

Applications

Note 224 | April 2010

Validation of the Eppendorf epMotion® 5075 TMX System for use with the Applied Biosystems PrepFiler™ Automated Forensic DNA Extraction Kit

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Abstract

The PrepFiler™ Automated Forensic DNA Extraction Kit provides comparable STR profiles to that of samples extracted with phenol-chloroform. The validation studies confirmed that the epMotion® 5075 TMX is a clean liquid handling system that provides a robust, reproducible and reliable method for obtaining genomic DNA from biological samples suitable for real-time qPCR and STR profiling. The epBlue software is user-friendly and scripts can be written easily and quickly. The software offers a “simulation” run, which allows the user to observe the robot movements (scripts) on the computer monitor prior to running the script on real samples. This simulation is a valuable tool in that it allows the script to be reviewed without wasting reagents and consumables. The epMotion 5075 TMX system can run a maximum of 96 samples in approximately 2.5 hours. Hands-on time is very minimal when compared to manual extraction which allows for timely processing of case-work samples and can cut down on the back-log of samples found in most crime laboratories.

Materials and Methods

epMotion 5075 TMX with integrated PC
Dispensing Tool TS 50
Dispensing Tool TM 50-8
Dispensing Tool TM 1000-8
Reservoir Rack
Gripper
epTIPS® Motion 1-50 µL
epTIPS® Motion Tips 40-1000 µL
Reservoir 30 mL
Reservoir 100 mL
96 well Deepwell Plate, 1000 µL
96 well Deepwell Plate, 2000 µL
96 well PCR plate, skirted
Thermomixer®
Deepwell Plate Adapter

Consumables and reagents from other vendors:

PrepFiler Automated Forensic DNA Extraction Kit (Applied Biosystems)
96-Well Magnetic Ring Stand 6 Tube Magnetic Stand
Heat Transfer Block (Promega)
DNA IQ™ Spin Baskets (Promega)
Vortex Mixer
Allegra x-22R Centrifuge (Beckman-Coulter)
Microfuge (Beckman-Coulter)
Ethanol (Anhydrous-Alcohol)
Isopropanol 99.5%
1.0 M dithiothreitol (DTT)
TE buffer (1X)
Harris Micro-Punch 3 mm or 6 mm tip
1.5 mL microfuge tubes

Sample Preparation

Biological samples covered a range of substrates commonly seen in forensic casework. All biological samples were from human donors (Table 1).

Samples for the Correlation, Precision, Reproducibility and Sensitivity studies were prepared for extraction by one of three methods:

A. Samples were placed in 1.5 mL microfuge tubes and lysed in 300 μ L lysis buffer with 3 μ L 1.0 M DTT. The tubes were incubated using a Thermomixer at 70 °C for 40 minutes at 900 RPM. If the sample matrix included a substrate, the lysate and substrate were transferred to a DNA IQ Spin Basket, and microfuged at 18,000 x g for 2 min.

B. Samples were placed in 1.5 mL microfuge tubes and lysed in 600 μ L lysis buffer with 6 μ L 1.0 M DTT. The tubes were incubated using a Thermomixer at 70 °C for 40 minutes at 900 RPM. If the sample matrix included a substrate, the lysate and

substrate were transferred to a DNA IQ Spin Basket, and microfuged at 18,000 x g for 2 min. The 600 μ L of sample lysate was split into two equal volumes of 300 μ L for manual and automated extraction.

C. Samples were placed in 1.5 mL microfuge tubes and lysed in 900 μ L lysis buffer with 9 μ L 1.0 M DTT. The tubes were incubated using a Thermomixer at 70 °C for 40 minutes at 900 RPM. If the sample matrix included a substrate, the lysate and substrate were transferred to a DNA IQ Spin Basket, and microfuged at 18,000 x g for 2 min.

Reagent Preparation

The PrepFiler Magnetic Particles tube was incubated at 37 °C for 30 minutes prior to use.

