Applications

Automated DNA Extraction of forensic samples with the DNA IQ™ System on the epMotion® 5075 LH

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Abstract

Forensic DNA laboratories are experiencing rapidly growing demand to process large numbers of evidence samples. In an effort to meet the rising needs for DNA analysis, liquid handling workstations are utilized for the automation of the liquid handling tasks. The epMotion 5075 LH (Eppendorf AG) is a flexible and extremely accurate automated pipetting system capable to extract DNA from forensic casework and reference samples. This study demonstrates that the epMotion 5075 LH used in combination with the DNA IQ System (Promega Corporation) is versatile enough to accommodate the whole spectra of samples encountered by a crime laboratory. The performance of the epMotion LH 5075/DNA IQ System with regard to DNA yields and potential cross-contamination for different sample types was evaluated.

Introduction

In order to automate DNA extraction it is necessary to use a suitable purification method. Organic extraction (phenol/chloroform) not only utilizes hazardous chemicals but also requires multiple centrifugation steps. Chelex extraction is a rapid and relatively cheap method but it can leave PCR inhibitors in the final extract. Purification on silica matrices seems to be the best candidate for automation as the extraction process does not require centrifugation, gives high yield and possible PCR inhibitors are efficiently removed (Greenspoon, 2004).

Promega's DNA IQ system has been chosen for the automation on the Eppendorf epMotion platform due to its ability to rapidly purify small quantities of DNA. It becomes more efficient with samples containing small amounts of DNA (less than 50 pg). The DNA IQ system is widely used for the manual extraction of a broad range of forensic samples, including buccal swabs, blood stains, cigarette butts, sexual assault samples and various types of tissues (Promega Technical Bulletin #TB296).

The procedure for automated DNA extraction using DNA IQ system described below is based on the standard Promega extraction protocol and was adapted for the Eppendorf epMotion 5075 LH automated liquid handling system. Buccal swabs and swabs of dry blood stains were taken using novel swabs introduced by MicroRheologics (Copan Innovation Group). Those swabs were specially designed and certified for forensic use. Magnetic resin employed by the DNA IQ System has a defined DNA capacity in the presence of excess DNA and does only bind a specific amount of DNA. The resulting DNA extracts from both manual and automated extractions were compared using a human specific qPCR. A contamination study revealed no signs of well-to-well contamination during the automated process (extraction blank and checkerboard tests).

Materials and Methods

Consumables

- 4N6 DNA Swab, 4N6 DNA Kit (MicroRheologics, Copan Innovation Group)
- 1,5 ml Eppendorf DNA LoBind tubes
- 2,0 ml Eppendorf DNA LoBind tubes
- Promega DNA IQ System
- Promega DNA IQ Spin baskets
- Absolute ethanol, isopropyl alcohol, DTT

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- Eppendorf epTIPS Motion Filter 1000 µl
- 1,2 ml Eppendorf DeepWell plates
- Bio-Rad iQ™ SYBR Green Supermix
- ABI Quantifiler™ Human DNA Standard (200 ng/µl)
- Promega PowerPlex® 16 System
- Eppendorf twin.tec PCR Plates

Equipment
- epMotion 5075 LH automated pipetting system with gripper
- Dispensing tool TS1000
- Dispensing tool TM1000-8
- Reservoir rack with 30 ml reservoirs
- Thermal module
- Thermorack for 24x 1.5 ml Safe lock tubes
- DeepWell plate Thermoadapter
- Promega Deep Well MagnaBot® 96 Magnetic Separation Device
- MagnaBot Spacer
- Promega MagneSphere® Technology Magnetic Separation Stand 12 position
- Eppendorf Mastercycler ep realplex S
- ABI PRISM 310
- Eppendorf Thermomixer comfort

