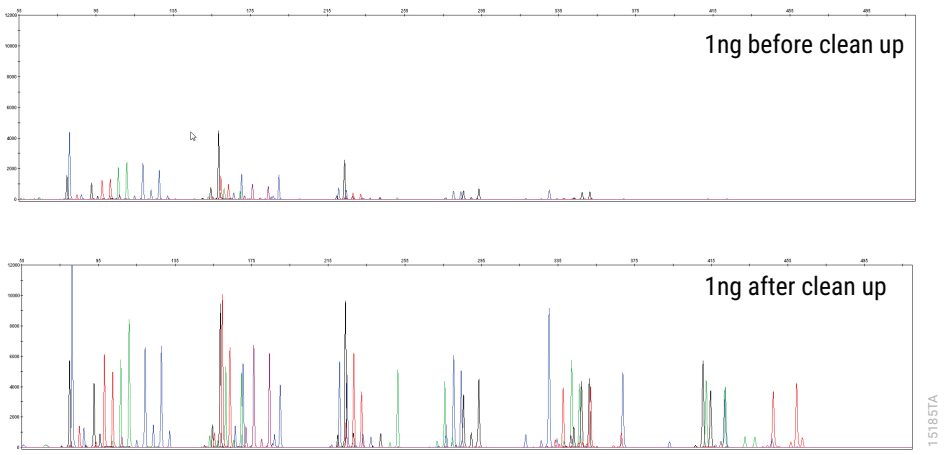

Figure 19. DNA profiles developed from blood/blood mixture samples before and after cleanup with the Maxwell® FSC DNA IQ™ Casework Kit (scaled to 12,000RFU). The top panel represents the profile from the sample extracted using the Casework Direct Kit, Custom, before cleanup. The bottom panel represents the profile from the sample cleaned up using the Maxwell® FSC Instrument with the DNA IQ™ Casework Extraction Kit. Samples were quantified using the PowerQuant® System on the Applied Biosystems® 7500 Real-Time PCR System. Amplification was performed using the PowerPlex® Fusion 6C System on a GeneAmp® 9700. One microliter of amplified product was electrophoresed on an Applied Biosystems® 3500xL instrument using a 1.2kv, 24-second injection.

and microscopic testing. The Casework Direct screening method was shown to also be suitable with touch DNA samples for use in deciding subsequent steps in a forensic laboratory workflow.

The Casework Direct Kit, Custom (Cat.# AX4560) is now available as Casework Direct System (Cat.# DC4560, DC4561).

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