180 μ L of Isopropanol 99.5 % was added to sample lysate and particles. The wash step was performed three times using 300 μ L of the PrepFiler Wash Buffer.

The DNA was eluted with 50 μ L of the PrepFiler Elution Buffer.

Sample Matrix	Sample volume	Lysis Buffer Volume [μ L]	Preparation Method	Study
Buccal Swab	One swab	300	A	Correlation
Fingerprint	One swab of fingerprint on pre-cleaned glass	300	A	Correlation
Plastic Bottle Swab	One swab	300	A	Correlation
Toothbrush	1/6 of bristles from one large toothbrush	300	A	Correlation
Liquid Blood	100 μ L	600	B	Correlation
Blood Stain on Denim	200 μ L placed onto denim, dried. 0.25 x 0.5 inch cutting taken	600	B	Correlation
Cigarette butt	1/3 of the paper that covers the filter	600	B	Correlation
Urine Stain	200 μ L placed onto white cloth, dried. 0.5 x 1.5 inch cutting taken	600	B	Correlation
Ear Wax	2/3 of a swab	600	B	Correlation
Feces	0.25 x 0.5 inch cutting	600	B	Correlation
Fingernail	2 mm x 0.5 mm clipping	600	B	Correlation
FTA (Blood and Buccal)	Four 3 mm punches	600	B	Correlation
Hair	Two roots	600	B	Correlation
Semen on Fabric	200 μ L placed onto white cloth, dried. 0.5 x 1 inch cutting taken	600	B	Correlation
Straw	Top 1 mm portion of 2 straws	600	B	Correlation
Tooth	0.25 g powder	600	B	Correlation
Liquid Urine	300 μ L of concentrated urine	600	B	Correlation
Extraction Blank	N/A	600	B	Correlation
Liquid Blood	120 μ L	900	C	Precision and Reproducibility
Liquid Semen	120 μ L	900	C	Precision and Reproducibility
Liquid Saliva	120 μ L	900	C	Precision and Reproducibility
Extraction Blank	N/A	900	C	Precision and Reproducibility
Liquid Blood-0.1 μ L	0.2 μ L	600	B	Sensitivity
Liquid Blood-0.2 μ L	0.4 μ L	600	B	Sensitivity
Liquid Blood-5.0 μ L	10.0 μ L	600	B	Sensitivity
Liquid Blood-30.0 μ L	60.0 μ L	600	B	Sensitivity

Table 1: Sample matrix, volume and sample preparation method for samples used in the Correlation, Precision, Reproducibility and Sensitivity studies

Sample preparation for measuring of precision

120 μL of liquid blood was placed into three, 1.5 mL microfuge tubes containing 900 μL lysis buffer. The same procedure was followed for liquid saliva and extraction blanks. 120 μL of liquid semen was placed into two, 1.5 mL microfuge tubes containing 900 μL lysis buffer. Samples were lysed and split into nine tubes (liquid blood, saliva and extraction blanks) or six tubes (liquid semen). Six of the liquid blood, saliva and extraction blanks tubes and four of the liquid semen tubes were utilized as six and four replicates respectively for the precision study, while three tubes of liquid blood, saliva and extraction blanks and two tubes of liquid semen were saved for the reproducibility study.

Automated extraction using the epMotion 5075 TMX

Sample lysate was transferred from the 1.5 mL microfuge tube to a 2000 μL deepwell plate, for automated extraction. The epMotion 5075 TMX is equipped with a gripper for plate movement, four positions for dispensing tools, twelve deck positions for plates, tips, reservoirs, etc., and a thermomixer (TMX) for heating and shaking plates or tubes. The deck layout design for the PrepFiler Automated Forensic DNA Extraction Kit on the epMotion 5075 TMX is displayed in figure 1.