MicroRheologics swabs are able to absorbe and release an increased volume of sample. More than 80% of the sample trapped is released (Fig.1A). Using conventional swabs, only about 30% is released (Fig.1B).
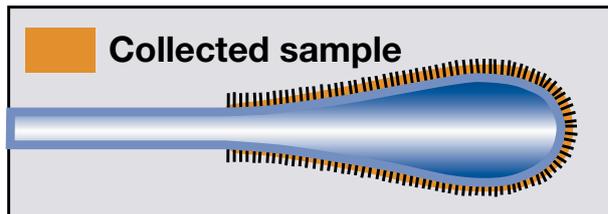
Figure 1A: MicroRheologics swabs for forensic use. More than 80% of the sample analyte is released.
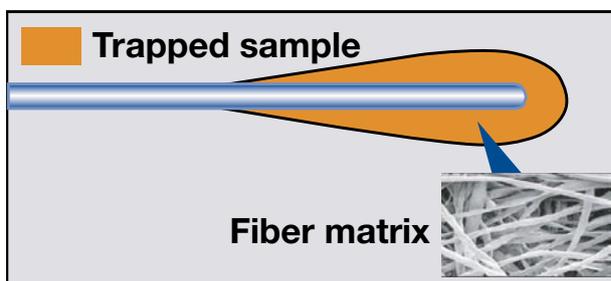
Figure 1B: Conventional swabs for forensic use. Sample dispersion, dilution and entrapment in the fiber matrix. 18 to 30 % of the sample is released.

Eppendorf consumables are PCR clean certified. Eppendorf DNA LoBind tubes significantly improve DNA recovery by reducing sample-to-tube binding (Fig.2).

Sample preparation

- Liquid blood used in this study was obtained from the laboratory staff.
- Cigarette butts used in this study were obtained from the laboratory staff. 6 mm cut was used for all experiments.
- Buccal swabs were taken using the 4N6 DNA Swab from the laboratory staff. Swabs were dried at RT for a minimum of 48 hours.
- Swabs of dry blood stains were taken using the 4N6 DNA Kit. Dry blood stains were prepared by placing 10 µl of the whole blood on the clean surface. Swabs were dried at room temperature for a minimum of 48 hours.
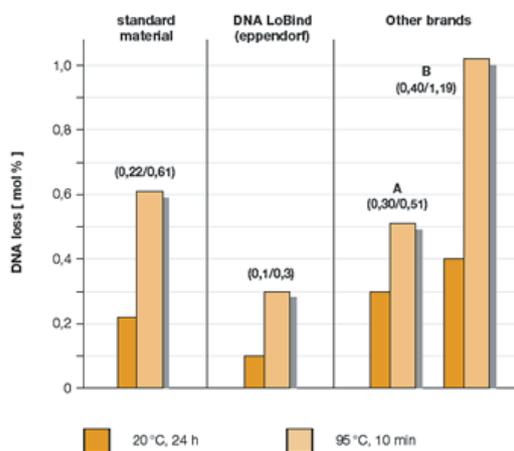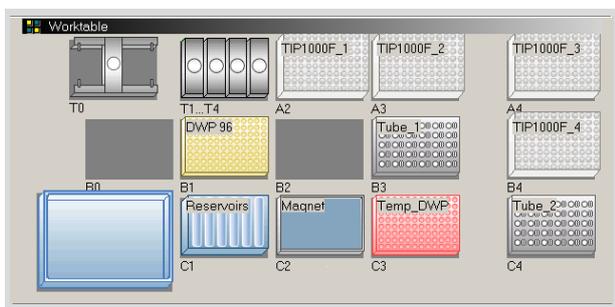
Important note:
4N6 DNA Swab and 4N6 DNA Kit are designed for forensic use and are certified to be free of human DNA, PCR inhibitors and DNase.

Figure 2: DNA loss of different materials under standard low salt conditions. Eppendorf DNA LoBind tubes show a loss of only 0.1 % at 20° C (nearly 100% recovery).

