

Automated DNA extraction by Eppendorf epMotion 5075 LH workstation



Hajkova J.¹, Saskova L.¹, Wehrhahn D.², Vanek D.¹



¹Forensic DNA Service, <http://DNA.com.cz>, Janovskeho 18, 170 00 Prague 7, Czech Republic

²Eppendorf AG, 22331 Hamburg, Germany
jana.hajkova@dna.com.cz

Introduction

As the forensic DNA laboratories are experiencing rapidly growing demand to process large number of evidence samples, robotic workstations are utilized for the automation of the liquid handling needs and enable to speed up the samples processing. The epMotion 5075 LH (Eppendorf AG) is flexible and extremely accurate robotic platform capable to extract DNA from forensic casework and reference samples. This study demonstrates the application of two manual protocols (Invitrogen – ChargeSwitch, Promega – DNA IQ) for DNA extraction from forensic samples on robotic liquid handling workstation epMotion 5075 LH (Eppendorf AG). Both extraction protocols are based on use of magnetic particles which bind DNA from the lysed sample. The aim was to compare and contrast these two protocols in terms of DNA extraction efficiency from different sample types, DNA yields and potential cross-contamination during automated extraction process.

Methods

Obtaining samples for testing using 4N6 DNA Swabs (buccal swabs) and cigarette butts

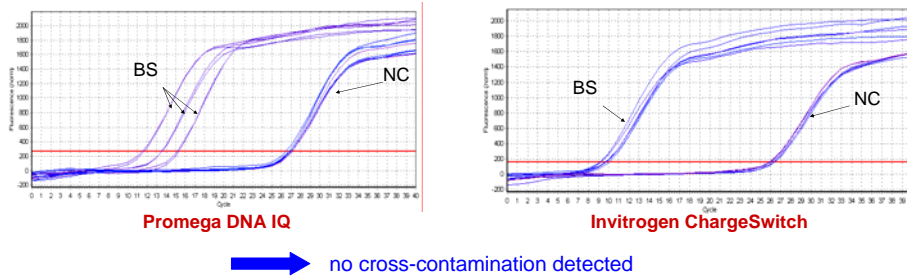
Automated DNA extraction from samples and negative controls using epMotion 5075 LH and Promega DNA IQ / Invitrogen ChargeSwitch extraction protocol

Quantitation of DNA using qRT-PCR Mastercycler ep realplex S, SYBR Green/Alu quantification system (Biorad); in duplicates

Results

Cross-contamination study – checkerboard test (Figure 1, Table 1)

Fig.1 – Amplification plot of DNA extracted from buccal swabs(BS) and negative control samples(NC); duplicates



Tab.1- Checkerboard test

| Checkerboard pattern | | | |
|----------------------|--------|--------|--------|
| ng/dl DNA | 1 | 2 | 3 |
| A | sample | blank | sample |
| B | blank | sample | blank |
| C | sample | blank | sample |
| D | blank | sample | blank |

| ng/dl DNA | 1 | 2 | 3 |
|-----------|-------|-------|-------|
| A | 0.074 | 0 | 0.244 |
| B | 0 | 0.023 | 0 |
| C | 0.075 | 0 | 0.278 |
| D | 0 | 0.028 | 0 |

| ng/dl DNA | 1 | 2 | 3 |
|-----------|------|------|------|
| A | 0.03 | 0 | 1.05 |
| B | 0 | 1.13 | 0 |
| C | 0.35 | 0 | 0.95 |
| D | 0 | 0.91 | 0 |

Promega DNA IQ Invitrogen ChargeSwitch

Promega DNA IQ x Invitrogen ChargeSwitch - comparison of extraction protocols (Figure 2, 3)

Fig. 2 – Amplification plot of DNA extracted from cigarette butts and negative control samples(NC); duplicates, 1- ChargeSwitch extraction protocol, 2- DNA IQ extraction protocol

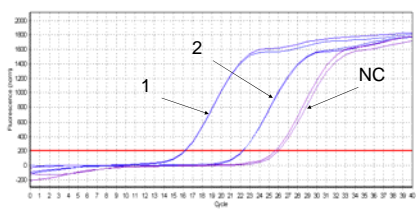
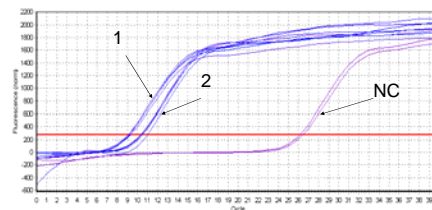


Fig. 3 – Amplification plot of DNA extracted from buccal swabs and negative control samples(NC); duplicates, 1- ChargeSwitch extraction protocol, 2- DNA IQ extraction protocol



big differences in ability of DNA extraction from samples with small amount of DNA (cigarette butts)

lower differences in ability of DNA extraction from samples with standard amount of DNA (buccal swabs)

Conclusions

Invitrogen ChargeSwitch extraction protocol is more flexible to isolate DNA from samples containing different amount of DNA – from pg to ng - in comparison with Promega DNA IQ extraction protocol. The differences between these protocols are more visible by isolating DNA from samples with little amount of DNA (cigarette butts). Both protocols successfully passed the checkerboard cross-contamination tests, that is why they are highly recommended for DNA extraction from forensic samples on robotic liquid handling workstation epMotion 5075 LH.

Acknowledgements

This work has been supported in part by Research grant No. FI-IM3/115 from Ministry of Industry and Trade, Czech Republic