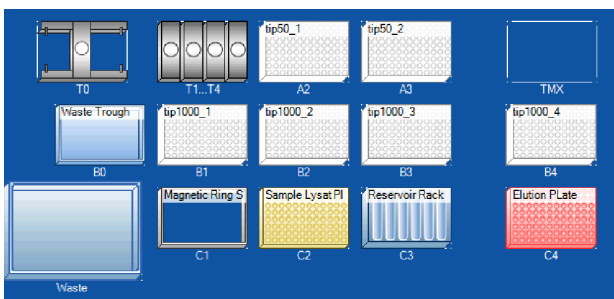


Fig. 1: Screenshot of the deck layout of epMotion 5075 TMX

The TS 50, 50 μL single channel dispensing tool with 1 - 50 μL tips was used to dispense 15 μL of the magnetic particles to each sample well in the sample lysate plate. The sample lysate plate was transferred to the TMX where the beads were mixed for 30 seconds at 800 RPM. The TM 1000, 40 - 1000 μL multi-channel dispensing tool with 40 - 1000 μL tips was used to multi-dispense 180 μL of iso-propanol 99.5 % to each sample well of the sample lysate plate. The plate was mixed for 10 minutes at 1000 RPM then transferred to the magnetic ring stand for 10 minutes to allow the magnetic particles to separate. The TM 1000, 40-1000 μL multi-channel dispensing tool with 40 - 1000 μL tips was used for the wash step (removing the supernatant to the waste trough and adding 300 μL of Wash Buffer to each sample well). After the supernatant was removed, the sample lysate plate was transferred to the TMX where the Wash Buffer was added and mixed for 1 minute at 1000 RPM.

The sample lysate plate was transferred back to the magnetic ring stand for 5 minutes to allow the magnetic particles to separate, prior to removing the supernatant. The wash step was performed a total of three times. The sample lysate plate was transferred to the TMX and incubated at room temperature for 5 minutes at 1000 RPM to allow evaporation of any remaining Wash Buffer. The TM 50, 1-50 μL multi-channel dispensing tool with 1-50 μL tips was used to dispense 50 μL of Elution Buffer to each sample well of the sample lysate plate. The plate was incubated at 75 $^{\circ}\text{C}$ at 1000 RPM for 7 minutes and 30 seconds. The plate was transferred to the magnetic ring stand for 7 minutes to allow the magnetic particles to separate before the TM 50, 1 - 50 μL multi-channel dispensing tool with 1 - 50 μL tips was used to transfer the 50 μL of eluate to the elution plate. The DNA extracts were stored at 4 $^{\circ}\text{C}$ for short term storage and -20 $^{\circ}\text{C}$ for long term storage.

Aspiration, dispense and mix speeds were all optimized to allow accurate volume transfer, minimum disruption to the magnetic particle ring, and to prevent cross-contamination from aerosolization of the liquids.

Manual Extraction

Samples were extracted manually in 1.5 mL microfuge tubes using the PrepFiler Automated Forensic DNA Extraction Kit in addition to using the phenol-chloroform method and QIAamp[®] Viral RNA Mini Kit (Qiagen) where mentioned. Manufacturer's recommended protocols were followed for each extraction kit, with the exception of the hair, tooth and fingernail samples which were lysed using the PrepFiler Automated Forensic DNA Extraction Kit with 300 μL of lysis buffer and incubated on the thermomixer for 40 minutes at 70 $^{\circ}\text{C}$ and 900 RPM.

Quantification of DNA

The Quantifiler[®] Human DNA Quantification Kit (Applied Biosystems) was used in conjunction with an Applied Biosystems 7000 Real-Time PCR System to quantify the DNA. Half reactions were utilized following manufacturer's recommended protocols. The data was analyzed using the SDS software v1.2.3 (Applied Biosystems).