Figure 3: Worktable DNA IQ of the epMotion 5075 LH

Preparing sample lysates

The initial lysis step (95 °C, mixing, 30 minutes) for both manual and automated process was performed on the Thermomixer comfort. 500 µl of DNA IQ Lysis Buffer (with added DTT) was used for all types of samples. Lysed cigarette butt (6 mm cut) samples, buccal swabs on 4N6 DNA Swab, and dry blood swabs on 4N6 DNA Kit samples were centrifuged in the Spin Baskets in order to achieve an optimum recovery.

Isolation of DNA from lysates

Manual extraction using the Promega DNA IQ System was performed according to the manufacturer (Promega Technical Bulletin #TB296).
The same amount of Resin (7 µl) as in the manual process was added to the 1,2 ml Deepwell plate prior to the automated DNA extraction.
The workspace of the epMotion 5075 was equipped with a Reservoir rack and 30 ml reservoirs, gripper tool, 1-channel dispensing tool TS1000, 8-channel dispensing tool TM1000-8, Thermal module, 2x Thermorack 24, DeepWell plate Thermoadapter, MagnaBot 96 Magnetic Separation Device with a spacer, and epTIPS Motion Filter 1000 (Fig.3).
The program starts with a sample transfer from 2 ml tubes into the DeepWell plate with Resin inside, mixing after dispensing, with an additional mixing step in the middle of the incubation.
The lysis buffer was aspirated after placing the plate on the Magnetic Separation Device. One additional wash step with Lysis Buffer and 3 steps with Wash Buffer were performed prior to the drying step (Magnetic Device). The final step comprised Elution Buffer addition, plate transfer on the Thermoadapter, incubation at 65 °C, plate transfer on the Magnetic Separation Device and sample transfer to the clean elution tube or plate.
Note:
To request the epMotion 5075 LH program for automated extraction using the DNA IQ System (Fig.4) please contact Eppendorf at support@eppendorf.com.

No.		Command	
1		NumberOfSamples	variable max:24 ENTER number of samples
2		SampleTransfer	TS_1000, 520µl, pipette, Tube_1 to DWP96_1
3		Temperature	TEMP3, on, keep, 90°C
4		Wait	2min 00sec
5		Mix	TM_1000_8, 2 * 440µl, DWP96_1
6		Wait	2min 00sec
7		Transport	DWP96_1 to C2
8		PoolOneDest	TM_1000_8, 520µl, pipette, DWP96_1 to Tubs_1
9		Transport	DWP96_1 to B1
10		ReagentTransfer	TM_1000_8, 200µl, pipette, Tubs_1 to DWP96_1
11		Transport	DWP96_1 to C2
12		PoolOneDest	TM_1000_8, 260µl, pipette, DWP96_1 to Tubs_1
13		Transport	DWP96_1 to B1
14		ReagentTransfer	TM_1000_8, 200µl, pipette, Tubs_1 to DWP96_1
15		Transport	DWP96_1 to C2
16		PoolOneDest	TM_1000_8, 260µl, pipette, DWP96_1 to Tubs_1
17		Transport	DWP96_1 to B1
18		ReagentTransfer	TM_1000_8, 200µl, pipette, Tubs_1 to DWP96_1
19		Transport	DWP96_1 to C2
20		PoolOneDest	TM_1000_8, 260µl, pipette, DWP96_1 to Tubs_1
21		Transport	DWP96_1 to B1
22		ReagentTransfer	TM_1000_8, 200µl, pipette, Tubs_1 to DWP96_1
23		Transport	DWP96_1 to C2
24		PoolOneDest	TM_1000_8, 260µl, pipette, DWP96_1 to Tubs_1
25		Wait	8min 00sec
26		Transport	DWP96_1 to B1
27		ReagentTransfer	TM_1000_8, 100µl, pipette, Tubs_1 to DWP96_1
28		Transport	DWP96_1 to TEMP3
29		Wait	13min 00sec
30		Transport	DWP96_1 to C2
31		SampleTransfer	TS_1000, 110µl, pipette, DWP96_1 to Tube_2
32		UserIntervention	! End of method

