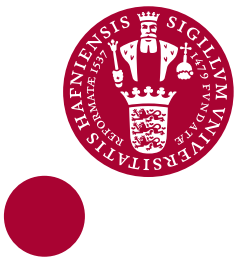

Purification and Concentration of PCR Products Leads to Increased Signal intensities with Fewer Allelic Drop-Outs and Artifacts

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Purification and concentration of PCR products leads to increased signal intensities with fewer allelic drop-outs and artifacts

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ABSTRACT

Capillary electrophoresis of amplified DNA isolated from trace evidence samples occasionally results in inadequate STR-profiles due to artifacts caused by, e.g. primers and dNTPs. Removal of artifacts by purification and subsequent concentration of the PCR products may increase the sensitivity and the quality of the DNA profiles without re-amplification of the sample. We have validated and implemented an automated method to purify and 2-fold concentrate PCR products resulting in allelic peaks with higher intensity (a median height across all loci from 130 to 404 RFU), fewer allelic drop-outs and a reduced number of artifacts compared to both an increase in injection time and increase in the number of amplification cycles.

1. Introduction

Occasionally, DNA isolated from trace evidence samples results in inadequate STR-profiles due to the interference of PCR related artifacts. Post-PCR purification will remove the non-reacted components and improve the analytical sensitivity, due to the relatively larger amount of PCR product injected into the capillary [1].

We have validated and implemented an automated method to purify PCR products enabling greater allelic intensities without the demand of additional sample material, which is often limited in crime case investigations.

2. Materials and methods

2.1. Sample material

Three sample set-ups were prepared: (1) DNA was extracted from 34 trace evidence samples. Samples were chosen due to their challenging initial STR-results, i.e. inhibition or weak signal intensities with allelic and/or locus drop-outs. Sample materials included gloves, paper, cigarette butts, finger nail scrapes, cotton swabs, etc. (2) A total of 100 pg DNA from a known control was amplified and subsequently diluted 20, 25, 27.5, 30 and 32.7 times with a negative PCR control. (3) DNA from two known persons was prior to amplification diluted to 56, 30 and 15 pg and amplified in duplicate.

2.2. PCR and purification

All samples were amplified with the AmpFℓSTR® SEfiler Plus™ Amplification Kit (Applied Biosystems (AB), Foster City, CA, USA). Samples were amplified with 29 amplification cycles in set-ups one and two, and both 28 and 29 amplification cycles in set-up three. Amplifications were performed on Gold-plated 96-well GeneAmp® PCR systems 9700 (AB). Amplificates were purified and 2-fold concentrated (subsequently denoted post-PCR purification) using the MinElute PCR Purification kit (Qiagen, GmbH, Hilden, Germany) on a QIAcube automated spin-column processing instrument (Qiagen) using an in-house optimized protocol. In brief, 20 μl of each amplificate plus 580 μl PB buffer (Qiagen) was transferred to spin-columns. Following centrifugation, samples were washed twice with 600 μl PE buffer (Qiagen). Finally, samples were eluted in 10 μl EB buffer (Qiagen).

2.3. Electrophoresis and data analysis

All amplificates were analyzed on ABI 3130*xl* DNA sequencers (AB) before and after purification. The injection time varied according to the nature of the experiment. Results were analyzed with GeneScan 3.7 and GenoTyper 3.7 (AB).

3. Results and discussion

Post-PCR purification of 34 trace evidence samples resulted in reduced primer peaks (Fig. 1A and B) and a 2-fold increase in the median number of detected allelic peaks from 12 to 25 (Fig. 1C and D). The signal intensities increased from a median of 130 relative

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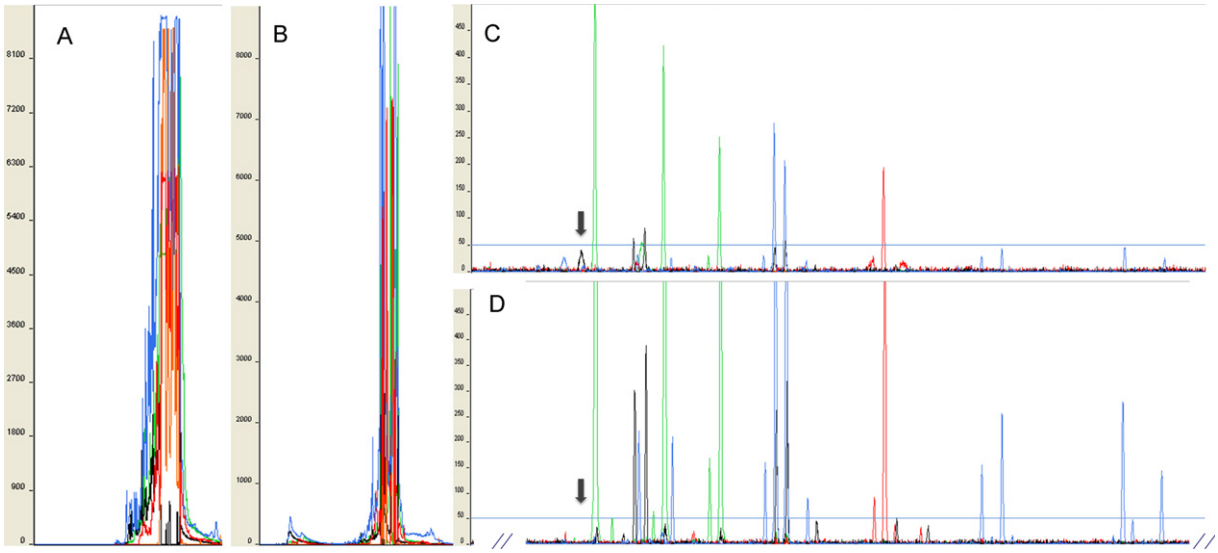