STR Analysis

The DNA extracts were amplified using the Identifiler[®] PCR Amplification Kit (Applied Biosystems) in conjunction with an Applied Biosystems GeneAmp[®] 9700 thermal cycler. One-fifth reactions were utilized following manufacturer's recommended protocols. An Applied Biosystems 3130xl Genetic Analyzer was used for electrophoresis. The resulting data was analyzed using GeneMapper[®] ID Software v3.2.1 (Applied Biosystems).

Results and Discussion

Correlation

The correlation study consisted of two different studies. The first study compared the STR profiles obtained from the PrepFiler Forensic DNA Extraction Kit to the STR profiles obtained from the phenol-chloroform method. The second study compared the DNA yields and STR profiles of samples manually extracted using the PrepFiler Automated Forensic DNA Extraction Kit to those extracted on the epMotion 5075 TMX.

PrepFiler Automated Forensic DNA Extraction Kit vs. Phenol-Chloroform Method

Sixteen different sample matrices plus extraction blanks were extracted in duplicate both manually and on the epMotion 5075 TMX using the PrepFiler Automated Forensic DNA Extraction Kit, in addition to the phenol-chloroform method. Fourteen of the sample matrices resulted in comparable STR profiles between all three extraction methods. One sample matrix, cigarette butt, resulted in a more complete STR profile using the epMotion in comparison to the other methods. One sample matrix, liquid urine, resulted in a

to determine if the performance of the epMotion 5075 TMX was comparably to that of a manual extraction. Samples lysed in 600 μ L of lysis buffer were split into two equal volumes of 300 μ L for manual and automated extraction. The DNA yields obtained from the automated method were comparable to those obtained from the manual method, with the exception of a single sample (“BS on Denim”). Results are summarized in Table 3/Figure 2.

The automated method was observed in its entirety and it was noted that on occasion, when the pipette tips are applied to the pipette tool, some of the tips are applied at a slight angle. In all instances with the exception of one, this did not cause a problem. In a single instance however, the angle of the tip was great enough that it disrupted the particles during the mix step and subsequently resulted in the loss of particles during each wash step. This particular occurrence was observed during the extraction of one of the “BS on Denim” samples (the duplicate “BS on Denim” sample was extracted without problems). Possible solutions to this problem would be: utilization of a different magnetic stand (peg vs. ring) or partial removal

Sample	Number of Alleles Observed								Number of Alleles Expected
	PrepFiler-Manual		epMotion 5075 TMX		Phenol:Chloroform		QIAamp		
	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2	
Liquid Blood	20	26	26	26	26	26	N/A	N/A	26
Blood Stain on Denim	26	26	26	0	26	26	N/A	N/A	26
Buccal Swab	29	29	29	29	29	29	N/A	N/A	29
Cigarette Butt	0	0	23	19	1	3	N/A	N/A	27
Urine Stain	6	2	2	6	4	7	N/A	N/A	26
Ear Wax	24	18	24	29	25	24	N/A	N/A	26
Fingernail	21	26	25	18	21	26	N/A	N/A	26
Fingerprint	0	2	0	0	0	0	N/A	N/A	27
FTA Blood	28	28	28	28	23	27	N/A	N/A	28
Hair	28	28	28	15	25	26	N/A	N/A	28
Plastic Bottle Swab	1	16	10	15	16	0	N/A	N/A	26
Semen on Fabric	29	29	29	30	29	29	N/A	N/A	29
Straw	26	26	26	26	26	23	N/A	N/A	26
Tooth	29	29	29	29	29	29	N/A	N/A	29
Toothbrush	26	14	26	0	21	26	N/A	N/A	26
Liquid Urine	4	2	2	0	2	1	22	25	26

Table 2: STR results for the PrepFiler Forensic DNA Extraction Kit vs. Phenol-Chloroform Method

more complete STR profile using the QIAamp® Viral RNA Mini Kit. Results are summarized in Table 2.

Manual vs. Automated Extraction Method (epMotion 5075 TMX)

DNA from two sets of various sample matrices were extracted both manually and on the epMotion 5075 TMX

of the wash buffer with the large (40-1000 μ L) tips, followed by removal of the remaining 50 μ L with the smaller (1-50 μ L) tips.

