# Appearance of allelic drop-out in STR-multiplex amplified samples after capillary electrophoresis can be avoided by removal of residual primers

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## Abstract

In this study, the effect of sample purification on total signal intensities of genRES ® MPX-2 (nine-locus multiplex) amplified DNA prior to capillary electrophoretic analysis has been investigated. Sample purification with the QIAQUICK PCR purification kit led to an increase of the relative fluorescent signal intensity by a factor of $3.8 \pm 0.8$. In addition, increase of injection time showed a linear increase of signal intensity between 3 and 10 s. Higher injection times may influence electrophoretic fragment separation and, therefore, lead to broad signals which may not be sized correctly.
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## 1. Introduction

Due to reproducibility of fragment sizing, high sensitivity and the possibility of considerable automatisation, capillary electrophoresis (CE) is a widely used technique for the sizing of PCR-amplified short tandem repeat (STR) polymorphisms [1–3]. Nevertheless, analysis of microspecimen containing limited amounts of DNA may lead to allelic drop-out phenomena or missing signal detection. Because it is well known that the conductivity of applied samples greatly influences the electrokinetic injection of fragments [4], it was obvious to postulate that purification of samples might enhance the efficiency of fragment injection. Therefore, in this study the influence of sample purification has been investigated systematically with regard to signal intensities of samples containing confined DNA amounts.

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## 2. Material and methods

PCR was performed using the genRES® MPX-2 Amplification Kit (Serac) with a 5 µl aliquot (<100 pg) of Chelex-extracted sample DNA in a total volume of 50 µl. Parameters were: Hot start (12 min, 95 °C), 30 cycles (1 min, 93 °C; 1 min, 59 °C; 1 min 30 s, 72 °C), final step (45 min, 60 °C). A total of 30 µl was purified using the QIAquick PCR Purification Kit (Qiagen) according to the manufacturer's instructions (eluted volume: 30 µl). Prior to electrophoresis samples were prepared as follows: 1 to 5 µl amplified DNA, 12 µl formamide and 0.5 µl of internal lane standard LS 500 ROX (Serac). Detection of signals was performed with the ABI Prism 310 Genetic Analyzer according to the manufacturer's instructions (run module GS STR POP-4 F).

## 3. Results

### 3.1. Sample purification

Purification of amplified samples resulted in an increase of allelic signal intensities by a factor of $3.8 \pm 0.5$, independent of the total fragment length. In Fig. 1, two identical
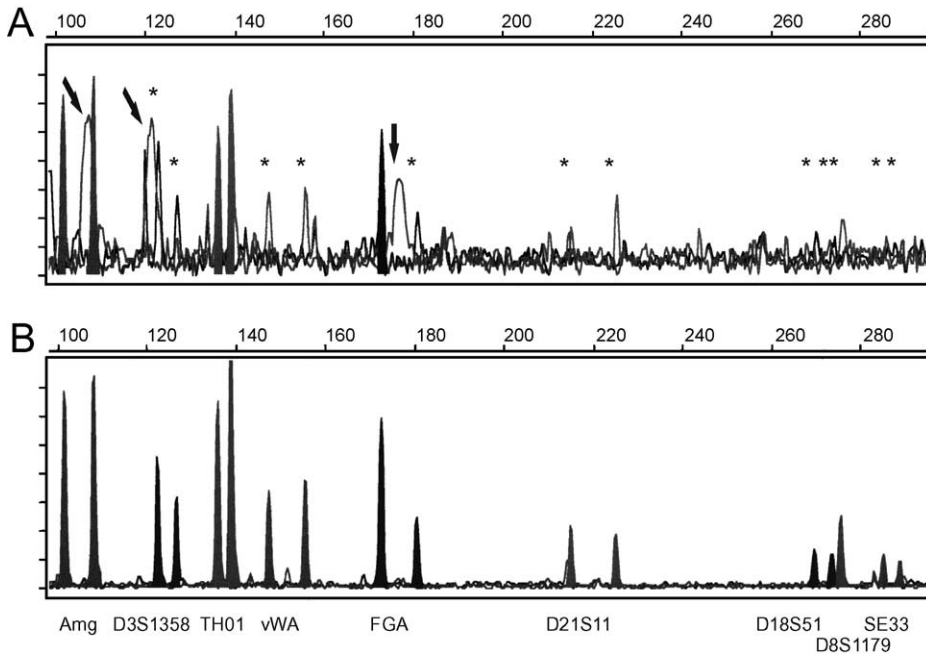


Fig. 1. Electropherograms of two identical blood samples. (A) Non-purified sample. (B) Purified sample. Allelic drop-outs (peak height threshold: 50 relative fluorescent units) are marked with an asterisk. Arrows depict non-specific signals, which may be due to residual fluorescent dye molecules. Runs were performed with a sample volume of 1 µl, each, and an injection time of 3 s. Amplified loci are indicated at the bottom.

samples, one of which was purified prior to CE, are shown. A total of 12 of the 17 allelic signals have not been detected by the analysis software in the non-purified sample, whereas all alleles were allocated in the purified one.

### 3.2. Injection time

The analysis of microspecimen often results in low signal intensities, suggesting an enhancing effect of longer injection times. In the range of 3 to 10 s, a linear dependence between signal intensity and injection time was observed in this study. Nevertheless, injection times longer than 10 s may influence electro-phoretic fragment separation and, therefore, lead to broad signals which may not be sized correctly.

## 4. Discussion

The presented experiments show that missing signal detection during electrophoresis may pretend allelic drop-out phenomena. A similar but weaker effect has recently been shown for amplified samples using AmpFlSTR Profiler Plus and COfiler [5]. The reason for a lack of detection can mainly be seen in the electrokinetic injection of the amplified fragments, which is greatly influenced by the ionic strength of the samples [4]. In addition, small DNA fragments, such as non-implemented primer molecules, compete with allelic fragments for sample injection due to their low molecular weight. In multiplex PCR systems, high amounts of residual primer molecules are present in the amplification mix and, therefore, may influence the overall sensitivity of the assay.

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