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Automated extraction of DNA from clothing

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ABSTRACT

Presence of PCR inhibitors in extracted DNA may interfere with the subsequent quantification and short tandem repeat (STR) reactions used in forensic genetic DNA typing. We have compared three automated DNA extraction methods based on magnetic beads with a manual method with the aim of reducing the amount of PCR inhibitors in the DNA extracts and increasing the proportion of reportable DNA profiles.

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1. Introduction

Common to the majority of all forensic genetic analytical methods performed today is the use of short tandem repeats (STRs) to provide discriminatory power [1]. However, major differences exist in the extraction methods of the DNA from trace evidence material prior to amplification. Clothing, and especially some dyes used in the manufacturing process, are known to give downstream problems following DNA extraction as the dyes may be co-extracted and act as inhibitors in the subsequent PCR reactions [2]. Here, we evaluate three different automated extraction protocols and compare the results with manual Chelex extraction [3].

2. Materials and methods

2.1. Samples

Clothing items from crime cases with at least two previously unprocessed stains of biological material were selected. From each item, two stains were isolated by manually cutting out the stains with scalpels. Each stain was subsequently divided into four even parts. Item parts from the two stains were combined in the same 1.5 mL sample tube (Eppendorf) resulting in four tubes each containing two item parts for each item. Stains from 120 items were collected.

2.2. Manual Chelex®

Following addition of 1 mL 20% Chelex solution (Chelex® 100 Resin, 100–200 mesh Na-form, BIO-RAD Laboratories), sample tubes were incubated for 1 h at 56 °C in shaking incubators (VorTemp 56, Labnet International, Inc.) followed by 8 min at 100 °C in a heating block (SBH200D, Stuart, Bibby Scientific Limited) and centrifuged for 1 min at 16,200 rpm in a Heraeus Biofuge Pico bench top centrifuge (Heraeus). The supernatant was transferred to Amicon Ultra 4 100K tubes (Millipore) containing 1.5 mL sterile RNase and DNase free water (Sigma–Aldrich) and centrifuged for 10 min at 4000 RCF in a centrifuge (Heraeus Megafuge). Following addition of 1.5 mL sterile RNase and DNase free water to the Amicon tubes, the tubes were centrifuged for 10 min at 3000 RCF. DNA extracts (30 μL) were transferred to 1.5 mL sample tubes (Eppendorf).

2.3. DNA extraction on QIAsymphony SP with Chelex pre-treatment

Samples were pre-treated according to the Chelex protocol with modifications. In brief, 800 μL 20% Chelex solution was added to sample tubes containing trace items. Following incubation and centrifugation with conditions as described above, the supernatants were transferred to 2 mL sample tubes (Eppendorf), loaded into the QIAsymphony SP (Qiagen) and extracted using the QIAsymphony DNA Investigator Kit (Qiagen) and the CW_500_H2O_V4 protocol. DNA extracts were eluted in 50 μL water.

2.4. DNA extraction on QIAsymphony SP

Samples were added 475 μL ATL buffer and 25 μL proteinase K (Qiagen). Following incubation for 15 min at 56 °C and 900 rpm in
a shaking incubator, supernatants excluding any solids were transferred to 2 ml Sarstedt tubes (Sarstedt) and extracted using the QIA symphony as previously described.

2.5. DNA extraction on AutoMate Express™

Trace items were placed in the LySep™ columns placed on top of lysate collection tubes included in the PrepFiler Express™ Forensic DNA Extraction Kit (Applied Biosystems (AB)) with sterile tweezers. To each sample, 500 µL freshly prepared lysis solution consisting of 500 µL PrepFiler Lysis Buffer and 5 µL freshly prepared 1 M Dithiothreitol (DTT) (Sigma–Aldrich) was added. Following incubation of the LySep columns for 40 min at 70 °C in a shaking incubator at 750 rpm, the columns were centrifuged for 2 min at 10,300 RCF. Following removal of the LySep column, the lysate collection tube was placed in the AutoMate Express instrument and extracted immediately using the PF Express program. DNA extracts were eluted in 50 µL water.

2.6. STR amplification and analysis

Extracted DNA was quantified using the Quantifiﬁer™ Human DNA Quantitation Kit (AB). Quantification reactions were prepared on Tecan HID EVOlution qPCR/STR systems using an in-house customized version of the Tecan supplied pre-verified Quantifiﬁer-Human tubes script as previously described [4].

Samples with DNA concentrations below 20 pg/µL were not processed. Samples with concentrations between 20 pg/µL and 50 pg/µL were ampliﬁed using 200 pg of DNA per reaction. Samples with DNA concentrations greater than 50 pg/µL were ampliﬁed using 500 pg of DNA. PCR setup was accomplished with Tecan HID EVOlution qPCR/STR systems using an in-house customized and validated version of the pre-verified SeﬁllerPlus Tubes_V1_SPS Seﬁller script and the AmpFiSTR™ Seﬁller Plus™ PCR Ampliﬁcation kit (AB). PCR with 29 cycles was performed in 96-well microliter plates (Eppendorf) on Gold-plated 96-well GeneAmp™ PCR systems 9700 (AB).

3. Results and discussion

Following quantification, some samples had undetermined Ct-values for the Internal PCR Control (IPC). These were considered inhibited. Inhibited samples were diluted fourfold and re-quantified. The number of inhibited samples for the different extraction methods was 70%, 1%, 8% and 0% for extractions with Chelex, Chelex-QIA symphony, PrepFiler Express and QIA symphony, respectively. Samples that were still inhibited following dilution and re-quantification were stopped from further processing. The number of inhibited samples following re-quantification was 51%, 0%, 3% and 0%.

Following analysis of the obtained STR proﬁles, the results were divided into categories based on the interpretation of the obtained proﬁle [Fig. 1]. The extraction method providing the highest amount of reportable proﬁles was the PrepFiler Express (90 out of 120). The extraction method providing the lowest amount of reportable proﬁles was the QIA symphony with the sample pre-treatment protocol recommended by Qiagen (49 out of 120). Interestingly, the modiﬁed sample pre-treatment protocol (Chelex-QIA symphony) resulted in a 47% increase of reportable proﬁles (49 vs. 72 out of 120) when compared to the recommended protocol.

4. Conclusion

The highest number of reportable proﬁles was achieved with the PrepFiler Express kit. The number of inhibited samples was highest for manual Chelex based extracts and lowest for samples extracted using the QIA symphony with the lysis recommended by Qiagen.

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Conflict of interest

None.

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References