It has been determined that the performance of the epMotion 5075 TMX is comparable to that of the manual extraction method.

Data Set 1			Data Set 2		
Sample	Concentration of DNA ng/μL		Sample	Concentration of DNA ng/μL	
	Manual	epMotion 5075 TMX		Manual	epMotion 5075 TMX
Extraction Blank	0.00	0.00	Extraction Blank	0.00	0.00
Fingerprint	0.00	0.00	Fingerprint	0.00	0.00
PlasticBottleSwab	0.00	0.00	PlasticBottleSwab	0.02	0.17
UrineStain	0.00	0.01	UrineStain	0.00	0.01
LiquidUrine	0.01	0.03	LiquidUrine	0.00	0.00
Feces	0.35	0.16	Feces	0.04	0.00
CigButt	0.15	0.12	CigButt	0.03	0.07
FTA Buccal 1	0.03	0.06	FTA Buccal 1	0.33	1.22
EarWax	0.32	0.45	EarWax	0.16	0.46
Hair	1.73	2.70	Hair	0.12	0.19
LiquidBlood	1.03	57.28	LiquidBlood	6.83	2.12
FTA Buccal 2	1.10	1.68	FTA Buccal 2	0.19	0.81
Toothbrush	3.22	5.99	Toothbrush	0.05	0.28
Straw	2.80	4.17	Straw	0.55	0.48
Fingernail	2.88	3.18	Fingernail	1.23	1.42
BS on Denim	21.26	4.91	BS on Denim*	11.38	0.08*
FTA Blood 2	5.19	5.19	FTA Blood 2	5.41	5.66
FTA Blood 1	14.05	17.69	FTA Blood 1	16.52	9.22
BuccalSwab	34.81	8.38	BuccalSwab	32.11	14.97
Tooth	62.69	41.26	Tooth	61.82	15.93
Semen On Fabric	652.52	1109.53	Semen On Fabric	876.02	803.68

Table 3. Concentration of DNA (ng/μL) for manual extraction method vs. automated extraction with the epMotion 5075 TMX.