Figure 4: epMotion 5075 LH program for automated extraction using the DNA IQ System

Methods for DNA quality and quantity assessment

The aim of this study was to compare the manual and automated extraction using DNA IQ System in terms of quality and quantity.
The magnetic resin used in DNA IQ System has a defined DNA capacity. In the presence of excess DNA it will bind a specific amount of DNA (100 ng for 7 µl resin). When DNA is eluted into 100 µl of elution buffer, the final DNA concentration thus is approximately 1 ng/µl.
The resulting DNA extracts from both the manual and automated extraction were compared using a human specific assay-qPCR. All sample types (liquid blood, cigarette butt, buccal swab, dry blood swabs) were extracted manually in triplicates and 24 times in the automated process. Quantifications of all DNA extracts were performed in duplicates using the method described by Sifis (Sifis et al., 2002) on Mastercycler ep realplex S. To reproduce the method of Sifis the ramp rates were set to 13% for the annealing step and to 21% for the elongation step.

Real-Time PCR DNA quantification on Mastercycler ep realplex S

oligo ALU (amplicon 229 bp) Alu primers (Sifis et al., 2002):
ALU1: 5'-tgg tgg ctc acg cct gta a-3'
ALU2: 5'-cga tct cgg ctc act gca a-3'

Real-Time PCR reactions:

Final volume 30 µl – 15 µl iQ SYBR Green Supermix
0.15 µl 100 µM primer ALU1 (500 nM)
0.15 µl 100 µM primer ALU2 (500 nM)
0.3 µl 50 mM MgCl$_2$
12.4 µl PCR H$_2$O
2 µl sample DNA

Real-Time PCR program for Mastercycler ep realplex S:

| Temp.	Time	Cycles
94° C	4 min	1
94° C	30 sec	
55° C	45 sec	40
72° C	30 sec	
72° C	7 min	1

Preparation of DNA standards:

• Standard DNA - Quantifiler Human DNA Standard (200 ng/µl). All measurements in duplicates. For dilution TE buffer was used (Tris 10 mM, EDTA 0.1 mM)
• Range of concentrations was from 50 ng DNA/µl (std.1) to 0.023 ng DNA/µl (std.8) (see Table 1)

The standard curve plots and parameters are illustrated in Fig. 5, Fig. 6 and in Tab. 2. The detected C$_t$ of approximately 15 cycles for the lowest DNA concentration (0.023 ng/µl)

demonstrates the extreme sensitivity of the assay at low DNA levels. Corresponding to the findings of Buel (Nicklas and Buel, 2003) for Alu-based quantitation, the no template controls (NTC) also show a C$_t$ in the late cycles. As an assay cutoff all samples with a calculated DNA amount of less than 15 pg/µl were considered to be negative.
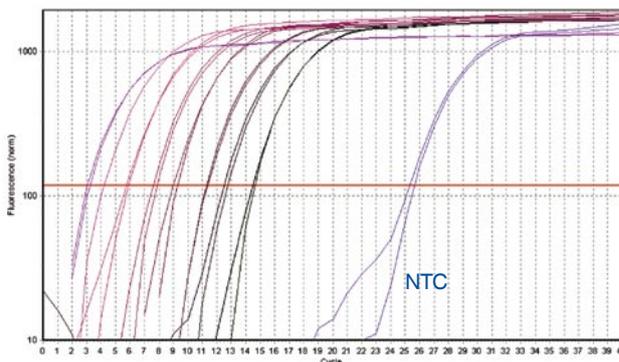
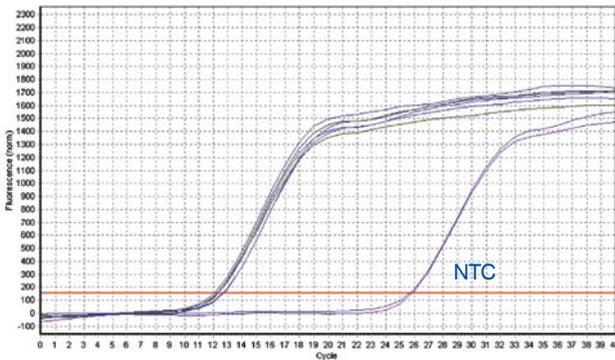**Figure 5:** Amplification Plot – Standard DNA dilutions and controls
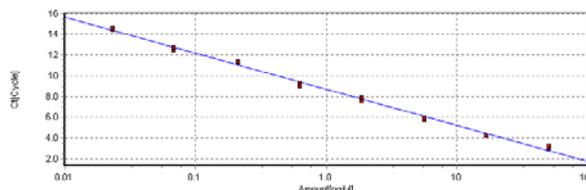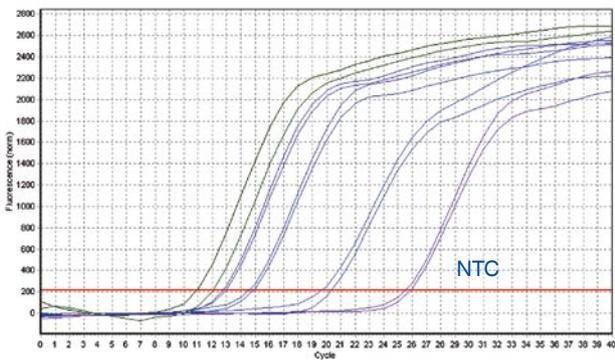