Fig. 1. Non-purified (A and C) and purified (B and D) amplificates from a trace evidence sample. A and B: Primer peaks. C and D: Electropherograms with signal intensities in RFU. The blue lines represent 50 RFU that is the lower threshold for STR-typing at our department. The arrow in C indicates the presence of a fluorochrome derived artifact. Arrow in D indicates the position of the artifact if not purified.

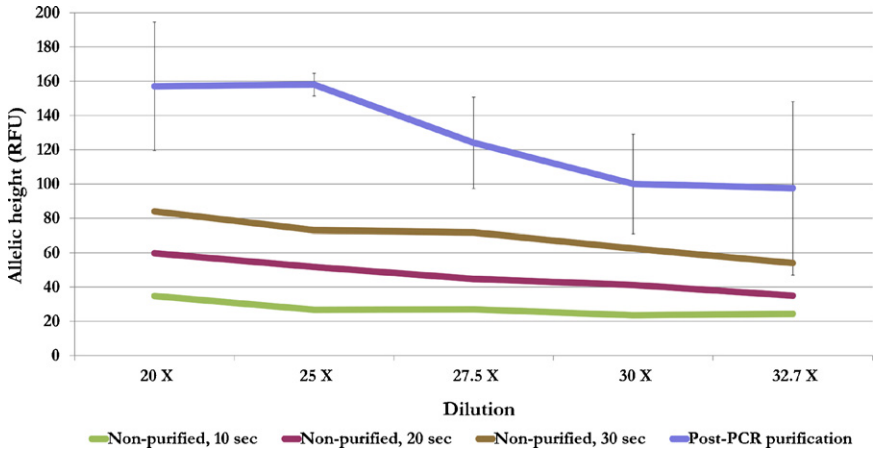

Fig. 2. The average signal intensities across all loci (RFU) pre- and post-PCR purification, ±std. dev. of non-purified ($n = 1$) diluted amplificates electrophoresed with injection times of 10, 20 and 30 s compared to purified ($n = 4$) amplificates electrophoresed with 10 s injection time.

fluorescence units (RFU) (ranging from 70 to 523 RFU) to 404 RFU (ranging from 128 to 1,941 RFU) across all loci. Post-PCR purification reduced fluorochrome derived artifacts as indicated by arrows in Fig. 1C and D.

An increase in the allelic intensities can be achieved by other approaches, as increasing the injection time and/or increasing the number of amplification cycles, wherefore post-PCR purification was compared to both of these approaches. Post-PCR purification electrophorated with an injection time of 10 s resulted in allelic peaks with higher signal intensities than non-purified amplificates electrophorated with injection times of 10, 20 and 30 s (Fig. 2). In addition, purified amplificates had fewer artifacts than non-purified (data not shown). Furthermore, samples amplified at 28 cycles and subsequently purified, yielded STR-profiles with greater allelic signal intensities with fewer allelic drop-outs compared to those of non-purified samples amplified at 29 cycles (data not shown).

4. Conclusion

We demonstrated that post-PCR purification and 2-fold concentration of amplificates reduced the primer peaks, increased the signal intensities of allelic peaks and resulted in fewer allelic drop-outs and fewer artifacts. The post-PCR purification method yielded better results compared to both an increase in the injection time and increase in the number of amplification cycles. Post-PCR purification and 2-fold concentration can be automated enabling reproducible and standardized processing and does not require the use of additional sample material. The in-house developed protocol for the QIAcube is freely available.

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None.

Conflict of interest statement

None.

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Reference

[1] P.J. Smith, J. Ballantyne, Simplified low-copy-number DNA analysis by post-PCR purification, J. Forensic Sci. 52 (2007) 820–829.