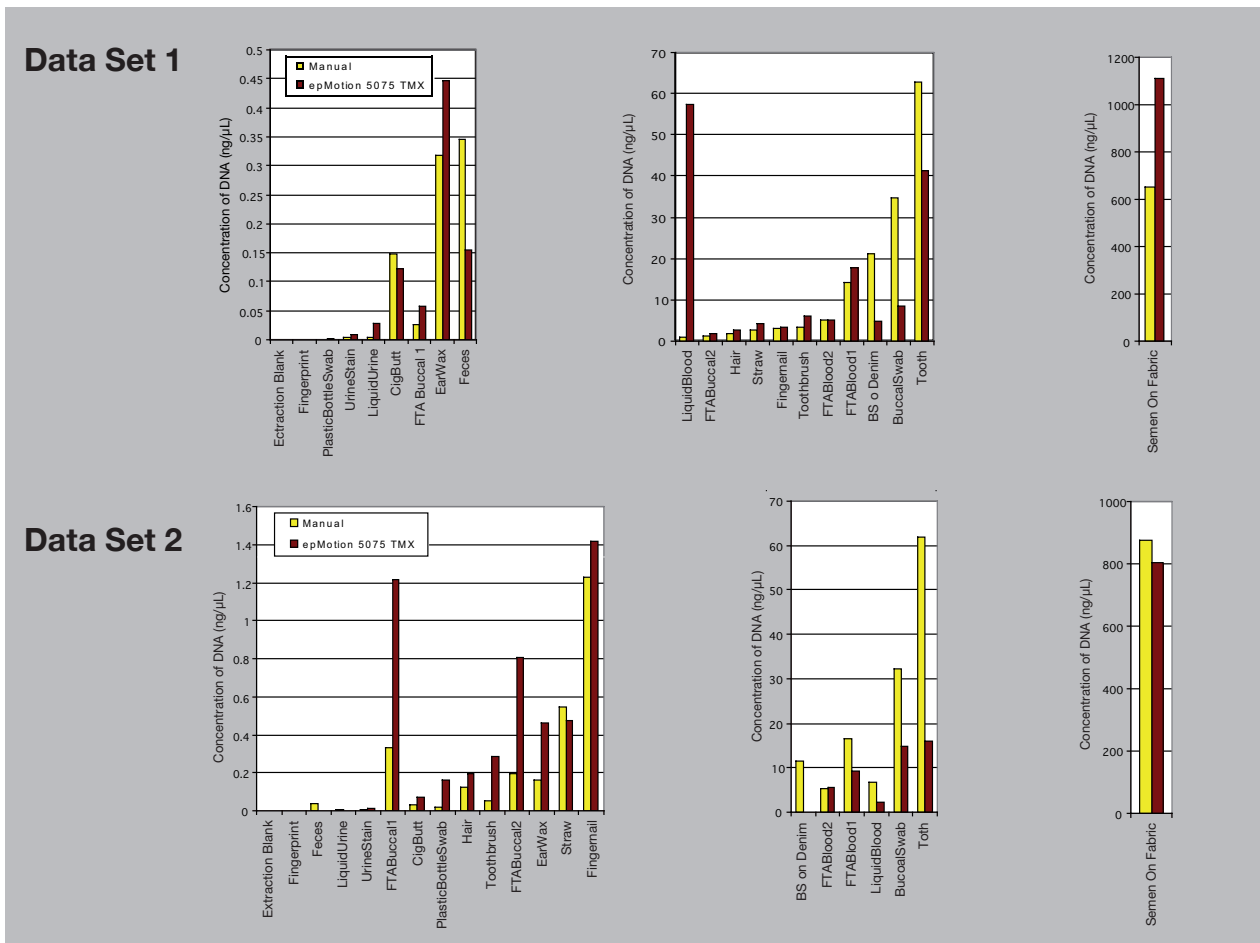


Fig. 2 Concentration of DNA (ng/μL) for manual extraction method vs. automated extraction with the epMotion 5075 TMX.

Precision

A precision study was performed to test the ability of the epMotion 5075 TMX to recover similar DNA yields within a sample set. Six replicates each of liquid blood, saliva and extraction blank, in addition to four replicates of liquid semen were extracted. The samples were quantified for human DNA in duplicate and the average of the two quantification results were utilized for statistical analysis. The results are summarized in Table 4.

Sample Type	No. of Replicates	DNA Concentration (ng/μL)			Standard Deviation
		Minimum	Maximum	Average	
Liquid Blood	6	31.02	42.54	37.32	5.28
Liquid Semen	4	361.71	522.37	413.60	73.52
Liquid Saliva	6	47.10	66.95	53.60	7.49
Extraction Blank	6	0.00	0.00	0.00	0.00

Table 4. Precision study: DNA Concentrations and Standard Deviation

Reproducibility

A reproducibility study was performed to assess the ability of the epMotion 5075 TMX to recover similar DNA yields from replicate extractions. Three replicates each of liquid blood, saliva and extraction blank, in addition to two replicates of liquid semen were extracted in a separate run following the precision study extraction. The samples were quantified for human DNA in duplicate and the average of the two quantification results were utilized for statistical analysis. The results are summarized in Table 5.

The concentration of DNA extracted using the manual method was 0.06 to 18.71 ng/μL, while the concentration for the automated method was 0.01 to 20.27 ng/μL for 0.1 to 30.0 μL of liquid blood, respectively. The results indicate that the magnetic particles are capable of binding/releasing low to high quantities of DNA. In addition, the results indicate that the performance of the epMotion 5075 TMX is comparable to that of the manual method.