Figure 6: Standard curve

Slope	-3.481
Y-Intercept	8.69
Efficiency	0.94
R^2	0.996

Table 2: Standard curve parameters

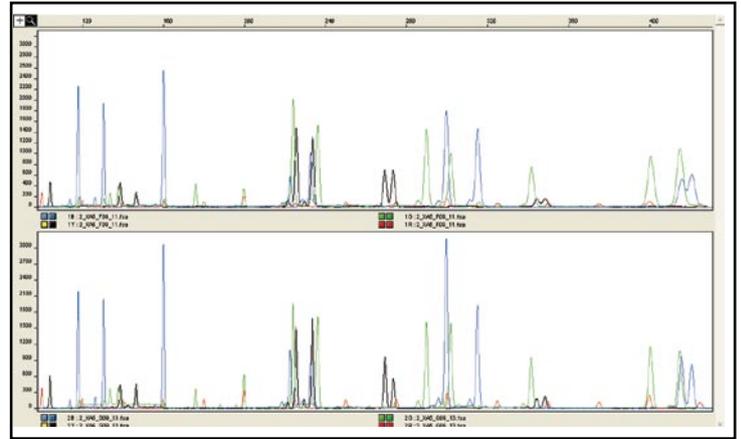| Standard	DNA concentration (ng/µl)	Amount	Dilution factor
std.1	50.000	10 µl (200 ng/µl pool)+30µl buffer	4x
std.2	16.700	10 µl (std.1)+20 µl buffer	3x
std.3	5.560	10 µl (std.2)+20 µl buffer	3x
std.4	1.850	10 µl (std.3)+20 µl buffer	3x
std.5	0.620	10 µl (std.4)+20 µl buffer	3x
std.6	0.210	10 µl (std.5)+20 µl buffer	3x
std.7	0.068	10 µl (std.6)+20 µl buffer	3x
std.8	0.023	10 µl (std.7)+20 µl buffer	3x

Table 1: Preparation of DNA standards

C_t values (corresponding to the expected final DNA concentration of 1 ng/µl) obtained for liquid blood, buccal swabs and dry blood stains showed no significant differences between the manual and automated extraction procedure (Fig. 7). DNA quantity variations were observed for cigarette butts in both manual and automated process (Fig. 8). This finding corresponds with our expectation as this sample type normally contains less than 100 ng of DNA. Diluted liquid blood gave proportionally lower DNA concentrations in extracts. No inhibition was observed for all samples quantified (data not shown).

One sample from each sample group (liquid blood, cigarette butt, buccal swab, dry blood stain) and each process (manual, automated) was typed using the PowerPlex 16 System. 1 µl of the final extract from liquid blood, buccal swab and dry blood stain gave a well balanced profile of similar peak heights and sister peak ratios across all loci (Fig. 9). Cigarette butt extracts were added normalized according to the measured DNA quantity.

Figure 9: PowerPlex 16 profile of DNA extracted from buccal swabs by manual and automated extraction. Top panel: DNA extracted manually using the Promega DNA IQ protocol. Bottom panel: DNA extracted using an epMotion 5075 LH and the automated Promega DNA IQ protocol

Figure 7: Amplification Plot – Liquid blood and controls. Automated and manual samples

Contamination study

All extraction batches contained one extraction negative control per 3 samples (column wise placed samples). Checkerboard contamination test was performed with samples containing high amount of DNA. The Checkerboard contamination test alternated wells containing liquid blood lysates with those containing reagent blanks across the plate in a checkerboard pattern (Fig. 10). This provided a very sensitive approach to detect both row-to-row and column-to-column contamination.

We observed no signs of well-to-well contamination during the whole automated process contamination study.

Figure 8: Amplification Plot – Cigarette butts and controls. Automated and manual samples

	1	2	3	4
A	sample	blank	sample	blank
B	blank	sample	blank	sample
C	sample	blank	sample	blank
D	blank	sample	blank	sample
E	sample	blank	sample	blank
F	blank	sample	blank	sample
G	sample	blank	sample	blank
H	blank	sample	blank	sample

Figure 10: Checkerboard pattern

Conclusion

Automated DNA extraction from forensic samples makes a real opportunity for the laboratories to speed up the processing time and thus to reduce backlogs. The System used for automation must be flexible enough to accommo- date different sample types, causing no risk of contamina- tion, and should be easy to operate. Automated DNA Extraction using the DNA IQ System on the Eppendorf epMotion 5075 LH meets all these criteria.

References

Literature
[1] Greenspoon SA., et al., Application of the Biomek 2000 Laboratory Automation Workstation and the DNA IQ System to the Extraction of Forensic Casework Samples. J Forensic Sci. 2004 Jan; 49(1)
[2] Sifis ME., Both K., Burgoyne LA., A more sensitive method for the quantitation of genomic DNA by Alu amplification. J Forensic Sci. 2002 May; 47(3)
[3] Nicklas JA., Buel E., Development of an Alu-based, Real-Time PCR Method for Quantitation of Human DNA in Forensic Samples. J Forensic Sci. 2003 September; 48(5)

Protocols
[1] Instrument Manual for the epMotion 5075 LH
[2] DNA IQ System – small sample casework protocol. Promega Technical Bulletin #TB296.

Ordering information

Article	Description	Order no. international	Order no. North America
epMotion® 5075 LH 230 V		5075 000.008	n/a
epMotion® 5075 LH 120 V		n/a	960020006
Mastercycler ep *realplex*^{4} S 230V		6302 000.601	n/a
Mastercycler ep *realplex*^{4} S 120V		n/a	950020318
Dispensing tool TM 1000-8		5248 000.050	920010521
Dispensing tool TS 1000		5280 000.053	960001036
Gripper		5282 000.018	960002270
Holder for Gripper		5075 759.004	960002211
Reservoir Rack		5075 754.002	960002148
Reservoirs 30 ml	(10 x 5 reservoirs in bags/cases, PCR clean)	0030 126.505	960051009
Reservoirs 100 ml	(10 x 5 reservoirs in bags/cases, PCR clean)	0030 126.513	960051017
epTIPS Motion 1000 µl Filter		0030 003.993	960050100
twin.tec PCR Plate 96, skirted, green		0030 128.664	951020443
Thermoadapter DWP 96		5075 751.054	960002391
Thermorack 24		5075 771.004	960002075

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