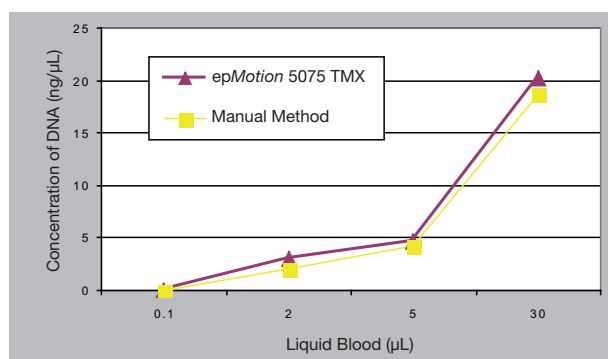


Fig. 3: Sensitivity Study

Sample Type	No. of Replicates	DNA Concentration (ng/μL)			Standard Deviation
		Minimum	Maximum	Average	
Liquid Blood	3	30.38	41.58	35.31	4.28
Liquid Semen	2	370.35	497.14	454.03	58.58
Liquid Saliva	3	40.13	57.17	49.13	6.13
Extraction Blank	3	0.00	0.00	0.00	0.00

Table 5. Reproducibility study: DNA Concentrations and Standard Deviation

Sensitivity

DNA from 0.1, 2.0, 5.0 and 30.0 μL of liquid blood was extracted both manually and on the epMotion 5075 TMX to both determine a range of DNA concentrations that can be reliably processed and to demonstrate that the performance of the epMotion 5075 TMX is comparable to that of the manual method. 600 μL of Lysis Buffer and 6 μL of 1.0 M DTT were added to each of four, 1.5 mL microfuge tubes containing 0.2, 4.0, 10.0 and 60.0 μL of liquid blood respectively. The sample lysate was then divided into two equal volumes, so it could be extracted both manually and on the epMotion 5075 TMX. The results from the manual and automated extraction methods are summarized in Figure 3.

Contamination

To evaluate the potential for cross contamination using the epMotion 5075 TMX, seventeen extraction blanks, across nine different runs, were processed throughout the validation study over thirty-five days. Generally, extraction blanks were placed in the last well after the samples, with the exception of the reproducibility study in which the three extraction blanks were placed in between samples (Figure 4). None of the extraction blanks resulted in detectable quantities of DNA. Extraction blanks that were amplified did not produce STR profiles. The results indicate that the epMotion 5075 TMX is a clean liquid handling system.

	1	2
A		
B	Sample	Sample
C	Extraction Blank	Sample
D	Sample	Sample
E	Sample	Extraction Blank
F	Extraction Blank	Sample
G	Sample	
H		

Fig. 4: Contamination study: Layout of extraction blanks within a plate of samples

Mixture Study

DNA from two known liquid blood samples was extracted on the epMotion 5075 TMX. The samples were normalized to an approximately equal concentration of DNA. The two liquid blood samples were then combined to create the following mixture ratios: 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1.

When the data was analyzed with an interpretation threshold of 50 RFU, 100 % of alleles were observed at all but one mixture (9:1), the mix ratio of 1:9 resulted in 100 % of alleles greater than 50 RFU.

When the data was analyzed with an interpretation threshold of 75 RFU, 100 % of alleles were observed at all but one mix ratio (both 1:9 and 9:1). When the data was analyzed with an interpretation threshold of 125 RFU, 100 % of alleles were observed at 4 mixtures (8:2, 7:3, 6:4 and 5:5).

When the data was analyzed with an interpretation threshold of 50 RFU, 100 % of loci had greater than two alleles observed with the exception of the 9:1 mixture which resulted in 89 % of loci with greater than two alleles observed. When the data was analyzed with an interpretation threshold of 50 RFU, eight of the nine mixtures analyzed resulted in 100 % of the minor alleles observed. The 9:1 mixture resulted in 80 % of the minor alleles observed. The results of the STR profile comparisons are summarized in Tables 6 and 7.

Mix Ratio	Percentage of Alleles >50 RFU	Percentage of Alleles >75 RFU	Percentage of Alleles >125 RFU
1:9	100 %	98 %	89 %
9:1	93 %	91 %	86 %
2:8	100 %	100 %	95 %
8:2	100 %	100 %	100 %
3:7	100 %	100 %	98 %
7:3	100 %	100 %	100 %
4:6	100 %	100 %	98 %
6:4	100 %	100 %	100 %
5:5	100 %	100 %	100 %

Table 6. Percentage of alleles observed above varying RFU levels.

Mix Ratio	Percentage of Loci with >2 Alleles Observed	Percentage of Total Minor Alleles Observed
1:9	100%	100%
9:1	89%	80%
2:8	100%	100%
8:2	100%	100%
3:7	100%	100%
7:3	100%	100%
4:6	100%	100%
6:4	100%	100%
5:5	100%	100%

Table 7. Percentage of loci with greater than 2 alleles observed and percentage of total minor alleles observed.

References

- [1] epMotion 5075 with integrated PC and epBlue Operating Manual Copyright 2008.
- [2] PrepFiler Automated Forensic DNA Extraction Kit *Getting Started Guide*. Part Number 4393917 Rev. B 12/2008.
- [3] PrepFiler Forensic DNA Extraction Kit *User Guide*. Part Number 4390932 Rev. 01 05/2008.
- [4] QIAamp Viral RNA *Mini Handbook*. Second Edition. December 2005.
- [5] Köchl, S., H. Niederstätter, and W. Parson. 2005. DNA Extraction and Quantitation of Forensic Samples Using the Phenol-Chloroform Method and Real-Time PCR, pp. 13-29, in A. Carracedo (ed.), *Methods in Molecular Biology*, Vol. 297, Forensic DNA Typing Protocols. Humana Press, Inc., Totowa, N.J.

Ordering Information Eppendorf

Product	Order no. international	Order no. North America
epMotion® 5075 TMX with integrated PC	5075 000.784	960020444
Dispensing Tool TS 50, 1-50 µL	5280 000.010	960001010
Dispensing Tool TM 50-8, 1-50 µL	5280 000.215	950020318
Dispensing Tool TM 1000-8, 40-1000 µL	5280 000.258	960050088
Gripper	5282 000.018	960002270
epTIPS® Motion 1-50 µL	0030 003.942	960050002
epTIPS® Motion 40-1000 µL	0030 003.985	960050088
Reservoir rack	5075 754.002	960002148
Reservoir 30 mL	0030 126.505	960051009
Reservoir 100 mL	0030 126.513	960051009
96 well Deepwell Plate 96/1000 µL, 20 pc.	0030 501.209	951033081
96 well Deepwell Plate 96/2000 µL, 20 pc.	0030 501.306	951033626
twin.tec PCR plate 96, skirted, 25 pc.	0030 128.648	951020982
Thermomixer® /compact	5350 000.013	022670000
Thermomixer® / comfort	5355 000.011	022670107
Deepwell Plate Adapter with lid	5363 000.012	022670565

Other Ordering Information

Product	Vendor	Order no.
Vortex Mixer	VWR Scientific Products	58816-121
PrepFiler™ Automated Forensic DNA Extraction Kit	Applied Biosystems	4393135
96-Well Magnetic Ring Stand	Applied Biosystems	4392342
6 Tube Magnetic Stand	Applied Biosystems	AM10055
Heat Transfer Block	Promega	V6741
DNA IQ™ Spin Baskets	Promega	V1221
Ethanol (Anhydrous-Alcohol)	VWR Scientific Products	IB15720
Isopropanol 99.5%	VWR Scientific Products	BDH1133-1LP
DL-Dithiothreitol	VWR Scientific Products	IC10059710
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Allegra x-22R Centrifuge	Beckman Coulter	
Microfuge	Beckman Coulter	

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